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January 2008

International Polar Year

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EMERGING INFECTIOUS DISEASES

January 2008

On the Cover

Fred Machetanz (1908–2002)
Quest for Avuk (1973)
Oil on board (81.3 cm x 130.8 cm)
Anchorage Museum at Rasmuson Center, Anchorage, Alaska.
1974.047.001 Gift of Mr. and Mrs. Elmer E. Rasmuson

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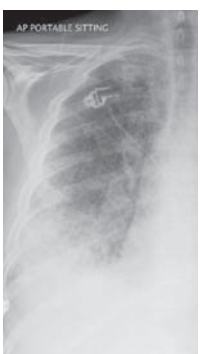

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January 2008

Another Dimension

Perspectives

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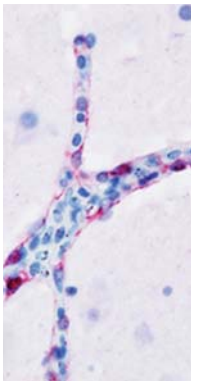

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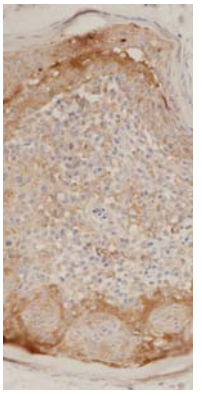

Dispatches

Letters

News & Notes

The International Polar Year, 2007–2008, An Opportunity to Focus on Infectious Diseases in Arctic Regions

Alan J. Parkinson*

On 3 occasions over the past 125 years, scientists from around the world have worked together to organize scientific and exploration activities in polar regions (www.ipy.org). The first International Polar Year (IPY) in 1881–1884 marked the first major coordinated international scientific initiative to collect standardized meteorological and geophysical data in polar regions. Fifteen expeditions led by 12 nations amassed a large amount of data, but the scientific value was diminished by disjointed publication efforts and lack of long-term institutional commitment; lessons were learned and corrected in subsequent polar years. The second IPY began in 1932. Forty-four nations led expeditions in the Arctic and Antarctic, resulting in greater understanding of the aurora, magnetism, and meteorology. Air and marine navigation, radio operations, and weather forecasting were greatly improved as a result. The third IPY, in 1957–58, was renamed the International Geophysical Year and capitalized on technologic advances developed during World War II. Technologic and scientific momentum was redirected toward research, particularly to studies of the upper atmosphere, a legacy that continues to the present day. Notable achievements included launching the first satellite, measurement of atmospheric greenhouse gases, delineating the system of mid-ocean ridges, and confirming the theory of plate tectonics.

The current 4th IPY covers the period March 2007 to March 2009, although it is officially designated IPY 2007–2008. It was established by the International Council for Science, the National Academy of Sciences and World Meteorological Organization. This period of focused scientific research promises to "... further our understanding of the physical and social process in Polar Regions, examine their globally-connected role in the climate system and establish research infrastructure for the future, and serve to attract and develop a new generation of scientists and engineers with the versatility to tackle complex global issues" (www.ipy.org). The 2007–2008 IPY also features human health as a research theme for the first time and thus presents an opportunity to do the following: 1) increase global awareness and visibility of health concerns of Arctic peoples, 2) foster human health research, 3) promote health protection strategies, and 4) ultimately improve the health and well being of Arctic peoples (www.arctichealth.org/ahhi).

The Arctic is unique in many respects. It has a sparse population, scattered over a very large geographic area; climate and latitude marked by seasonal extremes of temperature and daylight; and a spirited history of cross-border

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cooperation on issues of concern to Arctic peoples. The Arctic is home to ≈4 million people; approximately one tenth (350,000) are of indigenous ancestry (*1*). Many live in remote, isolated communities and are, as depicted by Fred Machetanz on the cover of this issue, still dependent on a traditional subsistence way of life that has little economic infrastructure. Health concerns of Arctic peoples include the remaining health disparities that exist between indigenous and nonindigenous segments of the population as well as the potential impact of a changing Arctic environment, characterized by rapid economic change and modernization, environmental pollution, alterations in the traditional subsistence food supply, and climate change (*2*).

Life expectancy in Arctic populations has greatly improved since the last IPY. For example, in 1950, the life expectancy for Alaska Natives, the indigenous people of Alaska, was 47 years at birth compared with 66 years for the general US population. By 2000, the life expectancy for Alaska Natives was 69.5 years, a gain of >20 years. Reductions in deaths from infectious diseases for Alaska Natives have been especially dramatic. In 1950, 47% of deaths among Alaska Natives were due to infections, as compared with only 3% for non-Native Alaskans. By 1990, infectious diseases caused only 1.2% of Alaska Native deaths, very similar to the 1% seen for non-Native Alaskans. Much of this improvement can be attributed to improved living conditions, provision of safe water and sewage disposal, implementation of vaccination programs, training of community-based health providers, and an integrated healthcare delivery system that provides improved access to better quality healthcare (*3*).

Despite improvements in these health indicators of Arctic residents, life expectancy is shorter and infant mortality rates are higher among indigenous Arctic residents in the US Arctic, northern Canada, and Greenland when compared with those of nonindigenous residents of Arctic countries. For example, life expectancy of Alaska Natives still lags behind that of the general US population, which was 76.5 years in 2000. Similarly, indigenous residents of the US Arctic, northern Canada, and Greenland have higher mortality rates from injury and suicide and as well as higher hospitalization rates for infants with pneumonia, meningitis, and respiratory infections (*4*–*6*). Some infectious diseases are linked to cultural practices of the indigenous population, such as botulism from ingesting improperly prepared traditionally fermented foods (*7*) and trichinosis from consuming meats from land and marine mammals (L.N. Moller, unpub. data). Many of these infectious disease health disparities can be eliminated through the focused application of existing public health strategies.

Many communities that were once isolated are now linked to major cities by air transportation and are only an airplane ride away from more densely populated urban centers. Consequently, these communities are now vulnerable to the importation of new and emerging infectious diseases (such as influenza, severe acute respiratory syndrome [SARS] or SARS-like infectious diseases and antimicrobial drug–resistant pathogens such as multidrug-resistant *Streptococcus pneumoniae*, methicillin-resistant *Staphylococcus aureus*, and tuberculosis).

The changing climate is already affecting Arctic communities. It is increasingly apparent that the most vulnerable will be those living a traditional subsistence lifestyle in remote communities; they are already facing health or economic challenges. The melting permafrost, flooding, and storm surges are progressively destroying village sanitation and drinking water infrastructures of many Arctic communities, paving the way for outbreaks of food- and water-borne diseases and respiratory infections (*8*). In addition, climate change may drive increased dissemination of zoonotic pathogens in water- and food-borne pathways (*Giardia*, *Cryposporidium*, *Toxoplasma*, *Trichinella*, and *Echinococcus* species), posing a direct threat to human health in communities that rely on wildlife as a source of food.

Temperature and humidity markedly affect the distribution, density, and behavior of many arthropod vectors and may increase the incidence and expand the northern range of many vector-borne diseases such as West Nile virus (*8*). Specific stages of the life cycles of many helminths and arthropods may be greatly influenced by temperature (*9*). For example, small changes in temperature can substantially alter the transmission of lung worms and muscle worms pathogenic to ungulates (caribou, muskoxen, thinhorn sheep, and moose). In other parts of the world, the convergence of population dynamics, environmental factors, and animal reservoirs has resulted in dramatic outbreaks of apparently new infectious diseases that constitute a considerable threat to global human health (most recently, SARS and avian influenza). The full impact of climate change on these host-parasite interactions, animal health population dynamics, and human health is unknown, but the known effects of climate change on these systems underscores the need for close monitoring.

In recognition of IPY 2007–2008, this issue of Emerging Infectious Diseases highlights infectious disease challenges faced by residents of Arctic regions. The IPY is a unique opportunity to increase awareness and visibility of infectious disease concerns of Arctic peoples. It can serve to reinvigorate cross-border collaborative infectious disease research networks that will focus on eliminating remaining health disparities caused by infectious diseases in these populations (www.inchr.org). Finally, the IPY can increase focus on development of sustainable international surveillance networks across the Arctic for monitoring infectious diseases of concern and evaluating the effectiveness of current intervention strategies (*10*). The establishment of these networks will be

essential for detecting the emergence of climate-sensitive infectious diseases in both human and wildlife populations and the design of effective interventions aimed at reducing risk and eliminating disease (*11*,*12*).

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Sexual Health and Sexually Transmitted Infections in the North American Arctic

Dionne Gesink Law,* Elizabeth Rink,† Gert Mulvad,‡ and Anders Koch§

Our objective was to describe the basic epidemiology of sexually transmitted infections for Arctic and sub-Arctic regions of North America. We summarized published and unpublished rates of chlamydial infection and gonorrhea reported from 2003 through 2006 for Alaska, Canada, and Greenland. In 2006, Alaska reported high rates of chlamydial infection (715 cases/100,000 population) compared with the United States as a whole; northern Canada reported high rates of chlamydial infection (1,693 cases/100,000) and gonorrhea (247 cases/100,000) compared with southern Canada; and Greenland consistently reported the highest rates of chlamydial infection (5,543 cases/100,000) and gonorrhea (1,738 cases/100,000) in the Arctic. Rates were high for both men and women, although the highest incidence of infection was predominantly reported for young women in their early twenties. We propose that community-based participatory research is an appropriate approach to improve sexual health in Arctic communities.

Four million people live in the Arctic (*1*), yet little is known about sexual health and sexually transmitted infections (STIs) in the circumpolar North. Arctic communities in North America comprise a large proportion of Native American, First Nation, Metis, Inuit, and other aboriginal peoples living in harsh climates, diverse landscapes, and a variety of community structures including urban, micropolitan, reserves or reservations, towns, villages, settlements, and remote fly-in communities. Access to healthcare varies by community and country and patient concerns about the preservation of confidentiality remain a barrier to accessing healthcare.

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STI intervention and prevention strategies have been developed primarily for urban and suburban environments (*2*,*3*), the rural South (*4*–*7*), Latino communities (*8*), and developing countries, primarily in Africa (*9*,*10*). Cultural differences alone will affect their generalizability to communities in the Arctic. This is further emphasized by Bjerregaard et al. (*11*) who stated: "Intervention models developed under quite different circumstances cannot be expected to work in Greenland and intervention studies are highly needed." However, combining the global knowledge gained from previous interventions involving other populations with the local knowledge and infrastructure of Arctic communities is important to develop innovative, culturally appropriate, and sustainable STI intervention strategies.

Our objective was to describe STI trends in the circumpolar Arctic, focusing on the North American continent (United States, Canada, and Greenland). We also propose a community-based participatory research approach to conducting research and planning interventions involving Arctic communities.

Methods

Data on chlamydial infection and gonorrhea in the United States, Canada, and Greenland were collected from a variety of sources. Rates for the United States and Alaska were obtained from federal (*12*) and state (*13*) reports. Rates for Canada were obtained in collaboration with the Public Health Agency of Canada STI Surveillance and Epidemiology Section (Public Health Agency of Canada, unpub. data; see also [*14*] for published summaries). Data reported for Canada's northern territories (Yukon, Northwest Territories, and Nunavut) were combined into 1 statistic and compared to data reported for Canada's southern provinces, which were also combined into 1 statistic. Data for Greenland were obtained from the Office of the Chief

Medical Officer in Greenland (*15*,*16*) and compared with data reported for Denmark by the Statens Serum Institut (www.ssi.dk). In situations where STI rates were not already available (primarily Greenland and Denmark), rates were calculated by dividing the number of cases by the total population and multiplying by 100,000. Population estimates were obtained from the US Census Bureau, Statistics Canada, Statistics Greenland, and Statistics Denmark (StatBank).

Chlamydial infection and gonorrhea rates reported for the year 2006 were standardized by age and sex to the year 2000 US population so that rates could be compared across countries after correcting for age and sex differences between the different populations. Rates were standardized to the year 2000 US population by using age- and sex-stratified counts available from the US Census Bureau (www.census.gov). Additionally, chlamydial infection and gonorrhea rates were stratified by the basic demographic characteristics of age, sex, and race (when available) to gain insights into target populations for community interventions. Rates by race were only available for Alaska.

Results

As expected, chlamydial infection was the most highly reported STI for the United States, Canada, and Greenland (Table 1). Compared with other states in the United States, Alaska reported the highest rates of chlamydial infection in 2003 and second highest in 2004 and 2005 (*12*). Canada's northern territories consistently reported the highest rates of chlamydial infection in Canada, which is consistent with the 1987–1994 rates measured by Orr and Brown (*17*) for the Keewatin District of the Canadian Central Arctic. Greenland reported chlamydial infection rates higher than Denmark and higher than any other country in the North American Arctic.

Co-infection with chlamydial infection and gonorrhea is common so we expected gonorrhea rates to be high for the Arctic regions. However, Alaska reported some of the lowest gonorrhea rates in the United States (*12*). As expected, however, the Canadian Northern Territories reported higher gonorrhea rates than their southern counterparts, and again, Greenland reported gonorrhea rates higher than those in Denmark and in any other country in the North American Arctic (Table 1).

Chlamydial infection rates reported for women were much higher than rates reported for men in Alaska (Table 2), Canada (Table 3), and Greenland and Denmark (Table 4). Compared to gonorrhea rates reported for men, however, gonorrhea rates were higher for women ≤30 years of age in Alaska (Table 2), ≤20 years of age in Canada (until 2006, when rates remained higher for women ≤24 years of age; Table 3), and ≤20 years of age or ≤30 years of age for women in Greenland (Table 4). Gonorrhea rates reported for men in Denmark were consistently higher than rates reported for women (Table 4). Reported rates of chlamydial infection and gonorrhea were consistently high for both men and women 15–30 years of age, particularly for those 20–24 years of age, regardless of country.

Discussion

Chlamydial infection rates were higher for Arctic and sub-Arctic areas in North America than for their southern counterparts. Gonorrhea rates reported for northern Canada and Greenland were also much higher than for their southern counterparts, although rates reported for Alaska were not very high. In 1741, Hans Egede, the first missionary to Greenland noted that "It is strange ... that even though [Greenlanders] have free intercourse with other people, these are not infected" (*11*). However, for the past several years Greenland has reported chlamydial infection rates

Table 1. Comparison of sexually transmitted infection rates reported for North America's Arctic countries, 2003–2006

Yearly rate*	United States	Alaska, USA	Canada	Northern territories,† Canada	Southern provinces,† Canada	Denmark	Greenland
Chlamydial infection							
2003	301.7	601.1	189.4	1,433	185	342	3,255
2004	316.7	609.4	197.1	1,805	195	401	3,208
2005	332.5	664.4	200.4	1,952	195	441	4,762
2006	347.8	682	202.2	1,922	197	458	4,527
2006 standardized‡	470.9	715	205	1,693	200	681	5,543
Gonorrhea							
2003	115.2	88.3	26.0	264	25	3.5	1,162
2004	113.5	87.4	28.9	215	29	7.7	1,148
2005	115.6	91.5	27.8	212	28	8.2	1,350
2006	120.9	95	33.1	281	32	7.5	1,418
2006 standardized	164.4	101	33	247	32	6.5	1,738

*Per 100,000 population. Data from Centers for Disease Control and Prevention, 2006 (*12*); Public Health Agency of Canada, 2007 (*14*); Office of the Chief Medical Officer in Greenland (*15,16*); Statens Serum Institute surveillance Epi-data online (www.ssi.dk).
†Canadian Northern territories: Yukon Territory, Northwest Territories, and Nunavut; Southern provinces: British Columbia, Alberta, Saskatchewan, Manitoba, Ontario, Quebec, Newfoundland, Nova Scotia, New Brunswick, and Prince Edward Island.
‡2006 standardized estimates are directly standardized to the year 2000 US population distributed by age and sex.

Table 2. Chlamydial infection and gonorrhea rates per 100,000 population by age, sex, and race reported for Alaska, 2006*

Characteristic	Chlamydial infection rates		Gonorrhea rates	
	M	F	M	F
Age, y				
15–19	966	4,158	78	346
20–24	2,673	4,990	344	496
25–29	1,250	2,253	185	309
30–34	607	854	162	162
35–39	269	420	134	81
Race				
White	235	389	28	41
Alaska Native/ American Indian	927	3,012	153	344

*Source: (*13*).

≈10× higher, and gonorrhea rates ≈100× higher, than rates reported for Denmark and the highest rates of both infections in the North American Arctic (Table 1).

Chlamydial infection and gonorrhea rates reported for the Arctic and sub-Arctic are very high for both men and women, although the highest incidence of infection is predominantly reported for young women in their early 20s (Tables 2–4). True rates could be higher than reported for a variety of reasons. As in other settings, asymptomatic infection is high for both men and women and can result in missed cases. How much knowledge exists in remote communities about STIs, their symptoms, and what to do if one suspects he or she has an infection is unclear. Even if a person suspects that he or she has an infection, accessing healthcare can be a challenge since many of the Arctic communities are remote fly-in communities with limited healthcare resources. Additionally, many Arctic residents spend their summers away hunting or whaling, usually at great distances from their communities, and certainly far away from a healthcare provider. Another barrier to care in small communities is the issue of confidentiality and the common perception that it can be breached easily. This results in delayed healthcare seeking or missed infections. Partner notification can also be hindered by cultural norms and taboos. For instance, in some communities, talking about something can be regarded as the same as wishing it upon the people. Therefore there can be a reluctance or even movement against talking about STIs or naming sexual contacts. Finally, reporting infections can become a challenge in an already overtaxed healthcare system with limited infrastructure.

STI rates are quite variable across the North American Arctic and sub-Arctic (Tables 1–4). Access to healthcare and reporting differences could explain some of the difference in rates. For instance, Greenland has universal healthcare. Canada has universal healthcare, but it differs for on-reserve and off-reserve aboriginal people. Alaska only has universal healthcare for indigenous people. These different healthcare coverage strategies could affect the healthcare-seeking behavior of the populations that live with them. Another nuance of northern rates is the small underlying populations from which cases arise. The addition of 1 new case can result in a large change in the rate of infection. Additionally, because no international surveillance system is in place to monitor STIs, the information collected is not standardized between the countries. For instance, in the United States, the only country that collects racial informa-

Table 3. Chlamydial infection and gonorrhea rates per 100,000 population by age and sex reported for northern territories (NT) and southern provinces (SP) in Canada, 2004–2006*

	2004				2005				2006			
	NT		SP		NT		SP		NT		SP	
Characteristic	M	F	M	F	M	F	M	F	M	F	M	F
Chlamydia, age, y												
<14	22	319	1	19	22	361	0.8	18	8	296	1	16
15–19	3,050	10,014	276	1,428	3,193	11,866	270	1,367	3,374	10,771	278	1,329
20–24	4,778	9,408	695	1,478	5,255	8,893	701	1,470	4,982	9,431	703	1,475
25–29	3,154	4,492	405	552	3,623	4,435	423	562	3,192	5,024	419	592
30–39	1,292	1,913	141	158	1,461	1,856	157	158	1,697	1,812	164	170
40–59	338	359	31	22	486	450	34	21	399	432	36	24
≥60	120	142	4	1	90	215	4	1	84	126	5	2
Total	1,190	2,451	128	260	1,339	2,595	132	256	1,312	2,556	134	258
Gonorrhea, age, y												
<14	0	23	0	2	7	38	0	3	0	23	0	4
15–19	376	761	57	124	437	737	53	112	671	1,473	63	132
20–24	820	763	126	99	968	650	118	102	1,038	1,246	132	118
25–29	738	332	91	43	689	433	93	41	557	526	104	57
30–39	306	169	65	14	311	86	61	15	267	234	67	21
40–59	137	15	23	3	71	45	23	2	86	25	27	0
≥60	24	0	4	0	22	0	4	0	42	25	5	0
Total	240	189	37	21	239	184	35	20	251	312	40	25

*See (*14*).

Table 4. Chlamydial infection and gonorrhea rates per 100,000 population by age and sex reported for Greenland (GLD) and Denmark (DK), 2004–2006

	2004				2005				2006			
	GLD		DK		GLD		DK		GLD		DK	
Characteristic	M	F	M	F	M	F	M	F	M	F	M	F
Chlamydia, age, y												
15–19	9,378	20,332	944	3,361	7,986	31,383	1,243	3,891	12,462	27,125	1,345	4,095
20–24	13,229	16,890	1,966	3,526	9,003	20,594	2,264	3,720	17,154	21,854	2,391	3,768
25–29	6,444	8,590	978	1,284	5,776	11,006	1,089	1,322	10,837	10,445	1,114	1,335
≥30	916	1,229	86	41	805	1,005	85	87	1,630	1,507	93	88
Total	2,481	4,158	287	511	3,852	5,597	324	554	3,704	5,468	343	571
Gonorrhea, age, y												
15–19	3,714	7,346	13.9	7.7	2,141	7,801	11.5	4.0	5,360	8,763	11.1	6.5
20–24	4,663	4,450	46.4	7.4	2,993	5,648	39.9	13.1	5,994	5,858	32.5	16.0
25–29	3,056	1,795	51.3	4.5	1,984	3,184	44.9	4.7	3,755	2,374	39.4	10.8
≥30	493	434	10.7	1.2	224	312	14.1	1.1	363	498	12.4	1.2
Total	1,047	1,301	13.6	1.9	1,174	1,550	14.6	2.0	1,252	1,609	12.5	2.6

tion as part of its surveillance program, chlamydial infection, gonorrhea, and syphilis rates reported for American Indians and Alaska Natives were recently reported to be 2–6× higher than rates reported for non-Hispanic whites (*18*). In Canada, STI rates are suspected of being higher for aboriginal people, but no data exist to confirm this hypothesis. In Greenland, 89% of the population is Kalaallit Inuit, making it of arguable importance to collect racial information for Greenland.

There is a dearth of research pertaining to the factors that contribute to sexual health and STIs among aboriginal people (*18*,*19*). Chlamydial infection and gonorrhea are major causes of ectopic pregnancy in the Canadian Arctic (*17*), and STIs are highest for Canadian aboriginal people 15–24 years of age (*20*). Westernization, culture, and identity have been suggested as possible factors influencing STI transmission among Inuit youth in northern Canada (*20*); however, research is still needed to provide evidence for this hypothesis. Most other studies have focused on HIV and AIDS (*19*,*21*).

In Greenland, much of the STI research has also focused on HIV/AIDs. HIV/AIDS came late to Greenland compared to the rest of Europe and has remained limited to a heterosexual, alcohol-abusing group of persons of low socioeconomic status living in 2 communities in western Greenland. One reason that HIV, unlike other STIs, has not become a widespread epidemic across Greenland is because the prevalence of needle sharing and men who have sex with men is limited, considerably affecting the modes of transmission (*11*). However, research on HIV transmission among heterosexual persons, as well as the increased risk for co-infection with STIs and HIV, suggests that chlamydial and gonorrhea rates in Greenland are a public health concern that warrant further investigation (*22*–*24*).

As suggested by Steenbeek et al. (*20*), colonization and westernization in the Arctic may be responsible for increased rates of STIs for Arctic communities. We further hypothesize that these factors are contributing to disparately high STI rates in the Arctic through individual, familial, social, cultural, and environmental domains. We also hypothesize that high STI rates may only be a marker of greater underlying public health concerns such as substance abuse, poor mental health, and the legacy of historic trauma.

Implications for Future Research

We propose that community-based participatory research (CBPR) is an appropriate approach to address sexual health and STIs in the Arctic. Sexual and reproductive health data for aboriginal populations are often not reported in national surveillance and survey reports (*25*). Also, indigenous communities have historically been reluctant to participate in research projects because traditional research methods, which emphasize the researcher as "the expert," have not engaged indigenous communities in designing and implementing research projects (*25*). CBPR has been identified as an effective and essential strategy for conducting research with indigenous peoples because of its emphasis on community participation to build ownership of research projects and community-based interventions as well as empowering the community to address its health disparities (*26*,*27*).

Several components of CBPR support its use as a methodologic framework for conducting research in aboriginal communities. First, CBPR engages aboriginal or indigenous people in full and equal partnership with those communities in efforts to observe and respect tribal sovereignty and the right to self-determination (*28*). Second, the growing interest in addressing the interrelatedness of historic trauma and health disparities in indigenous populations and the inherent complexities of unraveling the interconnected components and concepts related to historic trauma and health can best be understood by discussions and conversations with indigenous communities (*29*,*30*). Third, a legacy of harm

from past research, as well as mistrust of researchers, warrants the use of CBPR as a means to ensure that all phases of a research project, from the development of research questions to research design and data collection methods to dissemination of results, have community input and approval (*26*,*31*). Fourth, CBPR provides a forum to ensure timely communication of research results to the community by using information dissemination mechanisms that best meet the community's needs. Finally, the limited research on sexual health among indigenous populations primarily focuses on problem theory that provides insights into the predisposing, enabling, and reinforcing factors related to engagement in high-risk sexual behavior among aboriginal communities. However, emerging evidence in the field of aboriginal sexual health suggests that a risk-based approach to understanding sexual behavior in these communities not only has a narrow and negative focus, with scant opportunities for indigenous groups to capitalize on their strengths, but also is not congruent with indigenous cultural and social beliefs and historical experiences (*32*). CBPR, because of its collaborative nature, empowers community members to capitalize on the strengths and resources available in their community.

Conclusion

The use of CBPR as a framework in which to conduct sexual health research with and among indigenous populations is a promising approach that joins the strengths and skills of researchers with local knowledge, wisdom, traditions, and resources. The CBPR approach is much like taking a Bayesian approach to study design, data collection, analysis, interpretation, dissemination, and follow-up. Researchers provide global (prior) knowledge that is then integrated and updated with local (likelihood) knowledge provided by the community to produce a more holistic model of health. This approach means that study designs can be more effective, data collection can be more accurate and complete, interpretation of the results can be more insightful and relevant, dissemination of the study results can be more efficient and translated at the appropriate level for the community by community members, and interventions can be more effective, culturally appropriate, and sustainable. Community involvement in the project can also help facilitate translation of the research findings into clinical and political practice.

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Integrated Approaches and Empirical Models for Investigation of Parasitic Diseases in Northern Wildlife

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The North is a frontier for exploration of emerging infectious diseases and the large-scale drivers influencing distribution, host associations, and evolution of pathogens among persons, domestic animals, and wildlife. Leading into the International Polar Year 2007–2008, we outline approaches, protocols, and empirical models derived from a decade of integrated research on northern host–parasite systems. Investigations of emerging infectious diseases associated with parasites in northern wildlife involved a network of multidisciplinary collaborators and incorporated geographic surveys, archival collections, historical foundations for diversity, and laboratory and field studies exploring the interface for hosts, parasites, and the environment. In this system, emergence of parasitic disease was linked to geographic expansion, host switching, resurgence due to climate change, and newly recognized parasite species. Such integrative approaches serve as cornerstones for detection, prediction, and potential mitigation of emerging infectious diseases in wildlife and persons in the North and elsewhere under a changing global climate.

Insights about environmental change and emerging infectious disease have been derived primarily from temperate and tropical systems (*1–3*), even though host–pathogen relationships at higher latitudes of the Northern Hemisphere are affected by rapid climate change and anthropogenic disturbance (*4–8*). The North, therefore, serves as a sentinel and a complementary window, relative to temperate and tropical environments, for assessing the cascading ecologic effects of global climate change. Within relatively simple northern ecosystems, it is possible to differentiate signals of climate change from those associated with nonclimatologic drivers. Consequently, the North is a vital frontier for the exploration of biotic, abiotic, and historical determinants that influence the distribution, host associations, and evolution of pathogens in wildlife and human populations (*4*,*6*,*9*,*10*).

Host–parasite systems are particularly sensitive indicators of climate change and other causes of ecologic perturbation (*3*,*11*). Many macroparasites (e.g., helminths and arthropods) undergo life cycles with free-living stages whose development and survival are influenced by temperature, among other abiotic factors (*2*,*6*). For example, small changes in absolute temperatures can have substantial effects on the transmission dynamics of protostrongylid lungworms and muscleworms (species of *Parelaphostrongylus*, *Protostrongylus*, and *Umingmakstrongylus*), which cycle among the environment, gastropod (slug and snail) intermediate hosts, and ungulate (caribou, muskoxen, thinhorn sheep, moose) definitive hosts (Figure 1). These parasites are pathogenic in wildlife important to northern communities for cultural, economic, and spiritual reasons (*4*). The importance of keystone wildlife (those critical for the function and continuity of ecosystems and northern peoples), the potential sensitivity of host–pathogen assemblages to environmental disturbance, and the present and predicted scale of accelerated climate change and cascading ecologic

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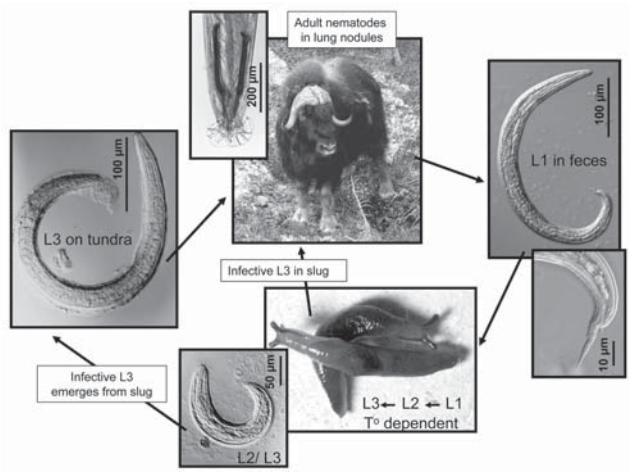

Figure 1. Life cycle of protostrongylid parasite: *Umingmakstrongylus pallikuukensis* in muskoxen definitive and gastropod intermediate hosts (*12*). Adult nematodes (for *U. pallikuukensis*, located in the lungs) lay eggs, which hatch to first-stage larvae (L1). L1 move up the airways, are swallowed, and pass in the feces, where they must invade the foot of gastropod intermediate hosts for further development to the infective third-stage larvae (L3). Development to L3 requires a minimum amount of heating and does not occur below a critical threshold; these development parameters vary among different protostrongylid species (*6,7,13*). Definitive hosts become infected by ingesting a gastropod containing L3 or, for some protostrongylids such as *U. pallikuukensis*, by ingesting L3 that have emerged from the gastropods and are free in the environment.

effects in the North give urgency to the need for a better understanding of these systems now and in the future. These environmental changes may presage substantial consequences for health, economic well-being, and continuity of culture and society at a global level.

The current International Polar Year, which covers the period March 2007 to March 2009 (although officially designated International Polar Year 2007–2008), offers a unique opportunity to develop a baseline, or snapshot, of the North and to explore the patterns and processes for infectious disease emergence in northern ecosystems that are globally relevant. As a contribution to this baseline, we outline approaches, protocols, and empirical models derived from a decade of exploration of pathogen biodiversity and disease in northern ecosystems (Table 1), illustrated with examples of drivers for emerging parasitic disease in northern wildlife (Table 2). Categories of emergence explored in our studies include 1) geographic expansion, 2) host switching, 3) resurgence due to climate change, 4) new introduction, and 5) new recognition of pathogens. This approach can be viewed as a broadly applicable framework for defining pathogen diversity, including distinguishing what is truly emerging from what is newly discovered, and the historical and contemporary drivers associated with emerging infectious diseases in the North and elsewhere in the world. This ecologic approach to understanding disease emergence in wildlife complements a growing recognition of the need for long-term epidemiologic datasets to detect the effects of climate change on human health (*8*). Collectively, these constitute a critical sentinel for the interaction of climate and infectious disease in the global arena.

The Approach and Tools

Defining Pathogen Diversity

Until recently, few baseline investigations had been carried out on the health of wildlife in northern North America (*4*,*14*–*18*). To develop an accurate and comprehensive baseline of diversity (parasite species and their host and geographic associations), we conducted opportunistic and targeted field surveys (Figure 2). Physical vouchers (representative specimens that are definitively identified and held in permanent museum repositories), frozen tissues, and associated data formed the basis for archival collections (*9*,*11*,*18*). Archival collections link surveys, informatics, phylogenies, history, biogeography, and ecology to form long-term baselines for exploring biodiversity and the evolutionary and ecologic determinants of infectious disease emergence (*3*,*9*,*18*). Such baselines allow tracking of changes in pathogen diversity and genetics, as well as differentiation between new detection versus true emergence of diseases.

Challenges associated with defining pathogen diversity in the North included a literature in which parasites of northern wildlife were often assumed to represent the same array of species as those commonly found in domestic animals from temperate regions. Further complications arose from the absence of properly preserved and documented parasite specimens and the specialized knowledge needed to identify such specimens to the species level. To identify parasite species in wildlife across extensive geographic ranges in remote northern locations, we developed molecular probes for the identification of larval parasites in feces. We used these techniques to redefine the distribution of a parasite (*Parelaphostrongylus odocoilei*) in multiple host species across northwestern North America (*15*) and to detect at least 1 new protostrongylid species in moose, muskoxen, and caribou in northern North America (*20*; Figure 2); these fell within the category of emergence of new recognition of a pathogen. In addition, by using a combination of morphologic and molecular methods of identification, we detected a host switch of a lungworm (*Protostrongylus stilesi*) from Dall sheep to sympatric muskoxen (*21*) (category of emergence: host switching).

A fine-scale understanding of these host and geographic associations allows us to identify naive wildlife populations and host species vulnerable to colonization by pro-

Table 1. Approaches and tools for exploring diversity and changes in complex host-parasite systems

Definition of pathogen diversity
1) Geographically extensive and site intensive survey and inventory
2) Determination of faunal diversity for species present, for which systematics is the foundation
3) Patterns of association for hosts
4) Geographic range for hosts and parasites
5) Numerical, abundance/ intensity data
6) Seasonal data for distribution and patterns of transmission
7) Survey linking parasite species diversity to population structure requiring integrated morphologic and molecular approaches for accurate and rapid diagnostics
8) Molecular prospecting for diversity
9) Distribution of parasites versus distribution of disease
Development of historical baselines
1) Archival museum collections
2) Host-parasite phylogenetic frameworks
3) Historical ecology and biogeography/phylogeography to clarify past abiotic and biotic determinants of distribution
4) Geographic information system applications
5) Analogue approaches to be applied where historical processes that have structured faunas are used to inform or predict the responses of contemporary systems under a regime of dynamic climate change
Exploration of environmental effects
1) Define thresholds and rates for development
2) Define tolerances for environmental parameters, e.g., temperature, humidity, precipitation
3) Define environmental limitations on distribution
Characterization of disease conditions
1) Laboratory-based experimental infections in parasite naïve hosts
2) Pathology and histology
3) Evaluations of natural mortality and associations with parasites
Establishment of surveillance networks and monitoring
1) Targeted strategic survey and inventory
2) Opportunistic networks linking wildlife managers and communities
Development and testing of predictive models
1) Integrative frameworks incorporating data from survey, parasite diversity, historical analogues, environmental thresholds, tolerances and constraints
2) Responses under scenarios for climatologic/environmental change
3) Validation through long-term monitoring

tostrongylids and other parasites. For example, exchange of protostrongylid parasites (*Umingmakstrongylus pallikuukensis* and *P. stilesi*) may occur between reciprocally naive populations of endemic and introduced subspecies of muskoxen at the ecotone (interface between ≥2 regions or habitats) formed by the Mackenzie River (*4*,*21*) (category of emergence: host switching and geographic expansion). In addition, our extensive survey across northern North America reinforces the hypothesis that Old World protostrongylids (i.e., species of *Elaphostrongylus*, *Neostrongylus*, and *Cystocaulus*) do not naturally occur in North America and have not yet been introduced (*20*), with the exception of *Elaphostrongylus rangiferi*, which has been translocated and established on the island of Newfoundland in eastern Canada (*19*) (category of emergence: new introduction of pathogen).

History of Northern Host–Parasite Systems

The diversity and distribution of hosts and pathogens in the North have been structured by dynamic climate change during the past 3 million years through alternating cycles of glaciation/deglaciation and isolation/expansion (*10*,*22*). Increasingly cold climate during the Pliocene and Pleistocene periods may have further influenced selection of an Arctic-adapted fauna with its own unique developmental constraints and environmental tolerances (*10*,*22*). Historical climate dynamics can serve as an analog for the effects of accelerated change currently experienced in the North (*9*,*18*,*22*), including the emergence of pathogens and disease. Based on our understanding of the past determinants for northern host–parasite systems, we predict increased host switching and range expansion as climate change removes ecologic barriers and developmental constraints for pathogen transmission and redraws the maps of host distributions and timing of seasonal movements by hosts (*10*,*23*).

A broader understanding of the drivers for infectious disease emergence is in part dependent on appreciating why pathogens occur in particular regional settings and host groups, which is in turn determined by evolutionary and ecologic constraints that have structured pathogen diversity in space and time (*11*,*22*,*23*; Table 2). Our interpretations of current host and geographic distributions of northern parasites have been guided by a strong foundation in historical processes, both recent and deep (10 thousand years before present [KYBP] to 5 million years before present [MYBP]) (*9*,*18*,*22*). For example, the current focal distribution of the Arctic-adapted lungworm *U. pallikuukensis* in a circumscribed population of muskoxen (Figure 2) is attributed to recent host extirpation and reduction to a remnant population in the early 1900s, primarily as a result of hunting (*12*,*14*). In contrast, deeper historical processes (600–300 KYBP) account for widespread distribution of the lungworm *P. stilesi* in wild sheep, which entered North America across Beringia, the land mass that historically joined North America and Siberia (*10*,*24*). The muscleworm *P. odocoilei* has a more heterogeneous distribution in wild sheep because these hosts were colonized relatively recently (late Pleistocene) in a host-switching event from deer (*Odocoileus hemionus*), probably when the 2 species were concentrated in glacial refugia, and later expanded northwards following deglaciation (categories of emergence: host switching and geographic expansion) (*15*,*24*; I. Asmundsson, E.P. Hoberg, E.J. Jenkins, unpub. data).

Knowledge of phylogeny of pathogens and co-evolutionary processes for parasites and hosts provides powerful insights about life cycles, host specificity, and site specificity within the host and the potential for emergence (*3*,*11*,*22*,*23*). For example, knowledge of the relationship and behavior of related parasites provided immediate information on the likely life history of *U. pallikuukensis* (*12*,*14*) and site specificity and potential hosts for *P. odocoilei* (*17*) and is now guiding our search for adults of a previously undescribed protostrongylid (*20*). Finally, we have a unique window into the past to examine historical diversity and phylogeny as pathogens in frozen feces of caribou and other northern wildlife species (both extant and extinct) emerge from receding ice packs—a natural cryoarchive. Whether these ancient pathogens represent a risk for reemergence of disease remains undetermined (*25*).

Table 2. Responses to climate warming and drivers for emergence of parasites and parasitic diseases in Arctic systems

Numerical responses (changes in abundance of parasites)
1) Temperature-mediated increases in rates of development for free-living stages, or those in intermediate hosts
2) Reduced parasite generation time, e.g., shifts from multiyear to single-year cycles, or from single to multiple within year
3) Environment-mediated changes (increases or decreases) in survival rates for developmental stages
4) Extension of season for parasite growth and transmission resulting from earlier thaw in spring and/or later freeze during fall
5) Amplification of parasite populations over time through accelerated development, increased rates of transmission, survival, and availability
6) Increases in parasite prevalence and abundance
7) Changes in density-dependant linkages for hosts and parasites leading to altered patterns of abundance for host populations
Functional responses (changes in host and geographic ranges)
1) Shifting patterns of geographic range for hosts and parasites including latitudinal and/or altitudinal shifts
2) Alterations in host range for parasites through geographic and host colonization, successful establishment in naive host species or host populations
3) Changing phenology (timing) for habitat use through alteration of migration and migratory corridors, relative changes in spatial and temporal overlap
4) Modification of ecotones and contact zones including northward or southward expansion for hosts and/or parasites if environmental tolerances are not exceeded
5) Local extirpation because conditions exceed developmental tolerances
Microevolutionary responses
1) Local adaptation through selection for optimal patterns of development
2) Directional changes in gene frequencies for parasites
3) Geographic mosaics or ephemeral patterns of local adaptation and emergence
Cumulative/synergistic responses
1) Breakdown in mechanisms for ecologic isolation promoting faunal interchange for hosts and parasites and cascading changes in ecosystems
2) Variable and cumulative synergy affecting the structure of entire parasite–host communities during episodes of climate change

Investigation of Natural and Experimental Disease

New host and geographic records for a pathogen do not constitute an emerging infectious disease unless the pathogen, either alone or in combination with other factors (e.g., weather, malnutrition, predation), actually causes disease. Disease associated with macroparasites is often sublethal and difficult to detect in free-ranging wildlife. This is especially true in the North, where the sheer size and diversity of the geographic areas, low densities and isolation of human communities, and remote study areas hamper year-round observation of illness and death in wildlife. Therefore, we recruited local community members and hunters to create a network of "wildlife health monitors," who are trained to collect standardized samples and data to develop baselines for wildlife health over time (http://wildlife1.usask.ca/sahtu/monitors.php). Even if a decline in a wildlife population is detected and documented, the role of disease can be difficult to tease from an array of complex ecologic relationships. Therefore, to isolate the effects of single pathogens and evaluate subclinical disease, we experimentally infected captive wildlife and monitored them by using a variety of veterinary clinical techniques (*24*,*26*).

Within the host, pathogenesis varies relative to parasite species, intensity of infection, and host factors such as age and immune status. In muskoxen, infections of *U. pallikuukensis* are cumulative with age, and adult parasites are associated with large, space-occupying lesions in the lungs. Infected animals exhibit epistaxis and may be more susceptible to predation (*12*,*14*). In wild sheep, *P. odocoilei* causes diffuse, pulmonary granulomas that can lead to respiratory failure in heavily infected or otherwise compromised animals. Experimentally infected sheep exhibited marked weight loss and transient neurologic signs, which, together with decreased respiratory function, would compromise their ability to escape predation at high altitudes and over rough terrain (*24*,*26*). Also, respiratory pathology may result from synergy of *P. odocoilei* with the lungworm *P. stilesi* because both parasites are present in almost all wild sheep in some regions (*24*,*26*). Infections of *Parelaphostrongylus andersoni*, possibly in combination with the new protostrongylid species, have been linked to verminous pneumonia in barren-ground caribou in Alaska and Canada.

The potential for protostrongylids to cause disease in individual hosts is apparent, but unequivocal evidence linking infection to declines in population health, numbers, or both remains to be demonstrated. Detection of population-level effects of protostrongylid parasites through experimental treatment of subpopulations is confounded by the broad-spectrum nature of anthelmintic treatment, which

would eliminate many other nematode species (*27*). Therefore, future work may be limited to modeling the effects of these parasites on host populations and monitoring causes of illness and death in natural populations.

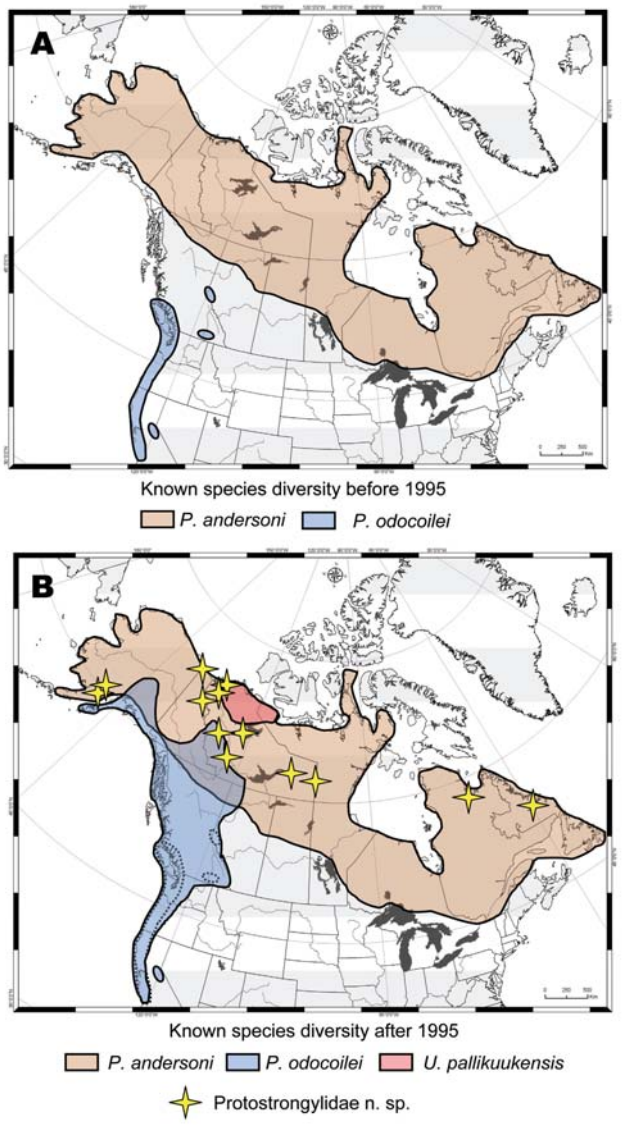

Figure 2. Geographic ranges for protostrongylid parasites in northern ungulates showing how survey and inventory have dramatically altered our understanding of diversity and distribution, before (A) and after (B) 1995. Distributions are depicted for *Parelaphostrongylus andersoni* in caribou (*19,20*); *P. odocoilei* in wild thinhorn sheep, mountain goat, woodland caribou, black-tailed deer, and mule deer (*15,17*); *Umingmakstrongylus pallikuukensis* in muskoxen (*12,14*); and a putative new species of Protostrongylidae in moose, caribou, and muskoxen (*20*). The range for *P. andersoni* in the North is presumed to coincide with caribou, although records substantiated by survey are few (*19,20*). Protostrongylids have not been detected in ungulates from the Arctic islands and Greenland and may be excluded from these high latitudes under current climate conditions.

Climate Change as a Driver for Infectious Disease Emergence

An evidence-based process is necessary to demonstrate a clear link between climate change and emergence of pathogens and disease, as the interactions are complex and nonclimate drivers may alter patterns of disease in both animals and humans (*2*,*8*,*28*,*29*; Table 2). To connect the dots, we must 1) collect baseline data on distribution, epidemiology, and effects of pathogens; 2) isolate the effects of temperature on pathogen transmission in laboratory and field; 3) provide regional evidence of climate change; 4) forecast temporal and spatial effects of climate change on parasite and host populations; and 5) detect epidemiologic and health consequences of climate change (*2*,*24*). In our study system, protostrongylid parasites in northern ungulates, we have accomplished much of steps 1–4 and continue to develop a detection system for step 5.

For step 1, an integrative research framework showed substantial new insights about the distribution, host associations, and behavior of pathogenic parasites in northern wildlife (Table 1). For step 2, field and laboratory experiments showed specific tolerances and thresholds and a direct relationship between temperature and development of *U. pallikuukensis* and *P. odocoilei* in intermediate hosts (*7*,*12*). For step 3, historical temperature data demonstrated that the core region for our studies (the Mackenzie District of the Northwest Territories) had the highest rate of warming in Canada (60-year warming trend of 2.2°C in annual temperature; www.msc-smc.ec.gc.ca/ccrm/bulletin/annual06/regional_e.cfm), and that warming available for parasite development had already increased at multiple sites in the Canadian Arctic (*4*,*6*,*7*). For step 4, a model for temperature and rates of larval development in gastropod intermediate hosts was validated for these protostrongylids and applied to climate warming scenarios (*6*,*7*). Based on these projections, we determined that climate warming led to substantial amplification of parasite populations in disease-endemic areas through reduction in generation times and broadened seasonal windows for transmission, contributing to heightened intensity of infection among individual hosts (*4*,*6*,*7*). Climate change also may result in release of some "generalist" parasites (e.g., *P. odocoilei*) from environmental constraints, which, in combination with a breakdown in mechanisms for ecologic isolation, could facilitate range expansion and colonization of naïve hosts (2 categories of emerging infectious disease) (*7*).

For step 5, we demonstrated that climate change precipitated a switch from a multiyear to single-year cycle of transmission for *U. pallikuukensis*, possibly starting in the late 1980s and coinciding with reports of clinically ill muskoxen in the disease-endemic region (category of emergence: resurgence due to climate change) (*4*,*6*). Following the warmest recorded annual temperature for this region

in 1998 (www.msc-smc.ec.gc.ca/ccrm/bulletin/annual06/rsummarytable_e.html?table=temperature&season=annual&date=2006&nyears=59), fatal pneumonia associated with *P. odocoilei* was first detected in wild sheep in the Mackenzie Mountains in 1999, although this may reflect increased vigilance rather than true emergence. Emergence of disease may follow climate change, but for macroparasites in particular, there are likely to be lag times determined by a period of development in the host population (*30*). Cascading and cumulative long-term effects of climate change, including shifts in host–pathogen relationships, may be among the factors contributing to large-scale changes in abundance and distribution observed in keystone wildlife (e.g., boreal woodland and barren-ground caribou) in northern North America.

Lessons Learned and the Way Forward

During the past decade, our investigations of emerging parasitic infections and diseases in northern wildlife have moved from opportunistic survey to targeted surveillance and hypothesis-driven research (Table 1). This work relied on field observation, laboratory experimentation, and enhanced diagnostic capacity by a network of collaborators with expertise in traditional parasitology, morphologic and molecular systematics, population genetics, epidemiology, wildlife biology, and traditional ecologic knowledge. Exchange of information relied on a system to collect and respond to community-based local knowledge and to respond to feedback in the form of community meetings and reports accessible to a lay audience. Meaningful engagement of aboriginal communities and integration of Western science with traditional knowledge has become a key part of any northern research (*31*; http://wildlife1.usask.ca/sahtu) and is a major focus of International Polar Year.

International Polar Year is a unique opportunity to address a critical need for baselines and archives needed to detect and explore drivers for infectious disease emergence in northern systems. While past efforts have been primarily opportunistic or specific to a particular issue, species, or location, we are seeing the development of long-term programs with a broad vision ensuring a comprehensive approach, such as the Beringian Coevolution Project (*9*,*18*; http://nix.msb.unm.edu; http://wildlife1.usask.ca/iwap/abstracts/galb.html) and the Circumarctic Rangifer Monitoring and Assessment Network (www.rangifer.net/carma/index.html; http://classic.ipy.org/development/eoi/proposal-details.php?id=162). Such programs depend on effective communication among research groups that have traditionally been on parallel but isolated trajectories; they also require a strong commitment to follow-up, often one of the biggest challenges in disease management and research on a global scale.

Another challenge is to recognize that neglected pathogens and legacy diseases, such as those caused by macroparasites, deserve equal research attention as "new" pathogens. Among sublethal effects of macroparasites are compromised physical and intellectual development and ability in humans (*32*) and reduced productivity and fecundity in domestic livestock (*33*). Examples of regulation of wildlife populations by macroparasites are increasing, often in combination with malnutrition or predation (*27*,*34*). Despite this, microparasites (generally prions, viruses, bacteria, fungi, and protozoans) often remain the singular focus of research on emerging infectious diseases (*35*,*36*). Greater effort needs to be dedicated to the effects of macroparasites on population health and demographics to ensure detection and proactive management of emerging diseases caused by these organisms. Our next challenge will be to understand the effects of interactions of multiple pathogens (both micro- and macroparasites), nutrition, stress, and other environmental factors on health of both individuals and populations.

Our work has resulted in a 10-year baseline for wildlife health across a region of northern North America increasingly vulnerable to climate change and other drivers of infectious disease emergence. Climate change will affect northern ecosystems by altering host range (i.e., expansions, shifts, increased or decreased overlap), abundance, and resilience, in addition to availability of environmental contaminants, free-living stages of pathogens, and intermediate hosts/vectors (Table 2). Climate change may also interact with other drivers for infectious disease emergence in northern North America. These include 1) development of new ecotones related to expansion of southern animal species, such as deer, red fox, and domestic livestock; 2) altered routes and timing of migration for wild birds and caribou herds; 3) habitat alteration and fragmentation due to resource extraction and development; and 4) historical and ongoing translocation of hosts and pathogens (e.g., historical introductions of reindeer and muskoxen, natural expansion of existing wood bison populations, proposed reestablishment of wood bison in Alaska, and increasing interest in livestock production in the North). The cumulative implications for health of wildlife that are culturally and economically important for northern communities are unlikely to be positive (*37*). In addition, environmental change is predicted to drive increased dissemination of zoonotic pathogens in water- and foodborne pathways (e.g., *Giardia*, *Cryptosporidium*, *Toxoplasma*, *Trichinella*, and *Echinococcus* spp.), posing a direct threat to human health in communities that rely on local water sources and country foods; environmental change may also lead to emergence of disease resulting from "spillover" of pathogens between persons and wildlife (*8*,*16*,*37*–*39*).

Effects of climate change on infectious disease emergence may be cumulative and play out over decades or may manifest as isolated and extreme events. Long-term cumulative processes may be the drivers for changing dynamics between hosts and pathogens (e.g., generation time, developmental rates, amplification, shifts in seasonal transmission) and create subtle effects that will be challenging to demonstrate in the absence of extensive baselines (*4*,*6*,*7*). Incremental and gradual increases in temperature may drive thresholds or tipping points influencing the interface for hosts and parasites (*6*). In contrast, extreme weather events of temperature and humidity, as an outcome of climate change, are predicted to result in a mosaic of ephemeral or explosive emergence of disease against this broader background. Signals for these patterns have already been demonstrated in the Arctic (*30*,*40*).

We seek to emphasize the generalities for processes of emergence for both animal and human pathogens, and offer an approach that may serve as a universal framework to understand, anticipate, and forecast change in these complex systems on global and regional scales. These drivers are not unique to the North; they are in action in scenarios all over the world (*8*). Wildlife pathogens, or diseases originating in wildlife, are emerging as a considerable threat to human health worldwide (e.g., severe acute respiratory syndrome, HIV/AIDS, Ebola, Hendra, Nipah, and avian influenza viruses) (*2*,*23*; www.cws-scf.ec.gc.ca/cnwds/intro_e.cfm). While our work focused on a nonzoonotic group of pathogenic nematodes, our integrated, multidisciplinary approach can be extrapolated to investigate a broader diversity of pathogens in the North and anywhere else where the animal-human interface is intact or expanding (Table 1). The North has emerged as a region of great interest because of the historical interaction between people and wildlife, the current impact of climate change on northern ecosystems, and the future importance of the natural resources (both renewable and nonrenewable) in this region.

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Dr Hoberg is chief curator of the US National Parasite Collection at the Beltsville Area Research Center, US Department of Agriculture, Agricultural Research Service. His interests include helminth systematics, biodiversity, and phylogeny, with an emphasis on coevolutionary processes, historical biogeography, and structure and dynamics of complex host–parasite systems.

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International Circumpolar Surveillance, an Arctic Network for Surveillance of Infectious Diseases

Alan J. Parkinson,* Michael G. Bruce,* Tammy Zulz,* and the International Circumpolar Surveillance Steering Committee[1]

Peoples of the Arctic and sub-Arctic regions live in social and physical environments that differ substantially from those of their more southern-dwelling counterparts. The cold northern climate keeps people indoors, amplifying the effects of household crowding, smoking, and inadequate ventilation on person-to-person spread of infectious disease. The emergence of antimicrobial drug resistance among bacterial pathogens, the reemergence of tuberculosis, the entrance of HIV into Arctic communities, and the specter of pandemic influenza or the sudden emergence and introduction of new viral pathogens such as severe acute respiratory syndrome are of increasing concern to residents, governments, and public health authorities. The International Circumpolar Surveillance system is a network of hospital, public health agencies, and reference laboratories throughout the Arctic linked together to collect, compare, and share uniform laboratory and epidemiologic data on infectious diseases and assist in the formulation of prevention and control strategies.

Arctic populations have historically endured the debilitating effects of both endemic and epidemic infectious diseases (*1*,*2*). The introduction of antimicrobial drugs and vaccines and the establishment of robust public health systems have greatly reduced illness and deaths caused by infectious diseases in many Arctic countries. Despite these interventions, high rates of invasive diseases caused by bacterial pathogens such as *Streptococcus pneumoniae* (*3–5*), *Haemophilus influenzae* (*6*), *Helicobacter pylori* (*7*,*8*), and *Mycobacterium tuberculosis* (*9–12*) continue to persist. In addition, the emergence of antimicrobial drug resistance among bacterial pathogens once easily treated with commonly used antibiotics (*10*,*13–15*), the entrance of HIV into Arctic communities (*10*,*16*), and the specter of pandemic influenza or the sudden emergence and introduction of new viral pathogens such as severe acute respiratory syndrome (SARS) are of increasing concern to residents, governments, and public health authorities of all Arctic countries.

Social and Physical Environment

Peoples of the Arctic and sub-Arctic regions live in social and physical environments that differ substantially from those of their more southern-dwelling counterparts (*17*). The circumpolar region can be defined as a region that extends north of 60° north latitude, borders the Arctic Ocean, and includes all or northern parts of 8 nations: the United States (Alaska), Canada, Greenland, Iceland, Norway, Finland, Sweden, and the Russian Federation (Figure 1). Climate in the Arctic varies geographically from severe cold in arid uninhabited regions to temperate forests bordering coastal agrarian regions.

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Approximately 4 million people live in the Arctic; almost half reside in northern regions of the Russian Federation. The populations of these countries comprise varying proportions of European and indigenous ancestry. The Arctic is the homeland of the Eskimo; the Inuit of Greenland, northern Canada, and northern Alaska; the Yupik of western Alaska and eastern coastal regions of the Russian Far East; and the Aleut of the Aleutian Chain. The interior and western coastal regions of northern Canada and Alaska are the homes of a wide variety of North American Indian, linguistically distinct groups, including the Athabaskan, Eyak, Tlingit, Gwich'in, and Metis. In Alaska the collective terminology for persons of indigenous ancestry is Alaska Native. Although the group is not registered in official statistics, the Saami people inhabit circumpolar regions of Norway, Finland, and Sweden. The Russian census recognizes (from West to East) the Saami, Nenets, Khanty, Sel'kup, Enets, Nganasan, Dolgan, Even, Chukchi, Chuvan, and Eskimo/Inuit-Yupik of the Russian Far East. Arctic populations have certain demographic characteristics that separate them from populations in more southern regions. Birth and mortality rates are higher, and life expectancy is lower; a high proportion of the population is thus of younger age (*1*). In remote regions of the North American Arctic, Greenland, and the northern Russian Federation, many residents live in small, isolated communities that are dependent on hunting and fishing with little or no economic infrastructure. In these remote regions, public health and acute-care systems are often marginal, sometimes poorly supported, and in some cases nonexistent.

The Arctic is well known as a vast source of natural resources such as fish, forests, oil, gas, and metal ores. Exploitation of these resources requires both infrastructure development and improved transportation. However, communities once isolated are now linked by air to larger urban centers, which provides daily access not only to secondary and tertiary healthcare, but also to national and international transportation, tourism, and trade. In Iceland, northern Norway, Finland, Sweden, and the more densely populated regions of the North American Arctic, Greenland, and the northern Russia Federation, a more affluent economic mixture has emerged because of light industry; oil, gas, and mineral development; transportation and agriculture; and more sophisticated systems of healthcare and public health.

Inadequate Housing/Crowded Living Conditions

In smaller isolated communities, inadequate housing is an important determinant of infectious diseases. The cold northern climate keeps persons indoors, which amplifies the effects of household crowding, smoking, and inadequate ventilation. Crowded living conditions increase person-to-person spread of infectious diseases and favor the transmission of respiratory diseases (*18–20*), tuberculosis (*12*), gastrointestinal diseases (*21*), and skin infections (*22*). In many smaller isolated communities, inadequate sewage disposal systems and water supplies pose a substantial risk to health, resulting in periodic epidemics of diseases transmitted by the fecal-oral route (*21*,*23*).

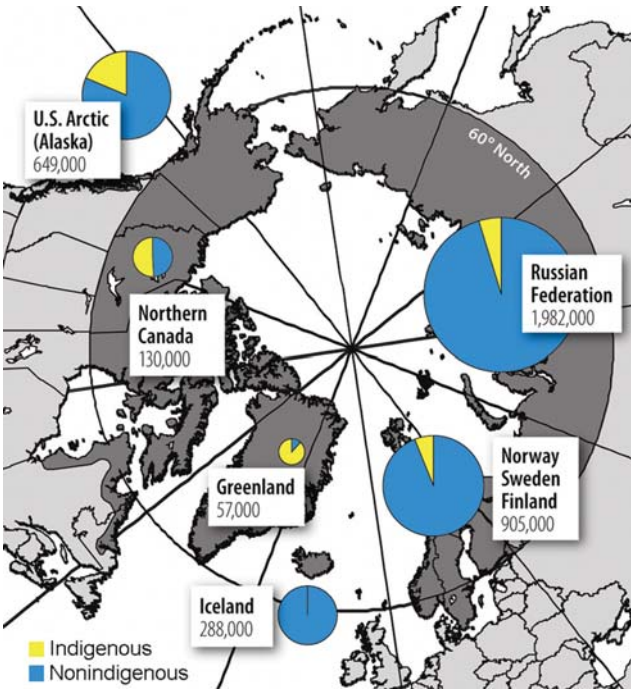

Figure 1. The circumpolar region and nonindigenous and indigenous populations of the Arctic. (Adapted from [*17*].)

Overuse of Antimicrobial Drugs

Empiric use, and possible overuse, of antimicrobial agents in remote Arctic regions has contributed to the emergence of bacterial strains now resistant to commonly used antibiotics. In the northern regions of the Russian Federation, underfunding of tuberculosis treatment programs have resulted in an unpredictable supply of antibiotics, which contributes to poor adherence and emergence of multidrug-resistant tuberculosis (*10*). In remote Alaskan villages, lack of ready access to laboratory confirmation of bacterial pathogens may contribute to overuse of antimicrobial agents. In addition, the presence of antimicrobial drug–resistant bacterial clones has led to an increase in infections with multidrug-resistant *S. pneumoniae* (*24*,*25*), methicillin-resistant *Staphylococcus aureus* (*13*), and clarthromycin- and metronidazole-resistant *H. pylori* (*14*).

Role of Surveillance

As in other parts of the world, a key component of prevention and control of infectious diseases in Arctic regions is surveillance. Effective surveillance can facilitate

timely control of outbreaks, inform public health officials' decisions on resource allocation, and provide data to adjust prevention and control strategies to maximize their effects. For example, population-based surveillance for invasive *H. influenzae* type b (Hib) disease in the US Arctic demonstrated prevaccine incidence rates of invasive disease of 601 cases and 129 cases per 100,000/population in Alaska Native and non-Native children <5 years of age, respectively (*26*). Immunization programs that use the Hib conjugate vaccine were implemented in the US Arctic in 1991 and resulted in a >10-fold decline in Hib cases in Alaska Native children. However, in May of 1996, continued surveillance detected 4 cases of invasive Hib in children <2 years, and during the next 12 months 10 cases occurred. Most cases were in infants who received 1–2 doses of Hib vaccine after a statewide change in 1996 to a vaccine that was less immunogenic after the first dose (*27*,*28*). This experience demonstrated the need for continued surveillance after the implementation of a successful vaccine program and the shortcomings of generalizing data from other countries or regions to develop public health policy in the Arctic.

Similarly, surveillance for invasive disease caused by *S. pneumoniae*, established in the US Arctic in 1986, showed that Alaska Natives had the highest reported average overall rate for invasive pneumococcal disease in the world (62 cases/100,000 population), which was 4× higher than the rate for non-Natives (16 cases/100,000) (*5*). Among Alaska Native children <2, the rate was 450 cases/100,000 versus 129 cases/100,000 among non-Native children. This surveillance system first detected decreased susceptibility to penicillin in the mid-1980s. Although these isolates were only moderately resistant to penicillin, resistance to multiple antimicrobial drugs was also found by 1989. Isolates fully resistant to penicillin detected in 1993 were first recovered from patients living in urban points of entry to the US Arctic and were indistinguishable from multidrug-resistant strains circumnavigating the globe (*24*,*25*). In the US Arctic, the proportion of pneumococcal isolates fully resistant to penicillin increased from 0% in 1991 to 6.1% in 1998, and the proportion of isolates that were resistant to ≥2 classes of antimicrobial agents increased from 4.7% in 1991 to 17.7% in 1998. In the US Arctic, the 23-valent pneumococcal polysaccharide vaccine is recommended for all persons >55 years of age; however, this vaccine remains underused. In 2001, the heptavalent pneumococcal conjugate vaccine (PCV7) was introduced to the childhood vaccination schedule, and by 2003, vaccine-type invasive pneumococcal disease rates had declined by 91% among Alaska Native children <2 years of age and by 80% among non-Native children <2 years of age. A 40% reduction of invasive pneumococcal disease in adults of all ethnicities suggests an indirect impact or herd effect of this vaccine in nonvaccinated persons. In addition, the use of PCV7 in this population has reduced the proportion of invasive disease caused by isolates resistant to penicillin, erythromycin, and cotrimoxazole (*29*).

These examples demonstrate the feasibility of conducting population-based surveillance to monitor the effects of implemented vaccination programs in reducing the extent of invasive disease caused by 2 common bacterial pathogens in an Arctic region. Population-based surveillance of diseases of concern, including invasive bacterial diseases, is conducted by public health agencies in Canada, Greenland, Iceland, Norway, Finland, and Sweden. Linkage of these surveillance systems would create the beginnings of a circumpolar network of hospitals, public health agencies, and reference laboratories throughout the Arctic to collect, compare, and share uniform laboratory and epidemiologic data on infectious diseases of concern, and assist in the formulation of prevention and control strategies.

International Circumpolar Surveillance

In 1998, the Arctic Investigations Program (AIP) of the Centers for Disease Control and Prevention, together with Health Canada's Bureau of Infectious Disease Laboratories Centres for Disease Control, now the Public Health Agency of Canada's Center for Infectious Disease Prevention and Control, proposed the establishment of an International Circumpolar Surveillance (ICS) system for the detection of infectious diseases of concern in the Arctic (*30*). The initial priority for ICS was the invasive bacterial diseases caused *S. pneumoniae*, *H. influenzae*, *Neisseria meningitidis*, and groups A and B streptococci.

ICS capitalizes on existing national infectious disease surveillance systems and existing long-standing circumpolar collaborative relationships forged through the Arctic Council (www.arctic-council.org) and the International Union for Circumpolar Health (www.iuch.org). The Arctic Council is a ministerial forum promoting cooperation and coordination between Arctic nations on common Arctic concerns and provides a unique opportunity to partner with Arctic nation ministries of health, nongovernmental organizations, and indigenous peoples' organizations to address health concerns of circumpolar communities. The International Union for Circumpolar Health is a nongovernmental organization comprising the memberships of 5 circumpolar health organizations that promote circumpolar cooperation on Arctic human health.

In 1999, a pilot surveillance system was established to monitor reported cases of invasive pneumococcal disease from 23 clinical laboratories in Alaska and 14 clinical laboratories in the northern Canadian Arctic above 60° north latitude, including the Yukon and Northwest Territories, Nunavut, northern Quebec, and Labrador (Figure 2).

Pneumococcal isolates from patients identified with invasive disease were forwarded to reference laborato-

ries in the US Arctic (AIP) and northern Canada at the National Center for Streptococcus (NCS), Edmonton, Alberta, or Laboratoire de Sante Publique, Quebec (LSPQ), respectively. Identified cases were also reported to local public health personnel, who reviewed and provided clinical, demographic, and vaccination history. Case and culture information was forwarded to the ICS coordinator at AIP for analysis, report generation, and dissemination. In 2000, Greenland joined ICS. Pneumococcal isolates from patients with invasive disease were forwarded from 15 district hospitals first to the Dronning Ingrids Hospital laboratory in Nuuk, Greenland, and then to the Staten Serum Institut (SSI), Copenhagen, Denmark, for serotyping and susceptibility testing. Iceland, Norway, and Finland joined ICS in 2001, reporting national pneumococcal disease surveillance and laboratory information to ICS annually.

Surveillance of other invasive bacterial diseases (*H. influenzae*, *N. meningitidis*, groups A and B streptococci) in the US Arctic, northern Canada, and Greenland was added to ICS in 2000. The northern region of Sweden, Norrbotten, joined ICS in 2003, reporting invasive diseases caused by *S. pneumoniae*, *H. influenzae*, *N. meningitidis*, and groups A and B streptococci.

An ICS quality control exchange program was instituted in 1999 among the 3 reference laboratories (AIP, NCS, LSPQ) for serotyping and antimicrobial susceptibility testing of *S. pneumoniae* (*31*). The program was extended to SSI in 2004. In 2005, an ICS quality control exchange program for serotyping of isolates of *H. influenzae* and *N. meningitidis* was implemented by the National Microbiology Laboratory, Public Health Agency of Canada, in Winnipeg, Manitoba.

The priorities and overall direction of ICS are governed by a steering committee consisting of 2 representatives from each participating country; representation from World Health Organization European regional office, Copenhagen; the Indigenous Peoples Secretariat; and Russian Association of Indigenous Peoples of the North. Other infectious diseases of concern identified by the steering committee , and therefore eligible for circumpolar surveillance, include hepatitis B, tuberculosis, HIV/AIDS, and acute respiratory virus diseases such as respiratory syncytial virus infections in infants. Surveillance of invasive bacterial diseases is coordinated by a subcommittee, the Invasive Bacterial Disease Working Group. As anticipated surveillance for other ICS priority diseases is implemented, similar coordinating working groups led by other partner countries will be established.

Almost half of the circumpolar region consists of northern regions of the Russian Federation, which to its west borders the Arctic regions of Norway and Finland, and to its east is within 2 miles of the US Arctic. The Russian Federation's communicable disease control systems

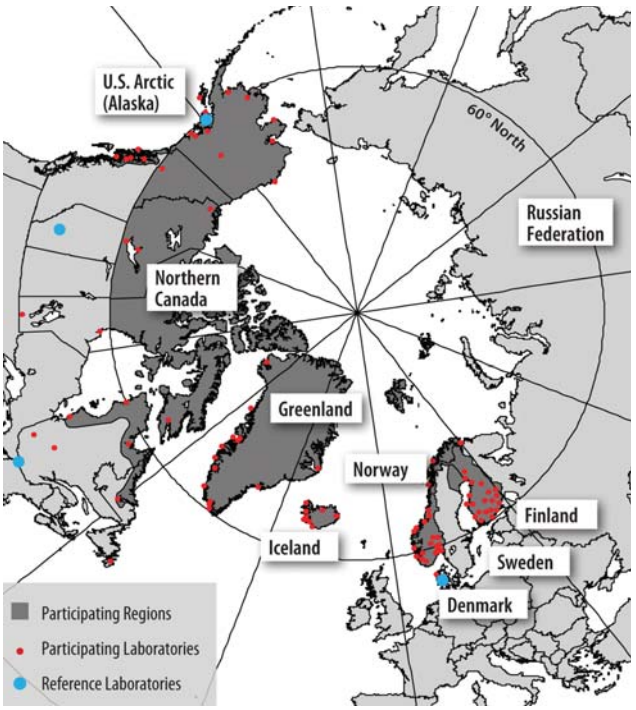

Figure 2. The International Circumpolar Surveillance system participating regions (dark gray), laboratories (small dots), and reference laboratories (large dots).

evolved separately from western public health systems and consist of relatively large federal, regional, disease-specific, sector-specific (prisons, military), and largely clinical case-based reporting systems (*10*). These differences, together with the relative isolation of northern and far-eastern regions of the Russian Federation, the language barrier, and absence of information exchange, have led to some difficulties in establishing cross-border cooperation in infectious disease prevention and control. However, because of a sharp rise in the 1990s in the incidence of communicable diseases such as HIV, sexually transmitted diseases, and tuberculosis (including multidrug-resistant tuberculosis) in the Baltic and Barents Sea regions, several initiatives are now aimed at improving cooperation in infectious disease prevention and control between countries of northwestern Europe and adjacent regions of the Russian Federation. For example, in 1999 the Norwegian Institute of Public Health, together with other Nordic state epidemiologists, established a program to strengthen infectious disease control in the Barents and Baltic Sea states and has since established a framework for communicable disease surveillance communication and training in northern Europe (www.epinorth.org). These activities provide a model for expanding cooperation and developing partnerships for the exchange of infectious disease surveillance information in other northern and far-eastern regions of the Russian Federation.

Impact of ICS

The ability of ICS to collect and share standardized, uniform laboratory and epidemiologic data on infectious diseases of concern in Arctic countries has already proved valuable in the formulation of prevention and control strategies in regions with small but at-risk Arctic populations. In 2000, ICS data were used to identify an outbreak of *S. pneumoniae* serotype 1 invasive disease that occurred among young adults in 2 northern regions of Canada (*32*,*33*). This investigation, together with data from Alaska that indicated that 78%–84% of invasive pneumococcal disease among children <2 years of age could be prevented by using PCV7, resulted in the implementation of vaccine programs in 3 northern regions of Canada using both the 23-valent polysaccharide vaccine and PCV7 in 2002. The introduction of PCV7 in the US Arctic (2001) and northern Canada (2002) has resulted in a rapid decrease in the proportion of isolates resistant to penicillin and other antimicrobial drugs, compared with the situation in countries not using the vaccine (Iceland and Finland) (*34*). Continued surveillance of invasive pneumococcal disease by ICS in these regions will monitor the impact and effectiveness of vaccine programs for preventing invasive pneumococcal disease and antimicrobial drug–resistant infections in these high-risk populations (*34*).

The reemergence of invasive disease caused by Hib in the US Arctic in 1996 following a change in conjugate vaccine type emphasized the need for continued surveillance to monitor vaccine impact, as well as to detect the potential emergence of disease caused by nonvaccine serotypes. Non–Hib (serotypes a, c, d, e, f) is uncommon as a cause of invasive disease in children; however, with the decline in Hib disease, the importance of infections caused by other nonvaccine serotypes has increased. In a 6-month period of 2003, 5 cases of *H. influenzae* type a (Hia) were detected in 3 infants in 1 remote region of the US Arctic (*35*). Between 2000 and 2004, 72 cases of serotype-confirmed *H. influenzae* infection were detected by ICS in Alaska and Canada (*36*). Of these, 34 (47%) were Hia, and 22 (65%) occurred in aboriginal people with a median age of 1.1 years. Hia is now the most common *H. influenzae* serotype seen in the North American Arctic, with the highest rates among indigenous children. Further research is needed to determine sequelae, risk factors, outbreak potential, and the utility of chemoprophylaxis for this disease.

Arctic Change and Infectious Diseases Surveillance

A common concern among peoples of the Arctic is the rapid pace of economic change and modernization occurring in many communities, which will bring new challenges to the health and well-being of Arctic residents (*19*,*37*). The increasing national and international travel by Arctic residents and increasing access to remote communities by national and international seasonal workforce and tourists have greatly increased the risk of importing infectious diseases to remote communities.

Climate change is also predicted to have major effects within the Arctic (*38*). The average Arctic temperature has risen at almost twice the rate of that in the rest of the world in the last 2 decades and could cause changes in the incidence and geographic distribution of infectious diseases already present in Arctic regions (*39*). For example, an outbreak of *Vibrio parahemolyticus*–related gastroenteritis was reported in July 2004 among cruise ship passengers that consumed raw, farmed oysters in the Prince William Sound area of Alaska (≈60° north latitude), >1,000 km further north than previous reported outbreaks. The July-August water temperature of the oyster farm had increased 0.21°C per year since 1997; 2004 was the first summer on record that the mean water temperature exceeded 15°C, the threshold temperature for the harvest of implicated oysters, which suggests that the ocean warming trend was responsible for this outbreak (*40*). Similarly, higher ambient temperatures in the Arctic may result in an increase in other temperature-sensitive foodborne diseases and influence the incidence of zoonotic infectious diseases by changing the populations and range of animal hosts and insect vectors. The melting of the permafrost together with an increase in extreme weather events such as flooding may result in damage to water and waste disposal systems, which may in turn increase community outbreaks of foodborne and water-borne infections. Temperature and humidity markedly influence the distribution, density, and biting behavior of many arthropod vectors, which again may influence the incidence and northern range of many vector-borne diseases (*39*). These examples emphasize the need for an established surveillance network in Arctic regions for monitoring emerging climate-sensitive infectious diseases.

Future Directions

ICS provides a model for international surveillance of infectious diseases and collaboration between clinical hospital and public health references laboratories and public health centers and institutes. The system currently provides standardized laboratory and epidemiologic data on invasive bacterial diseases that are comparable across borders and can be used to evaluate intervention strategies. However, the system also provides an infrastructure that can be used to monitor and respond to other emerging infectious disease threats. Tuberculosis presents a continuing challenge to the public health communities of the US Arctic, northern Canada, Greenland, and the Russian Federation. The establishment of an ICS tuberculosis working group would enhance ongoing efforts to reduce the rates of disease in these regions by sharing knowledge, methods, and surveillance

data. Because more than half of the circumpolar region is contained within the Russian Federation, efforts should be made to engage and develop partnerships with public health authorities in these regions to learn more about infectious diseases of concern, systems of surveillance, and interests in sharing infectious disease surveillance information.

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International Circumpolar Surveillance System for Invasive Pneumococcal Disease, 1999–2005

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The International Circumpolar Surveillance System is a population-based surveillance network for invasive bacterial disease in the Arctic. The 7-valent pneumococcal conjugate vaccine (PCV7) was introduced for routine infant vaccination in Alaska (2001), northern Canada (2002–2006), and Norway (2006). Data for invasive pneumococcal disease (IPD) were analyzed to identify clinical findings, disease rates, serotype distribution, and antimicrobial drug susceptibility; 11,244 IPD cases were reported. Pneumonia and bacteremia were common clinical findings. Rates of IPD among indigenous persons in Alaska and northern Canada were 43 and 38 cases per 100,000 population, respectively. Rates in children <2 years of age ranged from 21 to 153 cases per 100,000 population. In Alaska and northern Canada, IPD rates in children <2 years of age caused by PCV7 serotypes decreased by >80% after routine vaccination. IPD rates are high among indigenous persons and children in Arctic countries. After vaccine introduction, IPD caused by non-PCV7 serotypes increased in Alaska.

The International Circumpolar Surveillance (ICS) project was established in 1999 to create an infectious disease surveillance network throughout Arctic countries and territories. The project initially focused on invasive bacterial diseases caused by *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Neisseria meningitidis*, and groups A and B streptococci. In 1999, the project integrated prospective population-based surveillance data for invasive pneumococcal disease (IPD) from the US Arctic (Alaska) and northern Canada (*1*). Subsequently, the ICS network expanded to include Greenland in 2000, Iceland, Norway, and Finland in 2001, and northern Sweden in 2003. All northern circumpolar countries (north of latitude 60°N), with the exception of Russia, participate in ICS.

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IPD was of interest to the ICS in part because it is one of the leading causes of pneumonia and meningitis among indigenous persons of the circumpolar north. Incidence rates of IPD are higher among indigenous persons than among nonindigenous persons (*2*–*6*). The proportion of indigenous persons in Alaska and northern Canada <5 years of age is 11% and 12%, respectively. In Alaska, the 7-valent pneumococcal conjugate vaccine (PCV7) was made available to all children through a statewide immunization program. Data indicate that 92.6% of indigenous children and 64.6% of non-Hispanic white children in Alaska received ≥3 doses of PCV7 from July 2003 through June 2004, respectively. Routine use of PCV7 began in some areas of northern Canada in 2002. Other areas of northern Canada implemented PCV7 programs during 2003–2006. Scant data exist on vaccine coverage in northern Canada. However, neighboring Canadian provinces outside the ICS network, such as Alberta, have vaccine coverage rates >90% (www.phac-aspc.gc.ca/publicat/ccdr-rmtc/05pdf/cdr3106.pdf). Routine use of PCV7 began in Norway in 2006. PCV7 is not currently included in the routine infant immunization schedule in Greenland, Iceland, northern Sweden, or Finland.

We analyzed IPD data collected from January 1999 through December 2005. The purpose of the study was to determine rates of disease by country, common clinical findings, risk factors, serotype distribution, antimicrobial drug susceptibility patterns, and changes in disease rates with vaccine use.

Methods

In the participating regions, clinical laboratories send *S. pneumoniae* isolated from a normally sterile site to regional reference laboratories (Table 1). Reference laboratories confirm the identity, determine the serotype, and test for antimicrobial drug susceptibility of each isolate. Laboratory, demographic, and clinical data are collected for each invasive case of *S. pneumoniae*, and these data are forwarded to the Arctic Investigations Program (AIP) of the Centers for Disease Control and Prevention (CDC) in Anchorage, Alaska, the coordinating center for ICS.

Population distribution and land area vary widely among participating regions. Populations range from ≈57,000 persons in Greenland to >5 million persons in Finland. Land areas range from 102,968 km^2 for Iceland to 4,506,600 km^2 for northern Canada (Table 1). Regions with the largest land areas (Alaska, Greenland, and northern Canada) have populations scattered in remote, small communities often not connected by road to urban centers. Remote populations in Scandinavia and Iceland are better connected to larger urban communities.

A case-patient with IPD was defined as a resident of the surveillance area from whom *S. pneumoniae* was isolated from a normally sterile site, including blood, cerebrospinal fluid, pleural fluid, peritoneal fluid, or joint fluid. Population denominator data for the regions were obtained from the Statistics Canada Website (www.statcan.ca), the Alaska Department of Labor and Workforce Development Website (www.labor.state.ak.us), the Statistics Greenland Website (www.statgreen.gl), the Statistics Iceland Website (www.hagstofa.is), the Statistics Norway Website (www.ssb.no), the Statistics Sweden Website (www.scb.se), and the Statistics Finland Website (www.stat.fi). This study covers a 7-year surveillance period from January 1999 through December 2005.

Epidemiologic Data

Alaska and Northern Canada

Initial identification of a case of IPD results in a report to local public health personnel who complete standardized data collection forms (bacterial disease surveillance form [BDSF]) that include demographic, clinical, and risk factor information, and pertinent immunization history (www.cdc.gov/ncidod/aip/research/ics/forms.html). All laboratory and case-related data are forwarded without identifiers by fax and mail to the ICS coordinator at AIP in Anchorage, Alaska, where they are entered into a database and analyzed.

Greenland, Iceland, Norway, Northern Sweden, and Finland

End of year summary data are submitted electronically to the ICS coordinator at AIP in Anchorage, where they are entered into a database. Greenland and Iceland use the BDSF. Norway, northern Sweden, and Finland use other instruments.

Table 1. Demographics of countries participating in the study

Characteristic	Alaska	Northern Canada	Greenland	Iceland	Norway	Northern Sweden	Finland
Mean population	641,720	132,956	56,617	288,035	4,565,943	252,729	5,215,791
% Indigenous	19	59	Unknown	Unknown	<1	<5	<1
Region size, km^2	1,518,807	4,506,600	2,131,863	102,968	323,760	160,580	339,290
No. participating laboratories	23	14	15	10	33	1	23
Location of reference laboratories	Anchorage	Edmonton, Montreal, Winnipeg	Nuuk, Copenhagen	Reykjavik	Oslo, Tromso	Stockholm	Oulu

Laboratory Data

Isolates were serotyped by the Quellung reaction (Alaska, northern Canada, Greenland, Norway, and northern Sweden), counter-immunoelectrophoresis (Finland), or coagglutination (Iceland). Antimicrobial drug susceptibility testing was performed by microbroth dilution (Alaska and northern Canada), agar dilution (Greenland and Finland), or disk diffusion (Iceland, Norway, and northern Sweden). A laboratory quality control program has been in place since the program's inception (*7*).

Statistical Analysis

Data were double-entered into Paradox version 10.0 (Corel, Ottawa, Ontario, Canada), and analyzed by using EpiInfo version 6.04b (CDC, Atlanta, GA, USA), SAS version 8.0 (SAS Institute, Cary, NC, USA), and StatXact version 6.0 (Cytel Corporation, Cambridge, MA, USA). For Alaska, we compared disease rates by using 1999–2000 as the baseline period and 2001–2005 as the postvaccine period. For northern Canada, we used 1999–2002 as the baseline period and 2003–2005 as the vaccine implementation period. Standardized incidence rates were reported by using World Health Organization 2000 population standard and the age groups of ≤1, 2–19, 20–64, and ≥65 years. Statistical differences in rates between periods and between countries were assessed by using a 2-sample Poisson test. Trends in IPD incidence rates among children <2 years of age were assessed by using Poisson regression; p values are exact when appropriate.

Results

Descriptive Epidemiology

Over the 7-year surveillance period, 11,244 cases of IPD were detected among the 7 participating countries; 4,921 (53%) were in male patients. Of the 5,896 case-patients for whom outcome was reported, 569 died (case-fatality rate 10%). Median age of case-patients was 57 years and varied by country (Table 2). Among countries that did not use PCV7 during the study period, overall crude IPD incidence rates ranged from 11.6 in northern Sweden (age standardized rate 9.1) to 21.0 in Norway (age standardized rate 16.2) (Table 3).

Annualized age-specific incidence rates over the entire surveillance period were highest in children <2 years of age and the elderly (Tables 3, 4). Within each country, rates were highest in children <2 years of age except for Norway and northern Sweden, where rates were slightly higher in the elderly.

Race and ethnicity data were only available from Alaska and northern Canada. Over the entire surveillance period, annualized rates of disease were higher in Alaska Native (indigenous) persons (43.1 cases/100,000/year) than in nonindigenous persons (9.8 cases) (relative risk [RR] 4.4, 95% confidence interval [CI] 3.8–5.1). Increased risk for IPD was similar in northern Canada (RR 3.6, 95% CI 2.6, 5.2) with an annualized overall rate of 36.0 cases/100,000 among Canadian indigenous persons and 9.9 cases/100,000 among nonindigenous persons. Among children <2 years of age, increased RR for indigenous versus nonindigenous children was 6.5 in Alaska (95% CI 4.5, 9.4; indigenous 249.9 cases/100,000/year, nonindigenous 38.3 cases/100,000/year) and 2.3 in Canada (95% CI 1.0, 5.2; indigenous 170.7 cases/100,000/year, nonindigenous 74.7 cases/100,000/year).

Incidence Rates (All Serotypes) in Countries Using PCV7

In Alaska, incidence of IPD among all age groups decreased from 20.6 cases in the prevaccine period (1999–

Table 2. Characteristics of persons with invasive pneumococcal disease, by country*

Characteristic	Alaska, 1999–2005, n = 769	Northern Canada, 1999–2005, n = 251	Greenland, 2000–2005, n = 69	Iceland, 2000–2005, n = 274	Norway, 2000–2005, n = 5,744	Northern Sweden, 2003–2005, n = 88	Finland, 2000–2005, n = 4,049
Median age (range)	41.6 (1 mo–100 y)	30.2 (0 mo– 83 y)	44.7 (0 mo–91 y)	53.2 (1 mo–98 y)	63.2 (0 mo–99 y)	65.8 (9 mo–98 y)	54.2 (0 mo–100 y)
No. males (%)	423 (55)	149 (60)	37 (54)	145 (53)	2,856 (50)	40 (45)	2,271 (56)
No. indigenous (%)	372 (48)	191 (84)†	NA	NA	NA	NA	NA
No. hospitalized (%)	585 (77)‡	201 (87)‡	62 (100)‡	NA	5,567 (99)‡	NA	NA
Duration of hospitalization, d, median (minimum–maximum)	4 (0–188)	5 (0–77)	9 (0–131)	NA	NA	NA	NA
No. deaths (%)	96 (13)§	11 (5)§	13 (20)§	30 (27)§	419 (9)§	NA	NA

*NA, not available.
†Ethnicity data missing from 24 (northern Canada) cases. Denominator = 227.
‡Hospitalization data missing for 9 (Alaska), 21 (northern Canada), 7 (Greenland), and 127 (Norway) cases. Denominators are 760, 230, 62, and 5,617, respectively.
§Death information missing for 10 (Alaska), 21 (northern Canada), 3 (Greenland), 161 (Iceland), and 1,1016 (Norway) cases. Denominators are 759, 230, 66, 113, and 4,728, respectively.

Table 3. Annualized crude and standardized incidence rates (per 100,000 persons) of IPD by countries not using 7-valent pneumococcal conjugate vaccine*

Statistic or age group	Greenland (2000–2005)	Iceland (2000–2005)	Norway (2000–2005)	Northern Sweden (2003–2005)	Finland (2000–2005)
Total no. cases	69	274	5,744	88	4,049
Age-specific annualized incidence rates (no. cases)					
<2 y	77.4 (8)	89.8 (45)	50.0 (355)	21.1 (3)	52.3 (367)
2–19 y	4.8 (5)	6.8 (32)	4.9 (312)	0.0 (0)	5.0 (346)
20–64 y	25.5 (53)	8.9 (90)	14.5 (2,352)	9.3 (41)	10.9 (2,057)
≥65 y	16.6 (3)	53.1 (107)	66.7 (2,725)	30.9 (44)	26.6 (1,279)
Crude annualized incidence (all ages)	20.3	15.9	21.0	11.6	12.9
Annualized age standardized incidence†	19.8	14.6	16.2	9.1	11.6

*IPD, invasive pneumococcal disease,
†Rates adjusted to 2000 World Health Organization world standard population estimates.

2000) to 15.8 cases/100,000 persons in the postvaccine period (2001–2005; p = 0.0004). Among Alaskan children <2 years of age, incidence of IPD decreased from 173.5 cases in the pre–conjugate vaccine period to 79.2 cases/100,000 children in the postvaccine period (p<0.0001). Similarly, in northern Canada, incidence of IPD among all age groups decreased from 31.0 cases/100,000 persons in the prevaccine period (1999–2002) to 21.6 cases/100,000 persons in the vaccine implementation period (2003–2005; p = 0.007) (Table 4). Among northern Canadian children <2 years of age, incidence of IPD had been decreasing in the 2 years before PCV7 use (Figure). The incidence of IPD decreased from 185.6 cases/100,000 children in the prevaccine period (1999–2002) to 110.0 cases/100,000 children in the vaccine implementation period (2003–2005), However, this decrease was not statistically significant (p = 0.10) (Table 4).

In Alaska, incidence of IPD among all indigenous persons decreased from 56.0 cases/100,000 persons in the pre–conjugate vaccine period (1999–2000) to 38.1 cases/100,000 persons in the postvaccine period (2001–2005; p = 0.0003). Among Alaskan indigenous children <2 years of age, incidence decreased from 440.6 in the pre–conjugate vaccine period to 177.5 in the postvaccine period (p<0.0001). Similarly, in northern Canada, incidence of IPD among indigenous persons decreased from 44.2 cases/100,000 persons in the prevaccine period (1999–2002) to 25.0 cases/100,000 persons in the vaccine implementation period (2003–2005; p = 0.0005). Among Canadian indigenous children <2 years of age, incidence decreased from 229.3/100,000 persons in the prevaccine period to 92.6/100,000 persons in the vaccine implementation period (2003–2005; p = 0.01) (Table 4).

PCV7 Serotype–specific Incidence Rates in Countries Using PCV7

In Alaska, the incidence of serotypes contained in PCV7 in all age groups decreased from 9.6 cases/100,000 persons in the pre–conjugate vaccine period (1999–2000) to 3.4 cases/100,000 persons in the postvaccine period (2001–2005; p<0.0001). PCV7 serotype–specific incidence among Alaska children <2 years decreased from 128.3 cases/100,000 persons in the pre–conjugate vaccine period to 15.5 cases/100,000 persons in the postvaccine period (p<0.0001). Among older age groups in Alaska (2–19, 20–64, and ≥65 years), decreases in PCV7 serotype–specific incidence also occurred. However, this decrease was not statistically significant in persons 20–64 years of age (Table 4). In northern Canada, incidence of serotypes in PCV7 in all age groups decreased from 12.6 cases/100,000 persons in the pre–conjugate vaccine period (1999–2002) to 3.8 cases/100,000 persons in the vaccine implementation period (2003–2005; p<0.0001) (Table 4). PCV7 serotype–specific incidence among northern Canadian children <2 years of age decreased from 128.9 cases/100,000 children in the prevaccine period to 20.6 cases/100,000 children in the vaccine implementation period (p = 0.0008; Table 4). Decreases in age-specific incidence of PCV7 disease also occurred among older age groups in northern Canada except among persons ≥65 years of age (Table 4). Among indigenous persons in Alaska and northern Canada, PCV7 serotype–specific incidence showed a statistically significant decrease from the prevaccine period to the postvaccine implementation period (Table 4).

In Alaska, non-PCV7 serotype–specific incidence among all age groups increased from 8.3 cases/100,000 persons in the pre–conjugate vaccine period (1999–2000) to 10.5 cases/100,000 persons in the postvaccine period (2001–2005; p = 0.04). Non-PCV7 serotype–specific incidence among Alaskan children <2 years of age increased from 27.7 cases/100,000 children in the pre–conjugate vaccine period to 59.0 cases/100,000 children in the postvaccine period (p = 0.03). In northern Canada, non-PCV7 serotype–specific incidence among all age groups remained relatively stable at 17.1 cases/100,000 persons in the prevaccine period (1999–2002) and 16.8 cases/100,000 persons in the vaccine implementation period (2003–2005; p = 0.94) (Table 4). Non-PCV7 serotype–specific incidence among northern Canadian children <2 years of age

increased from 41.2 cases/100,000 children in the pre–conjugate vaccine period (8 cases) to 75.6 cases/100,000 children in the vaccine implementation period (11 cases). However, this increase was not statistically significant (p = 0.25; Table 4). Among indigenous persons in Alaska, non-PCV7 serotype–specific incidence demonstrated a statistically significant increase from the prevaccine to the postvaccine period. However, this trend was not seen among Canadian indigenous persons (Table 4).

Incidence Rates (All Serotypes) in Countries Not Using PCV7

IPD rates among children <2 years of age increased in Norway and Finland (p<0.01 and p = 0.04, respectively). A slight increase was also seen in Iceland, but this increase was not statistically significant (p = 0.56). Because of low numbers of cases, rates in Greenland were unstable but showed no statistically significant change (Figure).

Serotype Distribution

Several serotypes were common among Arctic countries participating in ICS, including 14, 4, 7F, and 6B. Among the 4 most prevalent serotypes in the Arctic, 3 are found in PCV7.

In Alaska, before vaccine use, the 5 most common serotypes were 14 (17%), 4 and 7F (9%), 9V (8%), 19F (6%), and 6B (6%). In the prevaccine period among children <2 years of age, 82% of serotypes in Alaska were in

Table 4. Rates/100,000 cases of IPD in Alaska and Northern Canada before and after introduction of PCV7*

	Alaska			Northern Canada		
Group	Prevaccine (1999–2000)	Postvaccine (2001–2005)	p value	Prevaccine (1999–2002)	Vaccine implementation (2003–2005)	p value
Total no. cases	257	512	NA	165	86	NA
All ages, y	20.6 (257)	15.8 (512)	0.0004	31.0 (165)	21.6 (86)	0.007
<2	173.5 (69)	79.2 (82)	<0.0001	185.6 (36)	110.0 (16)	0.10
2–19	10.7 (40)	6.6 (64)	0.02	22.9 (41)	9.7 (13)	0.009
20–64	13.7 (104)	14.1 (278)	0.82	23.8 (74)	18.8 (44)	0.24
≥65	57.9 (44)	44.5 (88)	0.17	64.4 (14)	73.5 (12)	0.84
Indigenous, all ages	56.0 (133)	38.1 (239)	0.0003	44.2 (134)	25.0 (57)	0.0005
<2 y	440.6 (47)	177.5 (50)	<0.0001	229.3 (33)	92.6 (10)	0.01
Nonindigenous, all ages	12.3 (124)	10.4 (273)	0.13	9.6 (20)	10.2 (16)	0.86
<2 y	75.7 (22)	42.5 (32)	0.05	65.4 (3)	87.2 (3)	1.00
PCV 7 serotypes (4, 6B, 9V, 14, 18C, 19F, 23F)†						
All ages, y	9.6 (120)	3.4 (110)	<0.0001	12.6 (67)	3.8 (15)	<0.0001
<2	128.3 (51)	15.5 (16)	<0.0001	128.9 (25)	20.6 (3)	0.0008
2–19	5.6 (21)	1.6 (16)	0.0003	8.4 (15)	1.5 (2)	0.01
20–64	4.1 (31)	2.8 (55)	0.09	7.1 (22)	1.7 (4)	0.005
≥65	22.4 (17)	11.6 (23)	0.05	23.0 (5)	36.8 (6)	0.55
Indigenous, all ages	24.9 (59)	4.9 (31)	<0.0001	17.1 (52)	3.5 (8)	<0.0001
<2 y	318.7 (34)	21.3 (6)	<0.0001	159.8 (23)	9.3 (1)	0.0001
Nonindigenous, all ages	6.0 (61)	3.0 (79)	<0.0001	6.2 (13)	3.2 (5)	0.24
<2 y	58.5 (17)	13.3 (10)	<0.0001	43.6 (2)	29.1 (1)	1.00
Non-PCV7 serotypes†						
All ages, y	8.3 (104)	10.5 (341)	0.04	17.1 (91)	16.8 (67)	0.94
<2	27.7 (11)	59.0 (61)	0.03	41.2 (8)	75.6 (11)	0.25
2–19	3.5 (13)	4.1 (40)	0.65	14.5 (26)	7.4 (10)	0.09
20–64	7.3 (55)	9.6 (189)	0.07	15.4 (48)	16.7 (39)	0.75
≥65	32.9 (25)	25.8 (51)	0.31	41.4 (9)	36.8 (6)	1.00
Indigenous, all ages	20.2 (48)	29.8 (187)	0.01	25.4 (77)	20.2 (46)	0.24
<2 y	65.6 (7)	145.6 (41)	0.05	48.6 (7)	74.1 (8)	0.44
Nonindigenous, all ages	5.5 (56)	5.9 (154)	0.71	2.9 (6)	7.0 (11)	0.09
<2 y	13.8 (4)	26.6 (20)	0.26	21.8 (1)	58.1 (2)	0.58
Penicillin nonsusceptible IPD, all serotypes‡						
All ages	4.0 (50)	2.1 (68)	0.0004	1.5 (8)	0.8 (3)	0.37
<2 y	62.9 (25)	21.3 (22)	<0.0001	10.3 (2)	0.0 (0)	0.51
Cotrimoxazole nonsusceptible IPD, all serotypes‡						
All ages	5.6 (70)	3.0 (96)	0.0003	2.6 (14)	1.8 (7)	0.50
<2 y	90.5 (36)	25.1 (26)	<0.0001	15.5 (3)	13.7 (2)	1.00

*IPD, invasive pneumococcal disease, PCV7, 7-valent pneumococcal conjugate vaccine; NA, not available. Values in parentheses are no. cases.
†Serotype available for 675 (88%) of 769 Alaska isolates and 240 (96%) of 251 Northern Canada isolates.
‡Antimicrobial drug susceptibility available for 677 (88%) of 769 Alaska isolates and 236 (94%) of 251 northern Canada isolates.

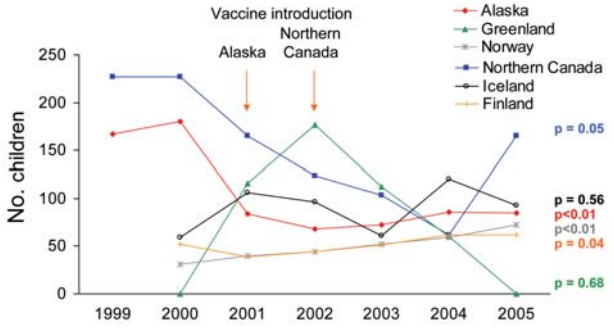

Figure. Annual invasive pneumococcal disease rates among children <2 years of age by International Circumpolar Surveillance System member country, 1999–2005. The p values are for trend.

PCV7. After introduction of PCV7 (2001–2005), 21% of serotypes were in PCV7 (Table 5), and among all ages 19A (11%) is now the most prevalent serotype, followed by 4 (8%), 12F (8%), 3/7F/8 (7%), and 14 (6%).

In northern Canada, the 5 most prevalent serotypes before PCV7 use were 1 (34%), 14 (11%), 4 (9%), 8 (8%), and 6B/9V (6% each). In the prevaccine period, among children <2 years of age, 76% of serotypes in northern Canada were in PCV7. During the vaccine implementation period (2003–2005), 21% of serotypes in northern Canada were in PCV7 (Table 5). Serotype distribution (all age groups combined) has changed; however, serotype 1 (24%) continues to be the most prevalent serotype, followed by 8 (11%), 3 (7%), 10A/18C/22F (6%), and 6B (5%). Two regions in northern Canada had outbreaks of serotype 1 during the surveillance period, which may have affected relative frequencies of serotypes (*8–10*).

Antimicrobial Drug Resistance

Alaska had the highest proportion of isolates nonsusceptible to antimicrobial drugs among ICS regions reporting these data. With use of PCV7, the proportion of isolates nonsusceptible to penicillin among children <2 years of age decreased from 40% to 29% and from 6% to 0% (prevaccine to postvaccine period) in Alaska and northern Canada, respectively (Table 6). Rates of IPD with penicillin-nonsusceptible isolates decreased from 62.9 cases/100,000 children to 21.3 cases/100,000 children ($p<0.0001$) in Alaska, and rates decreased from 10.3 cases/100,000 children to 0 cases/100,000 children ($p = 0.51$) in northern Canada (Table 4). However, in 2 countries currently not using PCV7, comparison of 2 periods (2000–2002 and 2003–2005) showed that rates of IPD with penicillin-nonsusceptible isolates increased from 0.6 cases to 1.7 cases/100,000 persons ($p = 0.04$ for all ages, Iceland), and from 0.5 to 1.0 ($p<0.0001$, all ages. Finland). In both of these countries, the proportion of isolates that were nonsusceptible to penicillin also increased (Iceland, 5% [5/106] to 12% [15/130], $p = 0.05$; Finland, 4% [82/1,850] to 7% [154/2,199]; $p<0.01$).

Since routine use of PCV7, the proportion of isolates nonsusceptible to cotrimoxazole, erythromycin, ceftriaxone, and penicillin decreased in Alaska. The same trend was not observed in northern Canada, where rates of antimicrobial drug resistance were much lower than in Alaska (Table 6).

Clinical Findings

Data on clinical findings were available for Alaska, northern Canada, Greenland, and Norway. Bacteremic pneumonia was the most common clinical finding (range 45%–65%), followed by bacteremia alone (range 16%–24%) and bacteremic meningitis (Table 7). Limited clinical data were available for Iceland and northern Sweden but were not included. Clinical data were not available for Finland.

Risk Factors and Medical Conditions in Persons ≥18 Years of Age

Data on risk factors and medical conditions were available for Alaska and northern Canada. Among adults with a

Table 5. Most prevalent serotypes in 6 countries reporting *Streptococcus pneumoniae* type to ICS and proportion of isolates covered by PCV7 and PCV13 vaccines*

	Alaska		Canada					
Rank	Pre-PCV7, 1999–2000, n = 224	Post-PCV7, 2001–2005, n = 453	Pre-PCV7, 1999–2002, n = 158	Post-PCV7, 2003–2005, n = 82	Greenland, n = 60	Iceland, n = 269	Norway, n = 291	Finland, n = 3,947
1	14 (17%)	19A (11%)	1 (34%)	1 (24%)	1 (22%)	7 (20%)	4, 14 (18%)	14, 4 (12%)
2	4, 7F (9%)	4 (8%)	14 (11%)	8 (11%)	12F (15%)	14 (12%)	9 (11%)	9V (8%)
3	9V (8%)	12F (8%)	4 (9%)	3 (7%)	4 (12%)	23 (12%)	6 (9%)	3, 23F, 7F (7%)
4	19F (6%)	3, 7F, 8 (7%)	8 (8%)	10A, 18C, 22F (6%)	22F (8%)	19 (10%)	23 (8%)	6B (6%)
5	6B (6%)	14 (6%)	6B, 9V (6%)	6B (5%)	3 (7%)	9 (10%)	7 (7%)	19A, 19F (4%)
Proportion of serotyped isolates covered by PCV7 and PCV13 vaccines (<2 y of age)								
PCV7	82% (51/62)	21% (16/77)	76% (25/33)	21% (3/14)	50% (3/6)	51% (23/45)	37% (10/27)	NA
PCV13	92% (57/62)	57% (44/77)	94% (31/33)	43% (6/14)	83% (5/6)	60% (27/45)	56% (15/27)	NA

*ICS, International Circumpolar Surveillance; PCV7, 7-valent pneumococcal conjugate vaccine (serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F); PCV13, 13-valent pneumococcal conjugate vaccine (7 PCV7sero types plus 1, 3, 5, 6A, 7F, and 19A); NA, not available.

Table 6. Proportion of isolates with nonsusceptibility to antimicrobial drugs in countries in the ICS reporting an antibiogram*

Antimicrobial drug	Age group, y	Alaska		Northern Canada		Iceland, % (n/N)	Northern Sweden, % (n/N)
		Pre-PCV7, % (n/N)	Post-PCV7, % (n/N)	Pre-PCV7, % (n/N)	Post-PCV7, % (n/N)		
Cotrimoxazole	<2	58 (36/62)	34 (26/77)	9 (3/33)	17 (2/12)	35 (13/37)	100 (1/1)
	All ages	31 (70/224)	21 (96/453)	9 (14/158)	9 (7/78)	18 (43/234)	12 (3/25)
Erythromycin	<2	42 (26/62)	13 (10/77)	0 (0/33)	8 (1/13)	26 (10/38)	0 (0/2)
	All ages	21 (47/223)	8 (37/452)	1 (1/157)	5 (4/79)	9 (21/235)	6 (3/53)
Ceftriaxone†	<2	23 (14/62)	5 (4/77)	6 (2/33)	0 (0/12)	0 (0/10)	NA
	All ages	11 (25/224)	1 (6/453)	4 (7/159)	0 (0/80)	0 (0/39)	NA
Penicillin‡	<2	40 (25/62)	29 (22/77)	6 (2/33)	0 (0/13)	13 (5/38)	0 (0/1)
	All ages	22 (50/224)	15 (68/453)	5 (8/159)	4 (3/81)	8 (20/236)	2 (1/52)

*ICS, International Circumpolar Surveillance; PCV7, 7-valent pneumococcal conjugate vaccine; NA, not available.
†Greenland reported nonsusceptibility of 0% (0/38) to ceftriaxone among all ages.
‡Greenland reported nonsusceptibility of 0% (0/41) and Finland reported nonsusceptibility of 6% (236/4,049) to penicillin among all ages. Finland reported nonsusceptibility of 9% (33/367) to penicillin among cases <2 y of age.

diagnosis of IPD in the North American Arctic, 40%–44% had a history of smoking, 37%–39% had a history of alcohol abuse, and 19%–27% had a history of chronic lung disease (Table 8).

Discussion

Our data show that cases of IPD continue to occur throughout Arctic countries with highest rates among children <2 years of age, adults ≥65 years of age, and indigenous persons of the North American Arctic. The rate of IPD with PCV7 serotypes in children <2 years of age decreased dramatically after routine vaccination in Alaska (128.3 to 15.5/100,000) and northern Canada (128.9 to 20.6/100,000). However, in Alaska, rates of non-PCV7 serotypes among children <2 years of age increased (27.7 to 59.0/100,000) during the same period. Although PCV7 serotype–specific IPD rates decreased among children <2 of age in countries that have implemented use of PCV7, overall rates of IPD among indigenous North American children remain high at 177.5 cases/100,000 and 92.6 cases/100,000 in Alaska and northern Canada, respectively, showing persistent health disparities.

Consistent with earlier studies, IPD rates were highest in the prevaccine period among indigenous persons in Alaska and northern Canada and high among children <2 years of age and the elderly (*1*,*5*,*11*–*18*). The relatively higher rates among children <2 years of age in Alaska and northern Canada (compared with other circumpolar countries) are primarily caused by increased rates in large indigenous populations (19% and 59% of the total population in Alaska and northern Canada, respectively), which tend to live under conditions of crowding, increasing environmental stress, and lower socioeconomic status.

IPD rates in the prevaccine period among indigenous children ranged from 229/100,000 (northern Canada) to 441/100,000 (in Alaska). These rates that are 4–6 times higher than those found among nonindigenous children in those regions. Indigenous persons in the circumpolar north have been shown to have high rates of IPD (*1*,*2*,*5*,*15*,*19*–*21*), as have other indigenous groups such as Aboriginal Australians (*22*), White Mountain Apaches (*23*) and Navajos in the southwestern United States (*24*), Maoris of New Zealand (*25*), and the bedouins of Israel (*26*).

Our data demonstrate that use of PCV7 in Alaska and northern Canada led to marked decreases in the incidence of IPD and PCV7 serotype–specific disease overall (all age groups combined) among indigenous persons (all age groups combined) and among indigenous children <2 years of age. Use of this vaccine has resulted in a near equalization of rates of PCV7 serotype–specific disease among indigenous children <2 years of age in Alaska and northern Canada. Our data also show decreases in disease among persons >2 years of age who were not targeted to receive vaccine. Although some children >2 years of age may have received vaccine as part of vaccine catch-up programs, older children and adults did not receive the vaccine. Our data show decreases in PCV7 serotype–specific disease among persons 20–64 years of age in northern Canada and among

Table 7. Clinical findings for invasive pneumococcal disease

Findings	Alaska, 1999–2005, no. (%)	Northern Canada, 1999–2005, no. (%)	Greenland, 2000–2005, no. (%)	Norway, 2000–2005, no. (%)
Pneumonia with bacteremia	466 (61)	162 (65)	36 (52)	2,598 (45)
Sepsis	154 (20)	41 (16)	14 (20)	1,404 (25)
Bacteremia	20 (3)	13 (5)	0	864 (15)
Meningitis with bacteremia	53 (7)	16 (6)	14 (20)	454 (8)
Other*	76 (10)	19 (8)	5 (7)	405 (7)
Total	769 (100)	251 (100)	69 (100)	5,725 (100)

*Empyema, cellulitis, necrotizing fasciitis, septic arthritis.

Table 8. Risk factors and medical conditions in persons ≥18 years of age with invasive pneumococcal disease*

Factor or condition	Alaska, 1999–2005, no. (%)	Northern Canada, 1999–2005, no. (%)
Cigarette smoking	223 (44)	54 (40)
Alcohol abuse†	201 (39)	50 (37)
Chronic lung disease/asthma	139 (27)	26 (19)
Diabetes mellitus	71 (14)	22 (16)
Immunosuppressive therapy	35 (7)	5 (4)
Injection drug use	11 (2)	3 (2)
Asplenia	9 (2)	4 (3)
Total	509 (100)	135 (100)

*Risk factors and medical conditions are not mutually exclusive. Each case may have >1 condition reported. Data were not available for Greenland, Iceland, Norway, northern Sweden, or Finland.
†Alcohol abuse was noted in the chart.

persons ≥65 years of age in Alaska. These data are consistent with decreasing rates of vaccine-type IPD among adults in the general US population (*27*).

The increased rate of non-PCV7–serotype disease in Alaska after introduction of PCV7, primarily among indigenous persons, is concerning. Increases of such magnitude have not been observed among the general US child population or elsewhere. However, continued vigilance is critical to monitor trends in pneumococcal disease and serotype distribution.

Incidence rates increased among children <2 years of age in 2 of 4 ICS members countries not using PCV7 over the study period (Norway and Finland). Norway began routine use of the vaccine among children in 2006. Continued collection of surveillance data will be critical in the coming years to assess the effect of pneumococcal vaccine, serotype shifts, and changes in antimicrobial drug susceptibility patterns.

Our data show that several serotypes (4, 6, 7, and 14) are common in northern circumpolar countries. Although serotype 1 was the most prevalent serotype in Greenland and northern Canada, it was not common in other circumpolar countries. Among countries that were not using PCV7 during the study period, ≈50% of all IPD cases in children <2 years of age were vaccine preventable (caused by serotypes present in PCV7). Use of 13-valent conjugate vaccine (which includes all PCV7 serotypes plus serotypes 1, 3, 5, 6A, 7F, and 19A) currently being evaluated for use in the United States could theoretically have prevented ≈70% of IPD cases among children <2 years of age.

In the 2 countries currently using PCV7 (Alaska and northern Canada), the proportion of penicillin-nonsusceptible isolates decreased. Published data on antimicrobial drug resistance in Alaska, the United States, and Canada showed an increasing proportion of *S. pneumoniae* drug-resistant isolates before vaccine implementation (*15*,*28–30*; www.cdc.gov/ncidod/aip/research/ar.html). Our data show a rapid decrease in the proportion of isolates resistant to penicillin and other antimicrobial drugs after PCV7 implementation. In the only 2 countries for which data on antimicrobial drug susceptibility were available and which were not using the vaccine (Iceland and Finland), the proportion of isolates resistant to penicillin increased over the study period.

To our knowledge, the ICS collaboration and data presented in this report are the first population-based assessment of IPD in the Arctic using similar case definitions and comparable laboratory methods. However, this study has several limitations. We did not collect detailed clinical and demographic information beyond what was available from medical record review and thus could not evaluate an extensive range of risk factors. Data on clinical findings, antimicrobial drug susceptibility, and risk factors are not collected consistently across the entire ICS network, and data on race/ethnicity are collected from only 2 ICS member countries. Because not all ICS member countries joined the network in the same year, the number of years of data available during the study period varied by country. In addition, limited information exists on diagnostic culturing practices of ICS member countries, which may lead to detection bias (milder cases more likely to be detected in some regions). This limitation may contribute to the wide variation in case-fatality rates across the ICS network. Finally, these data do not represent a complete picture of pneumococcal invasive disease in the far north because the Russian Federation does not participate in ICS, and data from only 1 region in northern Sweden are included.

The ICS project provides a broad view of IPD and the utility and status of prevention efforts in the Arctic. Demonstration of the effectiveness of PCV7 in Alaska and Canada and identification of issues relevant for future vaccine development are critical for decision making. Surveillance data on serotype and antimicrobial drug susceptibility distribution in Arctic countries provide necessary information for assessing the potential effect of current and future pneumococcal vaccines. Continuing evaluation of IPD in the ICS network will provide data necessary to maximize IPD prevention efforts throughout the region.

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Invasive Bacterial Diseases in Northern Canada

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International Circumpolar Surveillance (ICS) is a population-based invasive bacterial disease surveillance network. Participating Canadian regions include Yukon, Northwest Territories, Nunavut, and northern regions of Québec and Labrador (total population 132,956, 59% aboriginal). Clinical and demographic information were collected by using standardized surveillance forms. Bacterial isolates were forwarded to reference laboratories for confirmation and serotyping. After pneumococcal conjugate vaccine introduction, crude annual incidence rates of invasive *Streptococcus pneumoniae* decreased from 34.0/100,000 population (1999–2002) to 23.6/100,000 population (2003–2005); substantial reductions were shown among aboriginals. However, incidence rates of *S. pneumoniae*, *Haemophilus influenzae*, and group A streptococci were higher in aboriginal populations than in non-aboriginal populations. *H. influenzae* type b was rare; 52% of all *H. influenzae* cases were caused by type a. Data collected by ICS contribute to the understanding of the epidemiology of invasive bacterial diseases among northern populations, which assists in formulation of prevention and control strategies, including immunization recommendations.

The circumpolar region of Canada is a sparsely populated area of 1.74 million square miles comprising 3 territories (Yukon, Northwest Territories, and Nunavut) and the northern regions of Québec and Labrador. The estimated population is 132,956, which represents 0.4% of the Canadian population (*1*). The circumpolar population is younger (Table 1) and has a larger proportion of aboriginal persons than the general Canadian population. Approximately 59% of the population in the region self-identify as Inuit, First Nations, or Métis, compared with 3.3% of the total Canadian population (*2*). Northern populations tend to have higher rates of invasive bacterial diseases, including those caused by *Streptococcus pneumoniae* and *Haemophilus influenzae*, with aboriginal persons at greatest risk for disease (*3–5*).

Canada has a universal healthcare system that includes access to both physician and hospital care. Publicly funded vaccination programs are a major component of disease control programs. Universal infant *H. influenzae* type b (Hib) vaccination programs were implemented in the Yukon, Northwest Territories, Nunavut, and northern regions of Québec and Labrador in the early 1990s. Pneumococcal polysaccharide vaccine, which protects against 23 serotypes of *S. pneumoniae*, has been available since 1983 and is recommended by the Canadian National Advisory Committee on Immunization (NACI) for all adults ≥65 years of

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Table 1. Total and age-specific population estimates for the Canadian circumpolar region and entire population, 2001 census (*1*)

Region	Total	Age, no. (%)				
		<2 y	2–4 y	5–19 y	20–64 y	≥65 y
Circumpolar*	132,956	4,849 (3.7)	7,414 (5.6)	37,431 (28.2)	77,823 (58.5)	5,439 (4.1)
Canada†	30,007,095	652,120 (2.2)	1,044,160 (3.5)	6,082,585 (20.3)	18,339,680 (61.1)	3,888,550 (13.0)

*Yukon, Northwest Territories, Nunavut, northern Québec, and northern Labrador.
†Includes the circumpolar region.

age and children ≥2 years of age at high risk for infection. The 7-valent pneumococcal conjugate vaccine (PCV7) protects against 7 serotypes of *S. pneumoniae* and has been available in Canada since 2001; NACI recommends it for all children <2 years of age and children <5 years of age at high risk for disease. Meningococcal C conjugate vaccine has been recommended by NACI for all children <5 years of age, adolescents, and young adults since 2001 (*6*). Implementation of these NACI recommendations has occurred at various times throughout the region.

In Canada, communicable disease reporting is mandated at the provincial or territorial level; the list of reportable diseases varies by region. Reporting to national notifiable disease surveillance is not mandatory, and timely submission of case-by-case data with epidemiologic, clinical, and laboratory information is variable. Therefore, to increase the understanding of the epidemiology of invasive bacterial diseases in northern populations, Canada has participated in International Circumpolar Surveillance (ICS) since its inception in 1999. ICS is a population-based invasive bacterial disease surveillance network of circumpolar countries that includes the United States, Canada, Greenland, Iceland, Finland, Norway, and Sweden. We describe Canadian ICS data from 1999 through 2005, including the effect of universal PCV7 programs on invasive *S. pneumoniae* disease in children <2 years of age.

Methods

Case Reporting and Data Collection

Surveillance of invasive disease caused by *S. pneumoniae* began January 1, 1999. Surveillance for invasive *H. influenzae*, group A streptococci (GAS), group B streptococci (GBS), and *Neisseria meningitidis* commenced January 1, 2000. Cases reportable to ICS are defined as persons from whom an organism under surveillance is isolated from blood, cerebrospinal fluid, or other normally sterile site. Patients with clinical epiglottitis from whom *H. influenzae* is isolated from an epiglottis swab are also reportable to ICS. Cases are reported to public health officials by physicians or laboratories serving regions under surveillance; this includes patients managed outside of the region. Unconfirmed cases are not included. Standardized case report forms are completed in the region by trained communicable disease officers and include demographic, clinical, vaccination, and risk factor information. For the vaccine-preventable diseases (caused by *S. pneumoniae*, Hib, and *N. meningitidis*), details on the type of vaccine received are not currently available; however, information on the number of doses received is available. Reference laboratory representatives and communicable disease officers from each region participate in quarterly and annual data audits to ensure completeness of case finding and reporting.

Laboratory Methods

A network of laboratories ascertains infection with any of the 5 organisms under surveillance within the region. Invasive isolates are submitted to 1 of 3 Canadian reference laboratories (National Centre for Streptococcus, National Microbiology Laboratory, and Laboratoire de Santé Publique du Québec). The reference laboratory confirms the isolate's identity, determines its serotype or serogroup, and tests for antimicrobial susceptibility. Laboratories also participate in an ongoing quality control program.

Isolates were confirmed as *S. pneumoniae* by using conventional methods of identification (*7*). Strains were classified by the capsular swelling technique (*8*,*9*) by using commercial antisera (Statens Serum Institut, Copenhagen, Denmark). Antimicrobial drug susceptibility testing was performed by using the broth microdilution method consistent with National Committee for Clinical Laboratory Standards guidelines current at the time of testing (*10*,*11*).

M typing was performed on all submitted GAS isolates according to standardized methods (*12*) by using M type–specific antisera prepared in-house. Antisera to 61 of 86 internationally recognized M types, representing the most common M types (*13*), were available; strains for which an M type could not be assigned were classified as M nontypeable. GBS serotyping was performed according to conventional serologic techniques (*14*) by using type-specific antisera prepared in-house. Antisera were available for all 9 internationally recognized serotypes (Ia, Ib, II, III, IV, V, VI, VII, and VIII). For both organisms, Lancefield hot-acid extracts were prepared from the clinical isolates and tested in Ouchterlony immunodiffusion agar slides with appropriate control strains.

H. influenzae was confirmed by standard biochemical tests (*15*), and biotypes were determined according to current nomenclature (*16*). Serotyping was conducted by using a slide agglutination assay with antisera from commercial sources (Difco, Oakville, Ontario, Canada, and Denka Seiken, Tokyo, Japan). *N. meningitidis* was identi-

fied by using standard biochemical tests (*17*). Serogrouping was conducted by using bacterial agglutination with rabbit antisera to the different serogroups. Serotyping and serosubtyping were conducted by using an indirect, whole-cell, ELISA with monoclonal antibodies (*18*).

Statistical Analysis

Statistical analysis was conducted by using SAS statistical package version 9.1 (SAS, Cary, NC, USA). The analyses were stratified by organism. Crude and age-specific annual incidence was calculated by using total and age-specific population estimates from the Demography Division of Statistics Canada for 2001 (*1*). Because of the small numbers of cases per year, 3-year period–based annual incidence rates were calculated for each organism to determine time trends. Rates were calculated for 2 periods (1999 for *S. pneumoniae* or 2000 for *H. influenzae*, GAS, and GBS to 2002 and 2003–2005). Regression analysis was conducted to detect trends in crude annual incidence rates over time. Crude incidence rates by ethnicity were calculated by using population data from the aboriginal population profile, which was developed from 2001 census data (*2*). Because of the lack of additional period estimates, no trend analyses were conducted on data by ethnicity.

Information about PCV7 program implementation was collected from the regions (Table 2). The prevaccination period was defined as 1999–2002, and the program implementation period was defined as 2003–2005. Because Labrador implemented its vaccination program during the second period, data from this region on the effect of the vaccine were excluded from the analysis. Sensitivity analyses that included data from Labrador in both arms was conducted to ensure this did not alter the results. Annual incidence of *S. pneumoniae* for all ages and the number of cases in children <2 years of age were compared for the 2 periods. Bivariate analysis was conducted by using χ^2 and Fisher exact tests.

Results

There were 251 confirmed cases of invasive disease caused by *S. pneumoniae* in northern Canada from 1999 through 2005. During 2000–2005, 62 cases of invasive disease caused by *H. influenzae*, 45 caused by GAS, 17 caused by GBS, and 6 caused by *N. meningitidis* were reported. Because of the small number of *N. meningitidis* cases reported, no further disease-specific analyses were conducted for this organism.

Cases and Incidence Rates

In the ICS region, the crude annual incidence rate for *S. pneumoniae* was highest in 2001 (38.4/100,000 population) and lowest in 2005 (17.3/100,000 population), but this downward trend was not statistically significant (p = 0.119 by F test for slope). The age-specific incidence rate in children <2 years of age decreased during 2000–2004 but increased in 2005. However, these incidence rates are based on a small number of cases and changes in rates over time should be interpreted with caution. The incidence rates in the population ≥65 years of age, who were eligible for the 23-valent polysaccharide vaccination, did not show any trend (Table 3). During 1999–2002 and 2003–2005, the crude annual incidence rates were 34.0 and 23.6/100,000 population/year, respectively. Although this finding suggests a decreasing incidence over the 2 periods, data from additional periods are necessary to determine if this is reflective of a trend.

Table 2. Implementation dates of infant vaccination programs with universal 7-valent pneumococcal conjugate vaccine, by circumpolar region, Canada

Region	Date
Northern Québec	Apr 2002
Nunavit	Sep 2002
Northern Labrador	Jul 2003
Yukon	Jun 2005
Northwest Territories	Jan 2006

Among the 240 (95.6%) of 251 *S. pneumoniae* cases with serotype information, the most common serotypes were type 1 (30.4%), type 8 (8.8%), type 14 (7.9%), type 4 (6.3%), and type 6B (5.8%). A total of 47 (60%) of 52 cases in children <2 years of age were caused by PCV7 serotypes. Among persons ≥65 years of age, 23 (88.5%) of 26 cases were caused by serotypes in the polysaccharide pneumococcal vaccine.

There were no trends in overall crude annual incidence rates of *H. influenzae* or GBS (Table 4). The crude annual incidence rate of *H. influenzae* was lowest in 2003 (4.5/100,000 population) and highest in 2001 (13.5/100,000 population). Among 59 cases with serotype information, 31 (59%) were *H. influenzae* type a (Hia); 73.3% of these cases were in children <2 years of age. Eight cases (13.6%) of Hib were reported during the surveillance period: 6 in infants <5 months of age, 1 in a child 18 months of age, and 1 in an adult. Two infants had no vaccine information. The adult and 1 infant had not been vaccinated; the remaining 4 children had received only 1 Hib dose. Thus, none of these cases were considered vaccine failures. GAS incidence in-

Table 3. Crude annual incidence of *Streptococcus pneumoniae* in the Canadian circumpolar region by age group, 1999–2005

Year	Incidence/100,000 population (no. cases)		
	Total population	Age <2 y	Age ≥65 y
1999	25.6 (34)	226.9 (11)	55.2 (3)
2000	33.8 (45)	226.9 (11)	36.8 (2)
2001	38.4 (51)	165.0 (8)	91.9 (5)
2002	26.3 (35)	123.7 (6)	73.5 (4)
2003	22.6 (30)	103.1 (5)	110.3 (6)
2004	24.8 (33)	61.9 (3)	36.8 (2)
2005	17.3 (23)	165.0 (8)	73.5 (4)

creased significantly during 2001–2005 (F = 229.371, p = 0.01). The largest number of cases (n = 14) was reported in 2005 with a crude incidence rate of 10.5/100,000 population. The increase in GAS cases was not clustered by region, period, or serotype. A total of 1–4 cases of GBS were reported in the region annually, for a crude annual incidence range of 0.8–3.0/100,000 population.

Demographic Characteristics

Infections with *S. pneumoniae*, *H. influenzae*, and GAS were more common in male patients (59.8%, 58.3%, and 62.2%, respectively). Seventy-one percent of all cases of GBS were among female patients; 17.6% (3/17) of cases of GBS were among newborns <1 month of age. All of the newborn cases occurred in the early neonatal period. These 4 organisms disproportionately affect children <2 years of age and persons ≥65 years of age. Although <4% of the surveillance population was <2 years of age, 21%–67% of the infections occurred within this age group. Similarly, adults ≥65 years of age had a higher proportion of cases than the surveillance population they represent (Figure).

Data on patient ethnicity was missing for 42 (11.0%) of 381 cases of invasive bacterial disease. Aboriginal persons represented 59% of the surveillance population and 84% of cases of *S. pneumoniae*, 92% of *H. influenzae*, 93% of GAS, and 53% of GBS. To assess changing incidence over time by ethnicity, the surveillance period was divided into 2 periods (1999–2002 and 2003–2005 for *S. pneumoniae* and 2000–2002 and 2003–2005 for all other organisms). For all but GBS, the crude annualized incidence rates were higher in the aboriginal population than in the non-aboriginal population (Table 5). For *S. pneumoniae*, the disparity between aboriginal persons and non-aboriginal persons decreased from 4.6-fold to 2.5-fold between the 2 periods. Among aboriginal persons, the GAS rate in the second period was nearly double that seen in the first period. Six of 8 case-patients with Hib and all 27 case-patients with Hia for whom ethnicity data were available were among aboriginal persons.

Clinical Findings and Outcomes

Information on clinical findings was available for 380 of 381 cases (Table 6). The most common primary clinical finding for invasive *S. pneumoniae* was pneumonia (64.5%), followed by bacteremia/septicemia (21.5%). Among invasive GAS cases, the most common primary clinical finding was cellulitis (31.1%); necrotizing fasciitis accounted for 11.1%. For cases with *H. influenzae* or GBS, the most common primary clinical findings were bacteremia/septicemia (33.9% and 47.1%, respectively). There were no reported cases of epiglottitis caused by *H. influenzae*.

Cases with GAS had the highest case-fatality rate (18.2%, 8 of 44 cases with outcome data). Two (40%) of 5 cases with GAS and necrotizing fasciitis resulted in death; however, this difference was not statistically significant (p = 0.065, by Fisher exact test). The case-fatality rates for infections with the other 3 organisms were 4.8% (11/230) for *S. pneumoniae*, 6.1% (3/49) for *H. influenzae*, and 7.1% (1/14) for GBS. The relative risk for death did not vary by ethnicity (p = 0.550, by Fisher exact test).

Table 4. Crude annual incidence of *Haemophilus influenzae*, GAS, and GBS in the Canadian circumpolar region, 2000–2005*

Year	Incidence/100,000 population (no. cases)		
	H. influenzae	GAS	GBS
2000	6.0 (8)	5.3 (7)	3.0 (4)
2001	13.5 (18)	1.5 (2)	3.0 (4)
2002	6.0 (8)	3.8 (5)	1.5 (2)
2003	4.5 (6)	5.3 (7)	3.0 (4)
2004	8.3 (11)	7.5 (10)	1.5 (2)
2005	8.3 (11)	10.5 (14)	0.8 (1)

*GAS, group A streptococci; GBS, group B streptococci.

Effect of PCV7 Immunization Programs

Fifty-two cases of S. *pneumoniae* occurred in children <2 years of age. Eight of these case-patients had received ≥1 dose of pneumococcal vaccination: 1 case-patient with of PCV7-preventable *S. pneumoniae* had received only 1 dose of vaccine (serotype 6b), 6 case-patients had serotypes that were not preventable with PCV7 (serotypes 19A, 20, 13, 15a, 22, and 22F), and 1 case-patient had no information on serotype. These findings suggest that there were no known cases of vaccine failure.

Numbers of cases of disease caused by *S. pneumoniae* in children <2 years of age were compared during the prevaccination and program implementation periods (Table 7). In regions where universal PCV7 infant programs were implemented in 2002 (Nunavut and northern Québec), 19 cases with PCV7 serotypes were reported during the prevaccination period and no cases were reported during the program implementation period. In the other Canadian ICS regions where universal PCV7 infant programs were implemented after 2002 (excluding Labrador), 6 cases of PCV7-

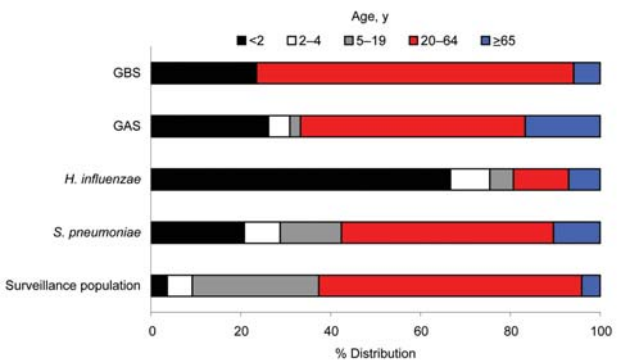

Figure. Age distribution of surveillance population and cases of infection with group B streptococci (GBS), group A streptococci (GAS), *Haemophilus influenzae*, and *Streptococcus pneumoniae* in the Canadian circumpolar region.

Table 5. Crude annualized incidence of *Streptococcus pneumoniae, Haemophilus influenzae,* GAS, and GBS by ethnicity in the Canadian circumpolar region, 1999–2005*

Organism, ethnicity	Incidence/100,000 population/ year (no. cases) 1999–2002	2003–2005
S. pneumoniae		
Non-aboriginal	9.6 (20)	10.2 (16)
Aboriginal	44.2 (134)	25.1 (57)
	2000–2002	2003–2005
H. influenzae		
Non-aboriginal	0.6 (1)	1.9 (3)
Aboriginal	11.9 (27)	8.4 (19)
GAS		
Non-aboriginal	0	1.9 (3)
Aboriginal	5.7 (13)	11.0 (25)
GBS		
Non-aboriginal	2.6 (4)	1.9 (3)
Aboriginal	2.2 (5)	1.3 (3)

*GAS, group A streptococci; GBS, group B streptococci.

preventable *S. pneumoniae* disease occurred in the prevaccination period and 3 cases in the program implementation period. A χ^2 analysis showed that the number of cases of PCV7-preventable illness by vaccination region was statistically significant (p = 0.019, by Fisher exact test). These results are conservatively biased because PCV7 was available in all regions in the program implementation period and a universal vaccination program was started during the later half of 2005 in the Yukon, which may have reduced the number of cases seen in the comparison area. Our findings suggest that early implementation of universal PCV7 programs was associated with a reduction in PCV7-preventable illness in children <2 years of age. A sensitivity analysis including Labrador in both arms (program or no program) did not change the statistical significance of the difference because 1 case in Labrador was not vaccine preventable.

Discussion

To our knowledge, this study is the first comprehensive surveillance report on invasive bacterial diseases in the Canadian Arctic. Disease caused by *S. pneumoniae* continues to be a serious problem in northern Canada. The annual rate for 2003–2005 was 23.6/100,000 population/year, which is more than twice the reported rate of invasive pneumococcal disease in the overall Canadian population (9.1/100,000 population in 2004) (*19*). Although this rate is lower than that seen in the earlier period (1999–2002, 34.0/100,000 population), additional data will be needed to determine if the decreasing trend is sustained. The decrease in the disease incidence may be partly attributed to PCV7 programs, as well as the mass pneumococcal polysaccharide vaccination campaigns launched in 2001 and 2002 in response to outbreaks of serotype 1 disease in parts of the region, which reduced the occurrence of this predominant serotype in subsequent years (*20*,*21*). Reduction in the number of cases among children <2 years of age in regions where universal infant PCV7 programs were implemented in 2002 (northern Québec and Nunavut) is an early indicator of the effect of the vaccination program. This finding is likely a conservative assessment of the effect of the program, given the staggered implementation of universal vaccine programs in the circumpolar region.

Although aboriginal persons represented 84% of invasive *S. pneumoniae* cases, a substantial reduction in disease incidence was demonstrated in the program implementation period. Progress toward elimination of this health disparity has also been reported for indigenous populations in Alaska (*22*) and Australia (*23*), where PCV7 has been available since 2001 to all indigenous children <2 years of age. The incidence of *S. pneumoniae* may be expected to decrease further among young children throughout northern Canada, particularly in aboriginal children, as universal PCV7 programs become fully implemented with sustained high coverage rates.

In contrast, there continues to be a health disparity for invasive *H. influenzae* disease. Annual period incidence rates for *H. influenzae* during 2003–2005 were >4-fold higher among aboriginal persons than among non-aboriginal persons. Although Hib disease is rare because of universal Hib vaccination, the greatest number of cases occurred among aboriginal persons, a group known to be at increased risk for Hib disease (*24*–*26*). Studies in Alaskan

Table 6. Primary clinical symptoms for patients infected with *Streptococcus pneumoniae, Haemophilus influenzae*, GAS, or GBS in the Canadian circumpolar region*

Symptoms	*S. pneumoniae* (n = 251), no. (%)	*H. influenzae* (n = 62), no. (%)	GAS (n = 44),† no. (%)	GBS (n = 17), no. (%)
Septicemia/bacteremia	21.5 (54)	33.9 (21)	22.7 (10)	47.1 (8)
Pneumonia	64.5 (162)	21.0 (13)	13.6 (6)	0
Meningitis	6.0 (15)	27.4 (17)	0	11.8 (2)
Cellulitis	0.8 (2)	4.8 (3)	31.8 (14)	11.8 (2)
Empyema	3.2 (8)	3.23 (2)	2.3 (1)	0
Necrotizing fasciitis	0	0	11.4 (5)	0
Septic arthritis	0.8 (2)	4.8 (3)	9.1 (4)	17.7 (3)
Other‡	3.2 (8)	4.8 (3)	9.1 (4)	11.8 (2)

*GAS, group A streptococci; GBS, group B streptococci.
†One patient had no information recorded for clinical symptoms.
‡Includes peritonitis, appendicitis, pericarditis, osteomyelitis, and endocarditis.

Table 7. Effect of universal PCV7 programs for children <2 y of age in the Canadian circumpolar region*

Location, period	No. cases with PCV7 serotypes	No. cases without PCV7 serotypes	Total
Northern Québec and Nunavut			
Prevaccination (1999–2002)	19	5	24
Program implementation (2003–2005)	0	8	8
Total cases	19	13	32
Northwest Territories and Yukon			
Prevaccination (1999–2002)	6	2	8
Program implementation (2003–2005)	3	3	6
Total cases	9	5	14

*PCV7, 7-valent pneumococcal conjugate vaccine.

aboriginal populations suggest that continued low-level nasopharangeal colonization facilitates transmission to susceptible children (*4*). Environmental and housing conditions, including overcrowding, are also potential contributing factors to these health disparities (*27–29*). The data also indicate an apparent emergence of type a disease, with all Hia cases occurring among aboriginal persons. Hia disease has also been reported in aboriginal populations in the United States and Australia (*30–32*). A possible shift in disease epidemiology to non-b serotypes has been suggested from findings in an adjacent Canadian region (*33*), whereas a sustained increase in non-b serotypes has not been detected in Alaska (*4*).

The incidence of GAS in the ICS region has been increasing since 2001. Although changes in rates over time should be interpreted with caution because the number of cases is small, this apparent increase in GAS disease is being monitored. The rate in northern regions is greater than that in the overall Canadian population. In 2004, the incidence of GAS in the ICS population was 7.5 compared with the Canadian rate of 2.7/100,000 population (Public Health Agency of Canada, unpub. data). Aboriginal persons represented the greatest proportion of GAS cases; this has also been observed among indigenous populations in the United States (*34*) and Australia (*35*). As with *S. pneumoniae* and *H. influenzae*, this may be partially attributed to poverty and crowded living conditions in these populations (*36*).

A major limitation of the data is that the number of reported cases is too small to permit analysis of smaller areas and subpopulations within the region. In addition, changes in rates over time should be interpreted with caution due to small numbers of cases. It is also expected that the number of reported cases of invasive bacterial diseases is an underestimate. However, the enhanced nature of the surveillance system and regular data audits represent improvements over routine passive surveillance. Laboratory specimens may not have been taken before initiation of empiric treatment, and collection and transportation of clinical specimens are difficult in remote areas experiencing extreme temperatures (*20*,*21*). Unfortunately, because vaccine registries have not yet been fully implemented in Canada, immunization coverage rates are not available. This situation limits our ability to evaluate the effect of vaccination programs.

Despite these limitations, data collected by ICS contribute to understanding the epidemiology of invasive bacterial diseases among northern populations in Canada and throughout the world. These data assist in formulation of prevention and control strategies, including immunization recommendations (*6*). ICS data have also been instrumental in identifying potentially emerging pathogens such as Hia. Continued collection of data will be used to assess the effect of vaccination in this population and monitor serotype replacement, antimicrobial drug resistance, and reductions in disparities in Northern populations.

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Sindbis Virus Infection in Resident Birds, Migratory Birds, and Humans, Finland

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Sindbis virus (SINV), a mosquito-borne virus that causes rash and arthritis, has been causing outbreaks in humans every seventh year in northern Europe. To gain a better understanding of SINV epidemiology in Finland, we searched for SINV antibodies in 621 resident grouse, whose population declines have coincided with human SINV outbreaks, and in 836 migratory birds. We used hemagglutination-inhibition and neutralization tests for the bird samples and enzyme immunoassays and hemagglutination-inhibition for the human samples. SINV antibodies were first found in 3 birds (red-backed shrike, robin, song thrush) during their spring migration to northern Europe. Of the grouse, 27.4% were seropositive in 2003 (1 year after a human outbreak), but only 1.4% were seropositive in 2004. Among 2,529 persons, the age-standardized seroprevalence (1999–2003) was 5.2%; seroprevalence and incidence (1995–2003) were highest in North Karelia (eastern Finland). Grouse may contribute to the epidemiology of SINV in humans.

Sindbis virus (SINV) was first recognized and isolated in 1952 from a pool of *Culex pipiens* and *Cx. univittatus* mosquitoes collected from a village in the Nile River delta in Egypt, after which the virus was named (*1*). SINV, a member of the western equine encephalomyelitis complex of the genus *Alphavirus* in the family *Togaviridae*, is an enveloped virus with a genome of single-stranded, positive-polarity, 11.7-kb RNA (*2*). SINV is present throughout the Old World but has never been found in the New World (the Americas). SINV seropositivity in humans has been reported in various areas, and antibodies to SINV have also been found from various bird (*3*–*5*) and mammal (*6*,*7*) species. The virus has been isolated from several mosquito species, frogs (*8*), reed warblers (*9*), bats (*10*), ticks (*11*), and humans (*12*–*14*).

Despite the wide distribution of SINV, symptomatic infections in humans have been reported in only a few geographically restricted areas, such as northern Europe, and occasionally in South Africa (*12*), Australia (*15*–*18*), and China (*13*). In the early 1980s in Finland, serologic evidence associated SINV with rash and arthritis, known as Pogosta disease (*19*). In 2002, SINV was confirmed as the causative agent of Pogosta disease by isolating the virus from acutely ill patients (*14*). The typical clinical picture of acute Pogosta disease consists of arthritis, itching rash, fatigue, mild fever, headache, and muscle pain (*20*). Since 1974 in Finland, the disease has occurred as epidemics of hundreds or even thousands of patients every seventh year (1974, 1981, 1988, 1995, 2002). Similar disease is also found in Sweden (Ockelbo disease) and Russian Karelia (Karelian fever).

Most clinical cases in Finland are reported during August and September; the ornithophilic late summer mosquito species, *Culex* and *Culiseta*, are presumed to be the primary vectors (*21*). Grouse (*Tetraonidae*) (*4*) and passerines (especially thrushes, *Turdidae*) (*5*,*22*) have been suggested as amplifying hosts for SINV in northern Europe. Grouse are of special interest because they have previously had a 6–7 year population cycle with population declines coinciding with SINV outbreaks (*4*,*23*,*24*). Migratory birds may also play a role in distributing SINV over long distances, as they do with West Nile and avian influenza viruses. Supporting information is provided by the fact that SINV is disseminated over vast geographic areas of Australia with

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isolates from widely separated locations sharing identical nucleotide sequences (*25*). Studies on antigenic relatedness of alphaviruses have also suggested that progenitor alphaviruses are spread over long distances by birds (*26*). Furthermore, phylogenetic studies have indicated that South African and northern European SINV strains are closely related (*14*,*27*). Therefore, the hypothesis is that migratory birds have carried the virus to northern Europe.

A previous human epidemiologic study on SINV from Finland (*4*) reported an annual incidence of 2.7/100,000 during 1989–1996 and a seroprevalence of 0.6% in women of reproductive age during 1992. In addition, SINV antibodies were found in a small number of game birds and mammals in Ilomantsi, eastern Finland, during 1981–1983 (*4*). We studied the human epidemiology of SINV during an additional 7-year period in Finland and the seroprevalence of SINV in resident grouse. Furthermore, we looked for SINV antibodies in migratory birds arriving in northern Europe. Our aim was to elucidate factors contributing to the epidemiologic pattern of SINV infections in humans in Finland.

Methods

National Surveillance for SINV and Testing of Human Serum Samples

We used data reported from January 1995 to December 2003 in our analysis of incidence of SINV in the Finnish population. Since 1995, all Finnish clinical microbiology laboratories have reported laboratory-confirmed (by antibody detection) diagnoses of SINV infection to the National Infectious Disease Registry, maintained by the National Public Health Institute in Helsinki. Most laboratory reporting is done electronically and includes date of specimen collection and patient's date of birth, sex, and place of treatment. Multiple reports for the same person received within a 12-month period are combined as a single case.

For our analysis of the seroprevalence of SINV in the Finnish population, we used all samples from October 6, 1999, to May 8, 2003, that were tested for SINV antibodies at Helsinki University Central Hospital Laboratory. This laboratory performs >70% of the SINV antibody testing in Finland; other testing is performed by the Department of Virology at the University of Turku. We included only the most recent sample from patients with multiple samples; we excluded samples from those who had acute SINV infection (immunoglobulin [Ig] M positive).

Testing of Resident Grouse

Blood samples from grouse were collected by hunters from September 10 through October 31, 2003, and the same dates in 2004. The blood was absorbed into small slips of filter paper, dried, and stored individually at –20°C. The age and sex of the grouse were determined by hunters.

Testing of Migratory Birds

In 2004, blood samples were collected from migratory birds in 2 bird observatories during their spring migration: on Jurmo Island (59°50′N, 21°36′E) on May 18 and 19 and in Tauvo (64°49′N, 24°37′E) May 24–27. In 2005, blood samples were also collected in 2 different bird observatories during the spring migration: on Lågskär Island (59°50′N, 19°55′E) May 22–25 and in Tauvo May 29–31. In addition, migratory bird samples were collected in Kokkola archipelago (63°52′N, 23°4′E) on July 30, 2005 (Figure 1, panel A). Birds were captured with mist nets and identified by certified bird ringers. Blood samples were obtained by absorbing blood from the veins of wings or feet into filter paper slips and then dried. When possible, samples from native birds were also collected into small glass capillary tubes. Samples from migratory birds were collected with the permission of the Animal Experiment Committee of the University of Helsinki (permission no. HY75-04). We used the English and scientific names of birds according to Cramp et al. (*28*).

Serologic Testing

The human serum samples were examined for SINV IgM and IgG antibodies by enzyme immunoassays (EIA) and for SINV total antibodies by hemagglutination-inhibition (HI) testing. The protocols for EIA (*29*) and HI (*29*,*30*), as well as the diagnostic criteria for acute infection and previous immunity (*29*), have been described. In the incidence analysis, ≈25% of the seropositive diagnoses were made by using EIA at the University of Turku, where HI testing was not used.

The bird samples were screened for antibodies by HI testing. Approximately 1 cm^2 of each blood-saturated dry filter paper slip was cut into small pieces, and 1 mL of Dulbecco phosphate-buffered saline plus 0.2% bovine serum albumin was added to elute the blood. The resulting dilution corresponded to an ≈1:10 serum dilution (*19*), as verified by parallel titrations with blood (on filter paper) and serum samples from a seropositive person, which were used as controls. A liquid dilution (250 μL) was used for HI analysis.

HI testing was performed with 2-fold dilutions (1:20–1:640). Only titers ≥40 were considered positive. Performance of the HI technique on bird samples was confirmed by comparison with a set of HI-positive and HI-negative human samples and with neutralization tests (NTs) in which endpoint neutralizing antibody titers inhibiting cytopathic effect on Vero E6 cells were determined ≈65 h after infection. All samples in the subset with an HI titer >40 (n = 7) showed NT titers >20, and all samples in the subset with an HI titer <20 (n = 8) showed NT titers <20. In addition, all samples with borderline HI results were determined by NT to be negative.

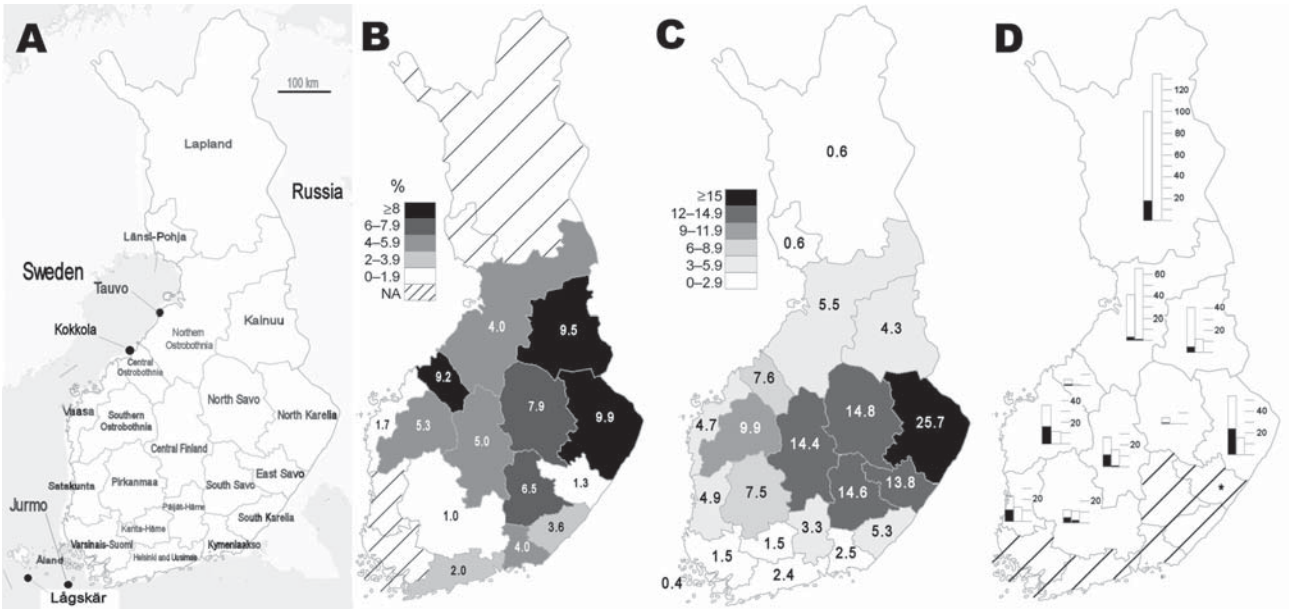

Figure 1. A) Map of Finland with the hospital district divisions. Migratory bird samples were collected from Lågskär, Tauvo, Jurmo, and Kokkola. B) Mean seroprevalence (1999–2003) of Sindbis virus (SINV) infection in human population (according to place of treatment) in the hospital districts of Finland. N/A, not available. C) Average annualized incidence (1995–2003) of SINV infection in human population (according to place of treatment) in the hospital districts of Finland. D) Prevalence of SINV hemagluttination-inhibition antibodies in resident grouse in Finland. The left bars represent the year 2003 and right bars 2004. The bars represent the total number of samples available; the black areas, the number of seropositive samples. *In 2003 in East Savo, the only sample collected was seropositive.

Results

Human Population

A total of 2,529 human specimens were included in our analysis. When the data were standardized according to the age distribution of the Finnish population, the estimated seroprevalence was 5.2% (Figure 2). Geographically, the seroprevalence was high in eastern Finland, especially in North Karelia and Kainuu, but also in central Ostrobothnia in western Finland (Figure 1, panel B). Seroprevalence was significantly higher for men (6.0%; mean age of all men studied 41.8 years) than for women (4.1%; mean age of all women studied 44.1 years) (χ^2 4,721, p<0.030). Seroprevalence increased with age, reaching 15.4% among persons 60–69 years of age (Figure 2).

The incidence of SINV during epidemic years was 25.6/100,000/year in 1995 and 11.5/100,000/year in 2002 (Figure 1, panel B); the average annualized incidence in nonepidemic years (1996–2001 and 2003) was 2.4/100,000. The rates for women and men were 8.7 and 6.6/100,000, respectively. The average annualized incidence was highest (13.5/100,000) among persons 50–59 years of age (Figure 2). Rates were higher for persons in the eastern parts than in the central part of the country and were highest in North Karelia (25.7/100,000; Figure 1, panel C); incidence peaked in North Karelia and in southern Ostrobothnia during the 1995 and 2002 outbreaks (Figure 3). However, a year after the outbreak in 2003, the rates were twice as high in southern Ostrobothnia than in North Karelia (Figure 3).

Resident Grouse

A total of 340 blood samples were collected from resident grouse in 2003, and 281 samples were collected in 2004 (Table 1; Figure 1, panel D). In 2003, a year after a human outbreak, the total prevalence of SINV HI antibodies in the grouse was 27.4%; in 2004, it declined significantly to 1.4% (Table 1; χ^2 76.8, p<0.001). In 2003, the prevalence was high in North Karelia (44%), western Finland (south-

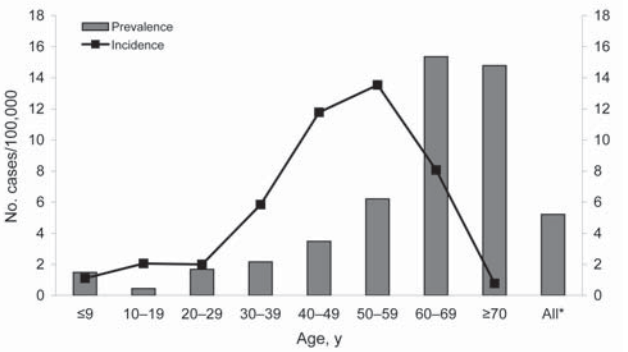

Figure 2. Mean seroprevalence (1999–2003) and average annualized incidence (1995–2003) of Sindbis virus infection in the human population, Finland, according to age groups. *Standardized according to the age distribution of the Finnish population in the respective period.

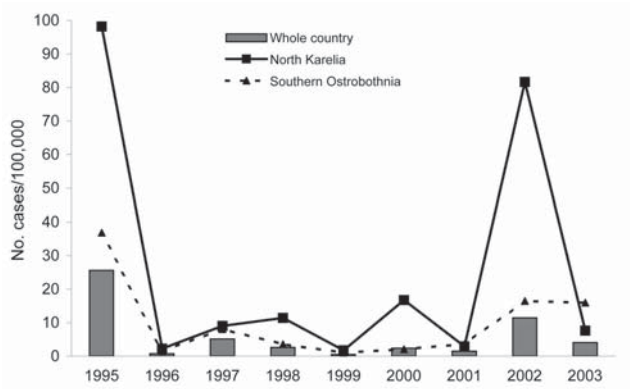

Figure 3. Average annualized incidence of Sindbis virus infection in human population, Finland, 1995–2003.

ern Ostrobothnia, Vaasa, and central Ostrobothnia) (44%), and Central Finland (41%), but pronounced also in Lapland in northern Finland (18%) (Figure 1, panel D). Also in 2003, 27.2% of the male grouse and 28.6% of the female grouse were seropositive; in 2004, 2.2% of the males and 1.3% of the females were seropositive (sex was unknown for 132/621 of the grouse). In 2003, 32.0% of the juveniles (born the same year) and 23.3% of the adults were seropositive; in 2004, none of the juveniles and 2.9% of the adults were seropositive (age was unknown for 96/621). The distribution of antibody titers in the positive samples was as follows: 40–120 (32.0%), 160–480 (42.3%), and 640–≥1,280 (25.8%).

Migratory Birds

A total of 836 blood samples were collected from migratory birds, of which 806 were collected during spring migration in May 2004 and May 2005. SINV HI antibodies were detected in 3 birds during spring migration: a robin (*Erithacus rubecula*) and a song thrush (*Turdus philomelos*) from Tauvo in 2004, and a red-backed shrike (*Lanius collurio*) from Lågskär in 2005 (Figure 1, panel A; Table 2). HI antibody titers were 240, 120, and 40, respectively. The song thrush was born the previous year; the age of the other 2 positive birds was unknown. SINV RNA could not be detected with reverse transcription–PCR (*20*) from the seropositive filter paper slip solutions. Virus isolation (*14*) from the available whole blood samples (kept at –70°C) from the seropositive robin and song thrush was not successful.

Discussion

Our findings show that SINV-seropositive migratory birds arrive in northern Europe during spring migration. Furthermore, after the 2002 outbreak in humans, SINV seroprevalence in grouse decreased markedly between the next 2 consecutive hunting seasons (2003 and 2004). These findings suggest that grouse may be involved in the epidemiology of SINV in humans.

The HI and EIA tests used in this study cross-react only poorly between alphaviruses in different antigenic complexes but may cross-react with other alphaviruses within the same antigenic complex. However, no viruses in the western equine encephalomyelitis complex other than SINV are known to circulate in northern Europe. Seroprevalence of SINV in the human population was analyzed for persons with suspected Pogosta disease; although this sample is not random and was recorded by the place of treatment (not residence), it does provide good representation of different geographic areas.

Although the incidence of SINV infection was higher in women than in men, the seroprevalence was higher in men. This unexpected finding might be explained by the possibility that infected men are more frequently asymptomatic than women, but more investigations are needed. The high seroprevalence but low incidence in Kainuu could be the result of considerable underdiagnosis.

Before 1965, no Finnish persons were found to be SINV seropositive (*31*); however, from 1981 to 1995, seroprevalence of SINV in the Finnish population rose considerably. In 1992, seroprevalence in pregnant women was 0.6% (*4*); in our study, seroprevalence in women 20–39 years of age was 2.3%, which further suggests a continuous increase in the seroprevalence of SINV in Finland. These data suggest that SINV may have been newly introduced to northern Europe during the 1960s to 1970s. As with West Nile virus, a candidate vehicle for the distribution of SINV is infected migratory birds.

Similar to the incidence we found, the incidence of SINV during 1980–1996 was highest in the provinces of North Karelia and Central Finland (*4*). In 2003, however, the incidence was highest in southern Ostrobothnia, possibly reflecting the high immunity to SINV in North Karelia after the 2002 outbreak.

In terms of age, 1 study found SINV seroprevalence in 1,850 hospital patients in Finland to be 19% in those

Table 1. Resident birds and Sindbis virus, Finland, 2003 and 2004

Common name	Taxonomic name	2003		2004	
		Samples, no.	Seropositive, %	Samples, no.	Seropositive, %
Capercaillie	*Tetrao urogallus*	67	31.3	41	2.4
Black grouse	*Tetrao tetrix*	144	31.3	84	1.2
Hazel grouse	*Bonasa bonasia*	49	22.4	58	3.4
Willow grouse	*Lagopus lagopus*	80	20.0	98	0
Total		340	27.4	281	1.4

Table 2. Migratory birds and Sindbis virus, Finland, 2004 and 2005*

Common name	Taxonomic name	No. samples (no. positive)					
		2004		2005			
		Jurmo	Tauvo	Lågskär	Tauvo	Kokkola	Total
Willow warbler	*Phylloscopus trochilus*	29	105	159	155	3	451
Garden warbler	*Sylvia borin*	1	1	46	2	3	53
Spotted flycatcher	*Muscicapa striata*	7	1	36	3		47
Pied flycatcher	*Ficedula hypoleuca*	7	19	13	7		46
Redstart	*Phoenicurus phoenicurus*	8	16	10	6		40
Whitethroat	*Sylvia communis*	1		34			35
Bluethroat	*Luscinia svecica*	1	30	1			32
Lesser whitethroat	*Sylvia curruca*	4		24			28
Sedge warbler	*Acrocephalus schoenobaenus*		3		5	11	19
Red-backed shrike	*Lanius collurio*	1		8 (1)		5	14
Robin	*Erithacus rubecula*	2	2 (1)	5		2	11
Song thrush	*Turdus philomelos*		11 (1)				11
Blackcap	*Sylvia atricapilla*	4		6			10
Redpoll	*Carduelis flammea*		3	1		2	6
Other (<5 individuals/species)†		5	5	19		4	33
Total		70	196	362	178	30	836

*Sampling was conducted during 2004 in Jurmo and Tauvo and during 2005 in Lågskär Island, Tauvo, and Kokkola. One of the birds sampled was not a migratory bird (willow tit), and 2 birds were not passerines (cuckoo and wryneck). Sindbis virus hemagluttination-inhibition antibodies were detected in 3 (a red-backed shrike, a robin, and a song thrush) of 836 samples.
†<5 birds were available from the following species: chaffinch (*Fringilla coelebs*) 4 samples, wood warbler (*Phylloscopus sibilatrix*) 4, icterine warbler (*Hippolais icterina*) 4, reed bunting (*Emberiza schoeniclus*) 3, siskin (*Carduelis spinus*) 3, red-breasted flycatcher (*Ficedula parva*) 2, trush nightingale (*Luscinia luscinia*) 2, chiffchaff (*Phylloscopus collybita*) 2, willow tit (*Parus montanus*) 1, wryneck (*Jynx torquilla*) 1, cuckoo (*Cuculus canorus*) 1, tree pipit (*Anthus trivialis*) 1, blackbird (*Turdus merula*) 1, grasshopper warbler (*Locustella naevia*) 1, rustic bunting (*Emberiza rustica*) 1, scarlet rosefinch (*Carpodacus erythrinus*) 1, and fieldfare (*Turdus pilaris*) 1.

patients <10 years of age (*32*). Our results are in contradiction to this because only 1.4% of persons <10 years of age were seropositive, and seroprevalence increased gradually by age, which we consider a logical finding. In those 60–69 years of age, almost one sixth of the population had immunity to SINV. Considering the high infection rate and that the infection may cause prolonged joint symptoms (*20*,*33*–*35*), even objectively observed by a physician (*36*), the disease is a potential public health concern.

In 1982 in Sweden, most of the 65 serologically diagnosed cases occurred in August. Incidence was highest for men 30–39 years of age and women 50–59 years of age, and prevalence was highest in central Sweden (*37*). Similar temporal and geographic findings were reported for 1981–1987; seroprevalence was 2.5% (*38*).

The last human outbreak in Finland occurred in 2002; ≈600 cases were serologically diagnosed. The next year, the seroprevalence of SINV in grouse was high. One third of the juvenile grouse were seropositive in 2003, which indicates active transmission of the virus to the grouse population that year. A large proportion of these grouse showed markedly high antibody titers. In 2004, the prevalence dropped dramatically; only a few adult grouse were seropositive. These data indicate that grouse become exposed to SINV and that the virus could have an endemic cycle involving grouse. However, other susceptible animals not screened in this study could be involved. Detection of high antibody titers in 2003 implies that the birds have almost certainly been infected and had marked viremia. In addition, the serologic methods that were used in this study (HI and NT) are stringent and can detect only relatively potent antibody responses; the true seroprevalence of the birds may be higher than that reported here.

Until the late 1980s, fluctuation in the grouse population densities in Finland was pronounced and regular, peaking every 6–7 years (*23*,*24*). During the 1970s and the 1980s, the human outbreaks and declines in the grouse population seemed to coincide (*4*). More recently, fluctuation in the grouse population has been less regular with less variation in population density (*39*). Whether the fluctuation of the grouse populations could be a cause or a consequence of the phenomenon behind the 7-year cycle of human outbreaks and whether SINV can be pathogenic to grouse remain unclear. Notably, in 2003, SINV antibodies were detected in various parts of the country, not only in areas where human infection was endemic.

During 1983–1985, Francy et al. (*21*) isolated 14 SINV strains from ≈60,000 female mosquitoes in Sweden. Most were isolated from *Cx. pipiens, Cx. torrentium,* and *Culiseta morsitans,* and seropositive birds were detected during a similar period, which suggests that these species may be important enzootic vectors in a bird–mosquito cycle. In 1983, neutralizing antibodies were not found in the few hundred migratory birds that arrived on the Swedish coast; in the same year, antibody prevalence in nesting birds (residents and migrants) was 3.4% in July and 10% in August (*21*). In the United Kingdom, SINV antibodies have also been demonstrated in resident and migrant birds and in poultry (*3*).

SINV-antibody prevalence in passerine birds sampled in Sweden between June and August during the 1990s (i.e., not during spring migration)—fieldfare (43.3%), redwing (37.0%), and song thrush (22.2%)—was markedly higher than the average (7.7%) of all species studied (*5*). The prevalence was significantly higher for birds sampled after the hatching year (13.9%) than for birds sampled during the hatching year (2.4%).

Of the seropositive migratory birds reported here, robin and song thrush mainly spend the winter in western Europe (some individuals migrate to northwestern Africa) (*28*), and red-backed shrike spend the winter in eastern tropical and southern Africa (*28*). Altogether, 806 of the 836 samples were collected during spring migration in bird observatories, which is usually where the birds first land when they arrive in the country from the sea; the other 30 were collected during midsummer. However, virus infection during the previous year in northern Europe cannot be excluded. Detection of viable SINV in the migrating birds would be the ultimate proof for their involvement in distributing SINV, but that remains to be shown. Extended longitudinal studies are needed to determine whether resident reservoir species are able to sustain SINV cycles endemically in northern Europe or whether the virus must be repeatedly introduced there by migratory birds from the southern hemisphere. Although the similarity of mosquito isolates of SINV from Sweden and Russia in 1980s and from Finnish patients in 2002 (*14*) favors the endemic cycle, larger analyses of SINV strains from Africa and northern Europe (*40*) suggest a continuous importation of South African strains to northern Europe or vice versa.

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Epidemiology of *Haemophilus influenzae* Serotype a, North American Arctic, 2000–2005

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Before the introduction of *Haemophilus influenzae* type b (Hib) conjugate vaccines, rates of invasive *H. influenzae* disease among indigenous people of the North American Arctic were among the highest in the world. Routine vaccination reduced rates to low levels; however, serotype replacement with non–type b strains may result in a reemergence of invasive disease in children. We reviewed population-based data on invasive *H. influenzae* in Alaska and northern Canada from 2000–2005; 138 cases were reported. Among 88 typeable isolates, 42 (48%) were *H. influenzae* type a (Hia); 35 (83%) occurred in indigenous peoples. Among Hia patients, median age was 1.1 years; 62% were male; 1 adult died. Common clinical manifestations included meningitis, pneumonia, and septic arthritis. Overall annual incidence was 0.9 cases per 100,000 population. Incidence among indigenous children <2 years of age in Alaska and northern Canada was 21 and 102, respectively. Serotype a is now the most common *H. influenzae* serotype in the North American Arctic; the highest rates are among indigenous children.

Haemophilus influenzae causes illnesses ranging from local respiratory infection to serious invasive disease, including meningitis, epiglottitis, septic arthritis, and septicemia (*1*). Among the encapsulated strains (a to f) that have been identified, *H. influenzae* serotype b (Hib) is the most virulent (*1–4*). Nonencapsulated (nontypeable) strains are usually associated with noninvasive infections but can cause invasive disease, including neonatal sepsis (*2–5*). Historically, Hib was the leading cause of bacterial meningitis in the United States and Canada (*6*). However, since the introduction of Hib capsular polysaccharide-protein conjugate vaccine in 1988, the incidence of invasive Hib disease has declined dramatically. Data from the Centers for Disease Control and Prevention (CDC) show invasive Hib disease in the United States has decreased by 99% to <1 case per 100,000 children <5 years of age (*7*). Similar declines have been documented in Canada (*8*).

Indigenous people, defined as the original people of Alaska (Alaska Native people) and northern Canada (aboriginal people), are at increased risk for Hib disease (*8–12*) than the general populations of the United States and Canada, and the risk for disease peaks at an earlier age (*12–14*). While Hib vaccination led to the rapid decline of Hib disease in all populations including indigenous groups, indigenous children continue to have higher rates of Hib disease than nonindigenous children (*12*,*15*).

With widespread vaccination against Hib, concern has been raised about the potential for replacement disease caused by non–type b encapsulated strains. Protection con-

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ferred by the Hib vaccine is specific to the type b polysaccharide capsule. It was suggested that reducing carriage of the vaccine type may open an ecologic niche, allowing increased colonization with non–type b strains of *H. influenzae* with the potential to become invasive (*6*,*16*).

Non–type b *H. influenzae* disease is uncommon in children; however, since the introduction of Hib vaccine, the relative importance of infections due to nonencapsulated and non–type b encapsulated *H. influenzae* has increased (*3*). Infections caused by nonencapsulated strains are more common in adults and are more likely to be associated with pneumonia, whereas infections caused by encapsulated strains tend to occur in younger children with a predominance of meningitis and bacteremia (*3*,*17*). Non–type b *H. influenzae* appears to be more common in persons with underlying medical illnesses, such as immunosuppressive conditions (*3*,*4*,*17*). The extent to which non-b *H. influenzae* causes invasive disease is not fully known (*17*). In some countries, only Hib disease is reportable; therefore, information on other serotypes is lacking. However, numerous case reports of invasive *H. influenzae* disease caused by encapsulated non-b serotypes, particularly types a, e, and f, have been published (*6*,*10*,*11*,*18–20*).

Although uncommon, *H. influenzae* serotype a (Hia) has been reported to cause invasive disease, meningitis, pneumonia, and sepsis (*3*,*20*). Hia disease may occur more frequently in indigenous populations (*10*,*11*,*21*). Reports of invasive *H. influenzae* disease have identified Hia in 7.8% of Australian aboriginal children and 16.7% of Apache children with invasive disease (*10*,*21–23*). The Navajo and White Mountain Apache populations have a higher rate of Hia disease than the general US population, and Hia is now a leading cause of invasive *H. influenzae* disease in these populations (*10*). Although high rates were discovered during the period of surveillance (1988–2003), significant increases in incidence were not found (*10*). Seasonal, temporal, and geographic clustering was not demonstrated. An outbreak of invasive Hia has recently been described in Alaska (*11*), and data from the International Circumpolar Surveillance (ICS) Program suggest that the number of cases of Hia has increased in both Alaska and northern Canada. The objectives of this study were to characterize cases of invasive Hia, to examine incidence rates over time, and to assess the relatedness of Hia isolates by molecular typing.

Methods

ICS, a population-based surveillance system for invasive bacterial diseases established in 1999, includes laboratory-based surveillance for *Streptococcus pneumoniae*, *H. influenzae*, *Neisseria meningitidis*, and groups A and B streptococci. Current member countries include the US Arctic (Alaska), northern Canada, Greenland, Iceland, Norway, northern Sweden, and Finland.

This study reviews data collected from 2000 through 2005 from Alaska and northern Canada. In Alaska, 23 laboratories throughout the state are asked to send any isolate of *H. influenzae* recovered from a normally sterile site to a reference laboratory in Anchorage at CDC's Arctic Investigations Program, which serves as the data repository for ICS. In northern Canada, a network of laboratories within the regions (Yukon, Northwest Territories, Nunavut, northern Quebec, and northern Labrador) participate, as well as 3 reference laboratories (2 national and 1 provincial). The laboratories are requested to send any isolate of *H. influenzae* recovered from a normally sterile site to the appropriate reference laboratory to confirm the identity, determine the serotype, and test for antimicrobial drug susceptibility. Laboratory, demographic, and clinical data are collected for each invasive case of *H. influenzae* occurring in Alaska and northern Canada, and these data are forwarded to ICS headquarters in Alaska.

A case of invasive *H. influenzae* disease is defined as illness in a resident of the surveillance area from whom *H. influenzae* is isolated from a sample obtained from a normally sterile site, including blood, cerebrospinal fluid, pleural fluid, peritoneal fluid, or joint fluid. Patients with clinical epiglottitis from which *H. influenzae* is isolated from an epiglottis swab are also reportable to ICS. The primary clinical manifestation of *H. influenzae* was determined by a review of the patient's medical record.

Population denominator data for Alaska and northern Canada were obtained from the Alaska Department of Labor and Workforce Development (www.labor.state.ak.us), Statistics Canada (www.statcan.ca), and the Demography Division of Statistics Canada. Estimates from Alaska and northern Canada reflect population figures from the 2000 and 2001 census years, respectively. Canadian indigenous estimates were calculated by using population data from the Aboriginal Population Profile, which is developed from 2001 Census data. This study covers a 6-year surveillance period, January 1, 2000–December 31, 2005. Alaska and northern Canada's estimated populations were 655,435 and 132,956, respectively. Indigenous peoples comprised 19% of the population in Alaska and 59% of the population in northern Canada.

Laboratory Methods

Isolates were streaked onto chocolate agar to check for purity and confirmed to be *H. influenzae*. Confirmation tests included a requirement for both X (hemin) and V (nicotinamide adenine dinucleotide) growth factors (Oxoid, Hampshire, UK), Gram stain, and serotyping by slide agglutination.

Antimicrobial Susceptibility Testing

Susceptibility testing in Alaska was performed by using Etest (AB Biodisk, Solna, Sweden). A direct colony suspension equivalent to a 0.5 MacFarland standard was prepared in Mueller-Hinton broth from an overnight culture. *Haemophilus* Test Medium (Remel, Lexna, KS, USA) was added to produce a confluent lawn of growth, and then Etest strips were placed onto the plate. The plates were then incubated for 20–24 hours at 35°C in 5% CO_2. The MIC was read at the point of intersection of growth and the strip. Susceptibility of *H. influenzae* to the following antimicrobial drugs was tested: ampicillin, ceftriaxone, meropenem, chloramphenicol, and trimethoprim-sulfamethoxazole (TMP-sulfa). Susceptibility data were not available from Canada.

Serotyping

Capsular serotyping was performed by slide agglutination with Difco antisera (Difco, Detroit, MI, USA) in Alaska and Remel antisera (Remel Europe Ltd, Dartford, UK) in northern Canadian laboratories (with the exception of the Laboratoire de Sante Publique in Quebec, which used PCR). If no capsular polysaccharide was present, the isolate was classified as nontypeable by slide agglutination. Known positive and negative controls were run weekly, and each culture was screened in saline alone to check for autoagglutination. Since 2005, laboratories have participated in an ongoing *H. influenzae* quality control program.

Pulsed-field Gel Electrophoresis (PFGE) and PCR

A selection of invasive isolates of Hia from cases in Alaska and northern Canada that tested positive for serotype a by both slide agglutination and genotype-specific PCR (*24*) were examined by PFGE with the use of the restriction enzymes *Sma*I and *Apa*I digestions (*18*,*25*) at the CDC laboratory in Anchorage, Alaska. The fragments were resolved with a CHEF DRII (Bio-Rad, Hercules, CA, USA) (2.2- to 35-s switch times) at 175V for 21 hours. DNA banding patterns were analyzed with BioNumerics version 3.0 software (Applied Maths, Sint-Martens-Latem, Belgium). Percentage similarities were identified on a dendrogram derived from the unweighted pair group method by using Dice coefficients and a band position tolerance of 1.5%. A similarity coefficient of 80% (≤3-band difference) was used to define related groups. The IS*1016*-bexA deletion was amplified from genomic DNA by PCR using sense IS*1016* (5′-ATTAGCAAGTATGCTAGTCTAT-3′) and antisense bexA (5′-CAATGATTCGCGTAAATAATGT-3′) primers (*26*).

Statistical Analysis

Data were double entered into Paradox v9.0 (Corel, Ottawa, Ontario, Canada), and analyzed by using Epi Info version 6.04b (CDC, Atlanta, GA, USA) and StatXact version 6.2 (CYTEL Software Corp., Cambridge, MA, USA). Statistical differences in rates between periods and between countries were assessed by using a 2-sample Poisson test; p values are exact when appropriate.

Results

Descriptive Epidemiology

We identified 138 cases of invasive *H. influenzae* disease from 2000 through 2005; serotype data were available for 132 (96%) of the isolates. Among these, 44 (33%) were nontypeable (Alaska 27; northern Canada 17). Of the remaining 88 isolates, 42 (48%) were serotype a, 27 (31%) serotype b, 12 (14%) serotype f, 4 (5%) serotype d, 2 (2%) serotype c, and 1 (1%) serotype e (Figure 1). The proportion of illnesses that resulted in death among Hia, encapsulated non-a, and nontypeable isolates was 5% (2/37), 14% (6/42), and 15% (6/40), respectively.

Among the 42 Hia isolates, 30 (71%) occurred in children <2 years of age; 4 (10%) occurred in children 2–5 years of age; the remaining 8 (19%) occurred in adults (range 21–73 years). Ethnicity data were available for 38 cases; 35 (92%) occurred in indigenous people. The median age among case-patients was 1.1 years (range 3 months to 73 years) and did not differ significantly by country. Overall, 62% were male (Table 1). Cases occurred in 3 Alaska regions and 3 regions in northern Canada. Most cases (60%) occurred in 1 northern Canadian region. No clear seasonal pattern of invasive Hia disease was observed; however, 5 (50%) cases of invasive Hia that occurred in indigenous Alaska children <2 years of age were clustered over a 5-month period in 2003 and occurred in 2 villages in western

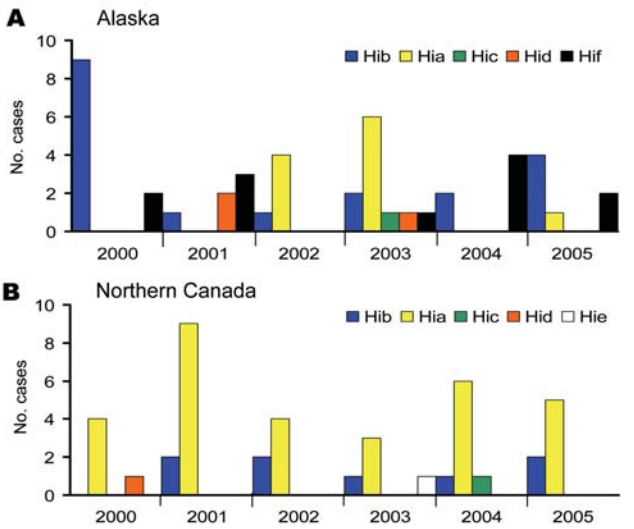

Figure 1. *Haemophilus influenzae* (Hi) cases by serotype in Alaska and Northern Canada, 2000–2005. A) Alaska; n = 42 typeable (27 nontypeable); 59% encapsulated non-b, 24% Hia. B) Northern Canada; n = 46 typeable (17 nontypeable); 81% encapsulated non-b, 74% Hia.

Table 1. Characteristics of Alaskan and northern Canadian persons with invasive *Haemophilus influenzae* type a disease, 2000–2005

Characteristic	Alaska (n = 11)	Northern Canada (n = 31)	Total (n = 42)
Median age (range)	10 mo (4 mo–73 y)	1.2 y (3 mo–31 y)	1.1 y (3 mo–73 y)
Male sex, no. (%)	7 (64)	19 (61)	26 (62)
Indigenous, no. (%)	8 (73)	27 (100)*	35 (92)
Age appropriately vaccinated for Hib, y (% vaccinated)	7 (88)†	21 (88)‡	28 (88)
Hospitalization, no. (%)‡	10 (91)	25 (96)§	35 (97)
Deaths, no. (%)¶	1 (9)	1 (4)	2 (5)

*Ethnicity data missing from 4 cases; denominator = 27.
†Vaccine history missing from 3 cases; denominator = 8.
§Hospitalization data missing from 5 cases; denominator = 26.
‡Vaccine history missing from 7 cases; denominator = 24.
¶Death data missing from 5 cases; denominator = 26.

Alaska (*11*). No clusters >2 cases in 1 village over a period of 2 months were observed in northern Canada. No pattern of increasing incidence rates was seen over the study period.

Incidence Rates by Age

Overall crude annualized incidence of invasive Hia for the 6-year study period was 0.9 cases per 100,000 population. In Alaska and northern Canada, crude annualized incidence rates were 0.3 and 3.9 cases per 100,000 population, respectively. Annualized incidence rates of invasive Hia in children <2 years of age were 19.7 cases per 100,000 population; annualized rates in Alaskan and northern Canadian children <2 years were 5.7 and 79.1 cases per 100,000 population, respectively (p<0.001, Table 2).

Incidence Rates by Ethnicity

The overall annualized crude incidence rates of invasive Hia over the study period in indigenous and nonindigenous people were 2.9 and 0.2 cases per 100,000 population, respectively. Among indigenous people, the overall annual crude incidence rates ranged from 2.0 to 4.0 cases per 100,000 population during the study period; annualized indigenous incidence rates in Alaska and northern Canada were 1.1 and 5.9 cases per 100,000 population, respectively (Table 2). Among indigenous children <2 years of age, the overall annualized incidence rate was 52.6 cases per 100,000 population, 20.9 and 101.9 cases per 100,000 persons in Alaska and northern Canada, respectively (Table 2).

Clinical Illness

Among all ages, the most common clinical syndromes were meningitis (33%) and pneumonia (29%), followed by septic arthritis (12%). Clinical manifestations differed between children and adults. As noted previously, all pediatric cases occurred in children <5 years of age. Adult case-patients were 21–73 years of age. Children were more likely to exhibit meningitis, and adults were more likely to exhibit pneumonia; septic arthritis was reported among 5 (15%) pediatric patients. Clinical features were similar in Alaska and northern Canada. No cases of epiglottitis were reported (Table 3).

Of the 37 case-patients with known hospitalization status, 35 (95%) were hospitalized with a median duration of 8 days. Case-patients in Alaska had a shorter median duration of hospitalization than those in northern Canada (6.5 vs. 9.0 days). Outcome information was available for 37 cases. Two patients (1 indigenous child, 1 nonindigenous adult) with invasive Hia died (Table 1). Both patients who died were diagnosed with pneumonia; neither had a history of immunodeficiency noted in the chart.

Recurrence

Two Alaskan Hia patients had recurrent disease. In 1 patient, the clinical manifestation was septic arthritis in both occurrences (4 months before recurrence). In the second patient, the initial clinical syndrome was pneumonia, followed by meningitis 4 months later. Both of these patients were <1 year of age when first brought for treatment. Neither child had documented immunodeficiency (*11*). No recurring cases of Hia were documented in northern Canada.

Antimicrobial Susceptibility Testing

All 11 Hia isolates from Alaska were susceptible to ampicillin, ceftriaxone, meropenem, and chloramphenicol. Ten of 11 Hia isolates from Alaska were tested for TMP-

Table 2. Cases and annualized incidence rate per 100,000 population of Invasive *Haemophilus influenzae* type a disease by age and ethnicity, 2000–2005*

Demographic group	Total no. (range/y); rate (range/100,000/y)	Alaska, no. cases (rate)	Northern Canada, no. cases (rate)	p value*
Overall	42 (4–9); 0.9 (0.5–1.2)	11 (0.3)	31 (3.9)	<0.001
<2 y of age	30 (1–8) ;19.7 (4.0–31.4)	7 (5.7)	23 (79.1)	<0.001
Indigenous	35 (4–8); 2.9 (2.0–4.0)	8 (1.1)	27 (5.9)	<0.001
<2 y of age, indigenous	29 (1–8); 52.6 (11.2–86.2)	7 (20.9)	22 (101.9)	<0.001

*p value for rate, Alaska vs. northern Canada.

Table 3. Clinical illness in Alaskan and northern Canadian children and adults with invasive *Haemophilus influenzae* type a disease, 2000–2005*

	Alaska (n = 11)		Northern Canada (n = 31)		North American Arctic (n = 42)	
Diagnosis	Children (n = 7), no. (%)	Adults (n = 4), no. (%)	Children (n = 27), no. (%)	Adults (n = 4), no. (%)	Children (n = 34), no. (%)	Adults (n = 8), no. (%)
Meningitis	2 (29)	0	12 (44)	0	14 (41)	0
Pneumonia	3 (43)	3 (75)	4 (15)	2 (50)	7 (21)	5 (62)
Bacteremia	0	1 (25)	6 (22)	1 (25)	6 (18)	2 (25)
Septic arthritis	2 (29)	0	3 (11)	0	5 (15)	0
Other†	0	0	2 (8)	1 (25)	2 (3)	1 (13)

*Children, <18 years of age; adults, ≥18 years of age.
†Includes osteomyelitis (1 child), pericarditis (1 adult), and cellulitis (1 child).

sulfa resistance; 2 of the 10 (20%) demonstrated intermediate resistance.

PFGE and PCR analysis

PFGE was performed on 9 Hia isolates from Alaska and 19 isolates from northern Canada. With 1 exception, all isolates were found to be closely related (<3-band difference) with a Dice correlation of ≥85% (Figure 2). All 28 isolates were negative for the IS*1016-bexA* deletion by PCR.

Discussion

Our data demonstrate that 69% of invasive *H. influenzae* disease in the North American Arctic is now caused by non-b serotypes (Alaska 51%; northern Canada 89%) with serotype a comprising almost half of cases (Alaska 24%; northern Canada 74%). Hia is now the most prevalent serotype in the North American Arctic. The clinical features of invasive Hia cases were similar to those of invasive disease caused by Hib. The overall annualized incidence of Hia in children <2 years of age living in the North American Arctic was 19.7 cases per 100,000 children (Alaska 5.7; northern Canada 79.1) in contrast to the rate of 0.83 cases of non–type b invasive *H. influenzae* disease among US children <5 years of age (*7*). The overall annualized incidence rate among indigenous children <2 years of age residing in the North American Arctic was particularly high at 52.6 cases per 100,000 children (Alaska 20.9; northern Canada 101.9).

Widespread use of Hib conjugate vaccine has resulted in a dramatic decline in invasive Hib disease in the United States and Canada (*7*,*8*), including among indigenous children in the North American Arctic (*7*,*8*,*14*) and the southwestern United States (*12*,*27*). However, the potential for serotype replacement remains a concern (*16*). Three population-based studies have documented small increases in the incidence of non–type b *H. influenzae* disease after the introduction of the Hib conjugate vaccine (*28*–*30*). Several recent studies conducted in different countries have demonstrated a predominance of serotype a disease. A study by Tsang et al. in Manitoba, Canada, found that most of the 52 *H. influenzae* cases reported during 2000–2004 were caused by serotype a (50%) or nonserotypeable isolates (38.5%) (*31*). Ribeiro et al. noted an 8-fold increase in the incidence of Hia meningitis from the prevaccine (0.02 per 100,000 population) to postvaccine period (0.16 per 100,000 population) in Brazil (*32*). However, more recently published data by this group demonstrate that the increase in incidence during the year following vaccine introduction was not observed in subsequent years (*33*). A study by Millar et al. has demonstrated high rates of invasive Hia disease among Navajo and White Mountain Apache children in the southwestern United States, although no increase in Hia incidence was noted after the introduction of Hib vaccine (*10*).

Clinical manifestations of invasive Hia disease were similar to those of invasive Hib disease in the prevaccine era (*29*). Meningitis was more common among Hia infections than in infections caused by encapsulated non-a and non-typeable Hi infections (37% vs. 24% and 12%, respectively, p = 0.05); conversely, pneumonia was less common (32% Hia vs. 41% encapsulated non–a *H. influenzae* and 40% nontypeable *H. influenzae*); however, this finding was

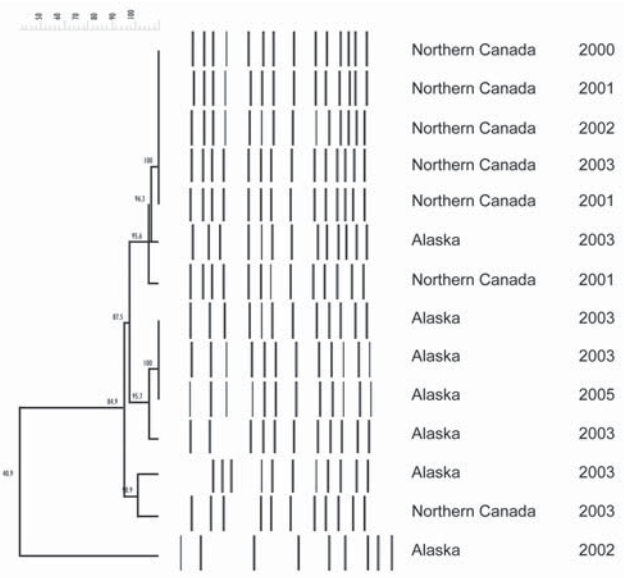

Figure 2. Pulsed-field gel electrophoresis of representative *Haemophilus influenzae* serotype a isolates from Alaska and Northern Canada, 2000–2005 (N = 14).

not statistically significant. Clinical disease varied with age when treatment was sought; meningitis was more common in children, and bacteremic pneumonia was more common in adults. The differing age distribution of Hia patients compared to patients with non-a and nontypeable infections may account for the differing clinical illnesses. Invasive Hia disease tended to occur among young children and nontypeable *H. influenzae* infections among adults (median age 1.1 vs. 39.2 years, respectively, p = 0.0003). The higher median age of patients infected with nontypeable *H. influenzae* is consistent with results of a recent US study that demonstrated an increase in the number of cases of invasive nontypeable *H. influenzae* among adults (*30*) in the postvaccine era, and a study by McVernon et al. in England and Wales that showed an increase in invasive non-b *H. influenzae* among older age groups (*34*).

There are several possible explanations for the high proportion of Hia among invasive *H. influenzae* disease in the surveillance population. First, an increase in virulence might explain the current predominance of serotype a. The IS*1016*-*bexA* deletion enables production of more capsule, which is thought to be the major virulence factor for invasive disease (*26*,*35*). However, Hia isolates from Alaska or northern Canada tested negative for the *bexA* deletion, and it appears unlikely that the high rates of invasive Hia disease, particularly among indigenous children in this region, are due to introduction of a particularly virulent strain of Hia. If a new highly virulent Hia strain were introduced into the North American Arctic in the postvaccine period, subtyping data may show a clonally restricted pattern. We found a high degree of relatedness with a predominance of 1 clone across the North American Arctic. However, these data do not directly support the introduction of a virulent clone because other studies suggest limited genetic diversity of Hia (*18*,*36*). Second, widespread use of Hib conjugate vaccine and the subsequent reduction in Hib colonization may have opened an ecologic niche for increased colonization with Hia or other non-Hib strains. Little data regarding carriage of non–type b strains of *H. influenzae* are available; however, an investigation of a cluster of 5 of the invasive Hia cases in Alaska found that among 31 close contacts of case-patients, 5 (16%) were colonized with Hia. Two of the 3 case-patients were infants with recurrent Hia disease; reexposure is the likely explanation for disease recurrence (*11*). Finally, a preexisting background rate of non-b serotype disease may have simply been uncovered due to the decreasing Hib rates. Further studies of invasive *H. influenzae* disease are needed to describe clinical and epidemiologic features, characterize the pattern and rates of colonization, determine risk factors for carriage, and further characterize the strains by using molecular techniques.

Hia disease raises many questions from a public health response perspective. While chemoprophylactic regimens are well described for contacts of persons with Hib disease (*37*), the utility of chemoprophylaxis or other public health prevention measures for non-b typeable disease such as Hia is not clear. Further research is needed to provide clear guidance to practicing physicians caring for patients with Hia disease.

This study has several limitations. Alaskan and northern Canadian data on non-b invasive *H. influenzae* disease were not collected in the pre-Hib conjugate vaccine era, making it difficult to determine baseline or prevaccine incidence of serotype a in this region. We did not collect detailed clinical and demographic information beyond what was available from medical record review, and therefore we were not able to assess other factors (e.g., *H. influenzae* carriage among case-patients, within the community, or among close contacts). In addition, most *H. influenzae* isolates are serotyped by using slide agglutination only. Because PCR is not yet routinely used throughout the ICS network for serotyping, nontypeable strains could have been misclassified as encapsulated strains.

This article analyzed population-based surveillance for invasive *H. influenzae* disease across the North American Arctic. We identified a high proportion of non-b serotypes over the 6-year study period, with particularly high rates of invasive disease caused by Hia. The reason for these high rates of invasive Hia disease is unknown and is likely multifactorial. While Hia incidence rates are high among particular groups in the North American Arctic, case numbers remain low (4–9 cases per year) and are lower than Hib rates in the prevaccine era. Hib vaccination remains one of the great public health success stories. Continued surveillance for *H. influenzae* disease is needed; however, to identify emerging problems and provide data necessary to develop effective prevention strategies.

The changing epidemiology of invasive *H. influenzae*-disease highlights the importance of continued surveillance for invasive *H. influenzae* disease in regions of the world where Hib conjugate vaccine is currently in use. ICS will continue to monitor invasive disease caused by all *H. influenzae* serotypes in the North American Arctic and other participating Arctic countries.

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Implementation and Effectiveness of Antiretroviral Therapy in Greenland

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Analyses from the Danish HIV Cohort Study showed that, despite comparable economic means and general education of healthcare personnel, antiretroviral treatment of HIV in Greenland began later and has been implemented at a slower pace with lower therapeutic effectiveness than in Denmark. However, implementation and quality of care improved considerably in recent years.

Like in Western Europe, the first case of HIV was observed in Greenland in the mid-1980s (*1*), but the epidemic in this isolated polar country has evolved differently compared with other industrialized countries (*2*). In a previous study we showed that most patients were infected through heterosexual contact and were middle-aged at the time of diagnosis. Many patients belonged to a socially marginalized group characterized by low income, unemployment, and heavy drinking. Even though highly active antiretroviral therapy (HAART) is tax-supported and free, we found an overall mortality rate of 11% per year for patients given HAART during 1997–2003 (*2*). In a molecular epidemiologic study, we showed that HIV was introduced at least 9 times into Greenland, and that one of these introductions has given rise to a circulating epidemic that has included 76% of all infected persons (*3*). Recently, we found 28% prevalence of transmitted drug resistance, corresponding well with the impression of low drug adherence and high risk behavior (T.V. Madsen et al., unpub. data). Contributing to the disappointing results could be the vast geography with often long distances to healthcare facilities, the short supply of specialized physicians, and the composition of the HIV-infected population. In 2002 the overall responsibility for treating HIV patients was transferred to the Department of Internal Medicine at Dronning Ingrids Hospital, Greenland's main hospital, located in the capital, Nuuk. The chief physician at that department takes care of HIV patients in Nuuk and supervises treatment of HIV patients in other areas. With access to data from all HIV-infected persons in Greenland and Denmark, we aimed to compare the Implementation and effectiveness of HAART during 1997–2007 in 3 areas: Nuuk; Greenland's remote districts (all towns and settlements except Nuuk); and the Western European country of Denmark, the former colonial power with which Greenland still has tight economical, social, and constitutional bonds.

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The Study

The population-based Danish HIV Cohort Study (DHCS) collects clinical and paraclinical data on all HIV-infected persons under care in Denmark and Greenland since 1995 (*2*,*4*), including antiretroviral treatment, HIV RNA (viral load), and date of death or emigration. Patients from DHCS were followed from first visit to an HIV clinic until date of death, emigration, or last visit to the clinic. To estimate viral loads and CD4 cell counts between measurements, we carried forward the last observed value. HAART was defined as combination antiretroviral treatment with at least 3 drugs, including at least 1 protease inhibitor (PI), or 1 non-nucleoside reverse transcriptase inhibitor (NNRTI), or abacavir. On January 1 for each year of the study we estimated the proportion of patients receiving HAART, each antiretroviral drug class, and selected antiretroviral drugs. National guidelines in both countries have recommended HAART initiation at a CD4 cell count <300 cells/μL, a threshold that has not changed since 1997. Among patients who had never received HAART, we estimated the proportion with a CD4 cell count <300 cells/μL, and among patients who had begun a HAART regimen at least 90 days previously, we estimated the proportion with a viral load <400 copies/mL. Annual mortality rates (MR) in the HIV population were estimated by person-years analysis; Poisson regression was used to test for trends over time.

Conclusions

Among 124 HIV patients in Greenland, 98 (79%) were infected through heterosexual contact, 78 (63%) were male, 111 (90%) were Inuit, and 98 (79%) were infected in Greenland. The median age at diagnosis was 50 years (interquartile range [IQR] 40–57 years), and the median CD4 cell count at diagnosis was 350 cells/μL (IQR 220–530 cells/μL). Among 4,702 HIV patients in Denmark, 2,114 (45%) were infected through homosexual contact, 1,745 (37%) through heterosexual contact, and 537 (11%) through intravenous drug use; 3,542 (75%) were male; 3,723 (79%) were Caucasian, and 650 (14%) black African. Half, 2,370,

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were infected in Denmark, 725 (15%) in Africa, and 1,046 (22%) unknown; the median age at diagnosis was 34 years (IQR 28–42). The median CD4 cell count at diagnosis was 284 cells/μL (IQR 108–490 cells/μL).

In Greenland only 3% had begun HAART on January 1, 1997, as opposed to 28% in Denmark (Figure 1). The proportion on HAART increased gradually up to 81% in 2006, but not until 2003 did the proportion in Greenland reach the level in Denmark. Further, as late as 2001, 96% of all treatment regimens in Greenland included an unboosted PI (26% in Denmark), and in 2002 only 7% were NNRTI based (40% in Denmark). At that time the International AIDS Society USA guidelines carefully encouraged the use of boosted PIs, and NNRTI-based regimens were considered an equally effective alternative to PI-based regimens (*5*). Only after 2002 did the pattern shift, and in 2006 it was similar to that in Denmark, with approximately half of the combinations being NNRTI based, the other half PI based, with ritonavir-boosted lopinavir used in 65% of all PI regimens on January 1. The newer PI atazanavir was used in only 9% of PI regimens in 2006 (28% in Denmark). NNRTI used in Greenland has almost exclusively been efavirenz, whereas in Denmark 24% of NNRTI use on January 1, 2006, was nevirapine. The difference between the curves "ever on HAART" and "currently on HAART" in Figure 1 reflects the number of persons currently interrupting their treatment; the proportion with interruption is higher in Greenland than in Denmark. Structured treatment interruptions have not been recommended in Greenland or Denmark, so these persons supposedly have interrupted their therapy because of compliance problems. There was no difference in the uptake of HAART between Nuuk and the remote districts in Greenland (data not shown).

Until 2002, >30% of patients not yet receiving HAART in Greenland had a CD4 cell count <300 cells/μL (Figure 2). In comparison, the proportion in Denmark has been <30% since 1998, with <5% having a CD4 cell count <200 cells/μL since 2001. Among patients ever starting a HAART regimen, the proportion with suppressed viral load in Greenland was <45% until 2003 but has increased to 73% in 2006 (Figure 2). Nuuk reached the 75% mark in 2004, whereas the increase in the remote districts started later and reached 69% in 2006. The proportions in Denmark were 62% in 1998, 81% in 2003, and 88% in 2006.

The overall mortality rate among HIV patients in Greenland decreased from 139 (95% confidence interval [CI] 81–239) per 1,000 person-years in 1995–1997 to 59 (95% CI 35–99) in 2004–2006, corresponding to a 9% decrease per year (mortality rate ratio [MRR] = 0.91, 95% CI 0.84–0.98, p = 0.014) (Table). The decrease was most marked in patients in Nuuk (MRR = 0.86, 95% CI 0.77–0.96, p = 0.006) and less in the districts (MRR = 0.96, 95% CI 0.86–1.08, p = 0.533).

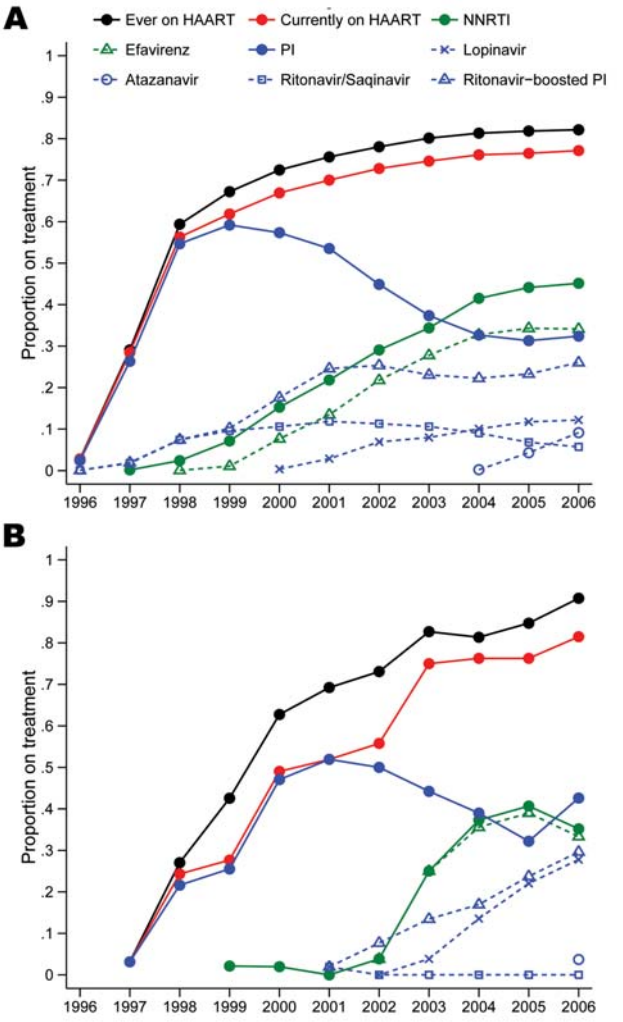

Figure 1. Proportion of patients receiving treatment on January 1, 1996–2006 in A) Denmark and B) Greenland. Numerator, patients who were receiving antiretroviral therapy as part of a HAART (highly active antiretroviral therapy) regimen. Denominator, all patients under observation. NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

Treatment of HIV patients in Greenland began at a later stage of disease and has been implemented at a slower pace with lower therapeutic effectiveness than in Denmark, despite comparable economic means, general education of healthcare personnel, and common therapeutic guidelines. From other studies we know that patient support and education improve adherence (*6*) and that guideline-recommended therapy is more likely to be chosen if the physician is specialized in HIV and has >20 HIV patients in care (*7*), regardless of whether this physician is a generalist or infectious disease specialist. We observed marked improvements in the choice of antiretroviral drug combinations and effectiveness of HAART from 2003 onwards. These advances coincided with the establishment of a dedicated

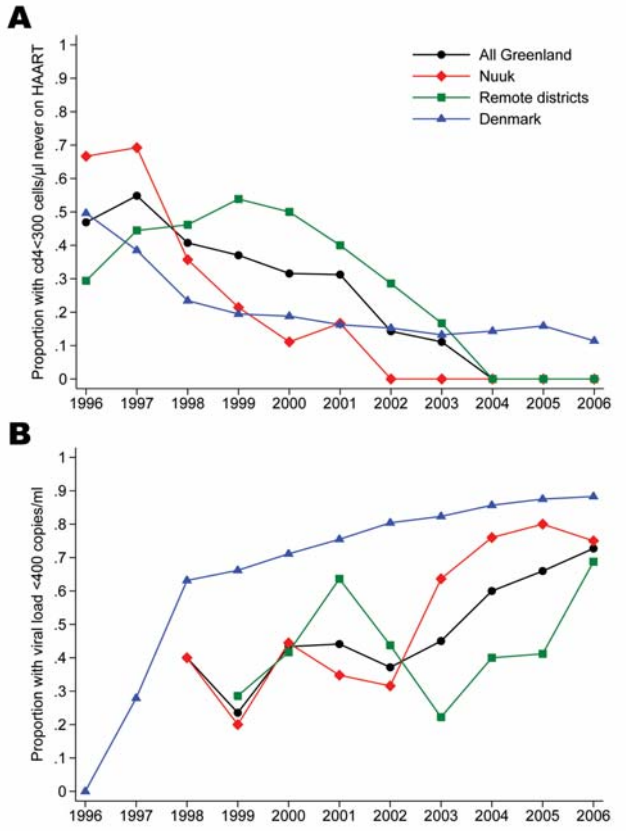

Figure 2. A). Proportion of patients with low CD4 cell count on January 1, 1996–2006. Numerator, patients with a CD4 cell count <300 cells/μL. Denominator, all patients who had not yet been prescribed a highly active antiretroviral therapy (HAART) regimen. B) Proportion of patients with suppressed viral load on January 1, 1996–2006. Numerator, patients with an HIV RNA <400 copies per mL. Denominator, all patients who had ever started a HAART regimen.

team in Nuuk and were most pronounced in that city when compared with the remote districts. Even though this temporal association does not prove causation, the improvements are likely to be partly attributable to the increased focus on HIV in the capital. The MR among HIV patients in Nuuk in recent years was higher than that in Denmark, but part of this difference may be attributable to a high background mortality rate among HIV-uninfected persons in Greenland (*2*,*8*) and an older HIV-infected population. As previously reported, sexually active persons in Greenland undergo frequent HIV testing (*2*), and CD4 cell counts were high at diagnosis, ruling out late testing as a contributor to the high MR. In conclusion, healthcare systems in the sparsely populated and isolated polar areas may be less fit to take on state-of-the-art care and treatment for HIV or other diseases previously unknown in the area, and an extra effort from the such providers may be needed to maximize control of the disease.

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Table. Mortality rate per 1,000 person-years among HIV patients in Greenland and Denmark, 1995–2007*

Variable	Greenland			Denmark
	All	Nuuk	Remote districts	
Years				
1995–1997	139 (81–239)	178 (89–356)	103 (43–247)	96 (88–105)
1998–2000	82 (47–144)	62 (26–148)	107 (51–225)	29 (25–34)
2001–2003	84 (50–141)	107 (58–199)	54 (20–144)	25 (22–28)
2004–2006	59 (35–99)	43 (19–96)	81 (41–162)	24 (21–28)
Mortality rate ratio change per year, 1995–2006	0.91 (0.84–0.98)	0.86 (0.77–0.96)	0.96 (0.86–1.08)	0.82 (0.80–0.84)
p value†	0.014	0.006	0.533	<0.001

*Ranges in parentheses are 95% confidence intervals.
†Test for trend.

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Dogs as Sources and Sentinels of Parasites in Humans and Wildlife, Northern Canada

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A minimum of 11 genera of parasites, including 7 known or suspected to cause zoonoses, were detected in dogs in 2 northern Canadian communities. Dogs in remote settlements receive minimal veterinary care and may serve as sources and sentinels for parasites in persons and wildlife, and as parasite bridges between wildlife and humans.

Throughout their long history of domestication, dogs have been sources of zoonotic parasites and have served as a link for parasite exchange among livestock, wildlife, and humans (*1*). Globally, dogs remain an important source of emerging disease in humans (e.g., eosinophilic enteritis caused by *Ancylostoma caninum*), a bridge for reemerging infections (*Echinococcus multilocularis*), and a source of parasites for immunocompromised persons (*1*).

Human disease and parasite infections in dogs in northern Canada have been recognized for some time (*2*–*5*). Historically, attention was focused on rabies virus, parvovirus, and canine distemper virus. However, dogs were also recognized as sources of zoonotic parasites such as *Echinococcus* spp. and as a possible bridge for rabies between wildlife and humans (*4*,*5*). Today, in many northern communities, veterinary services are absent or restricted, and disease surveillance programs and routine preventive health measures such as vaccination and parasite control are rare. These conditions have limited our understanding of disease interactions at the dog-human-wildlife interface and our ability to detect and respond to emerging diseases.

Northern environments and socioeconomic systems are changing rapidly and altering interactions among humans, animals, and their pathogens (*6*,*7*). In this study, we examined parasite diversity among dogs in 2 northern Canadian communities and evaluated the role of dogs as sentinels and sources of zoonotic infections in this changing landscape.

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The Study

Canine preventative healthcare clinics were available in Fort Chipewyan, Alberta, and Fort Resolution, Northwest Territories, in August 2006. Dogs were presented by their owners voluntarily and a detailed history, blood, and fresh fecal samples were obtained. Feces were stored at 4°C until examined within 6–12 days by quantitative sugar flotation and light microscopy (*8*). Fecal samples positive for *Giardia* spp. were genotyped (*9*). Serum samples were tested for antibodies against *Toxoplasma gondii* and *Neospora caninum* by using modified direct agglutination and immunofluorescence assays, respectively, at the US Department of Agriculture (Beltsville, MD, USA). Dilutions ≥1:25 were considered positive. Associations between parasitism and host (sex, age, community) and husbandry factors (housing, food type, community) were examined for adult dogs by χ^2 analysis and Fisher exact test by using analytical software (Statistix, Tallahassee, FL, USA).

The study population consisted of a variety of breeds and cross-breeds, including Siberian husky, Laborador retriever, German shepherd, terriers, and other types. Most dogs were housed outdoors and many were fed fish and game (raw, frozen, fresh, cooked, or dry). Of dogs eating wild game, they ate moose (95.8%), muskrat (53.5%), caribou (54.9%), bison (45.1%), rabbit (28.2%), beaver (25.4%), elk (15.5%), and deer (14.1%) (Figures 1, 2).

A minimum of 11 parasite genera were detected and 47% of dogs had ≥1 gastrointestinal parasite (Tables 1, 2). Taeniid eggs were either from *Echinococcus* spp. or *Taenia* spp. Dogs housed outdoors were more likely to have housing-associated parasites such as *Toxocara* spp., *Toxascaris*

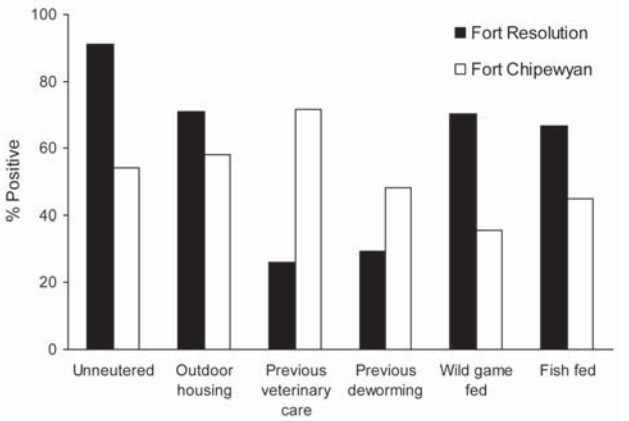

Figure 1. Husbandry practices for adult dogs (>6 months of age) in Fort Resolution and Fort Chipewyan, northern Canada. Results of all comparisons were significantly different between the 2 communities ($p<0.05$).

Figure 2. Northern dog with a typical meal. (Photograph provided by Susan J. Kutz.)

spp., *Cystoisospora* spp., and *Uncinaria* spp. ($p<0.0001$). Dogs who ate wild game were more likely to have game-associated parasites such as *Sarcocystis* spp., *Taenia* spp., and *Echinococcus* spp. ($p<0.05$). No statistically significant associations were found between food or housing, and *T. gondii* or *N. caninum* infections and previous veterinary care or deworming had no effect on parasite prevalence.

Conclusions

In the Northwest Territories, harvesting country foods is a key cultural activity and is important for sustenance; 75% of persons eat harvested meat and fish (*10*). Dogs fed fish and game can serve as indicators of parasites in these human food sources. Diet-associated zoonotic parasites detected in dogs included *Diphyllobothrium* spp., cestodes acquired by eating undercooked or inadequately frozen fish (found in humans throughout northern Canada); *Alaria* spp., trematodes acquired by eating frogs or paratenic hosts; and *T. gondii*, tissue protozoans acquired by eating oocysts from felid feces or tissue cysts in intermediate hosts (a worldwide human pathogen). In aboriginal persons in northern Canada, seroconversion for *T. gondii* during pregnancy has been associated with diets that include caribou (*11*). High seroprevalence in dogs indicates that *T. gondii* is common in the study area; however, the source of exposure was not identified. Given potential consequences for infection of parasite-negative pregnant women, further research is warranted on the association of human toxoplasmosis with a diet of country foods in northern regions.

Toxocara spp. are nematodes that cause visceral and ocular migrans in humans, particularly children. Although *Toxocara* spp. are considered limited to more southern regions (*3*), their presence in puppies and adults in Fort Resolution suggests that completion of their life cycle at northern latitudes is possible. Continuing warming trends may lead to increased occurrence of this parasite in the north. *Giardia* sp. Assemblage A is a protozoan that causes gastrointestinal disease in humans. Isolation of this zoonotic strain was unexpected because dogs are typically infected with Assemblage D, and Assemblage A suggests transmission from humans to dogs (*9*,*12*). This finding highlights a need to further investigate the apparent emergence of Assemblage A in domestic and wild animals in remote northern regions and transmission patterns among dogs, wildlife, and humans (S.J. Kutz, unpub. data). *Echinococcus* spp. are cestodes that cause hydatid (*E. granulosus*) or alveolar cysts (*E. multilocularis*) in the lungs and livers of humans. Although a reduction in dog teams in northern Canada has resulted in decreased prevalence of *E. granulosus* spp., the distribution, epidemiology, and role of the more pathogenic *E. multilocularis* spp. are not well understood in this region. *Uncinaria* spp. and *Toxascaris* spp. are also occasionally reported as zoonoses; however, evidence for these findings remains equivocal.

Dogs can also be sources of disease in parasite-naïve wildlife populations. They were the source for devastating distemper outbreaks in lions in the Serengeti (*13*), and lice of presumed dog origin are causing serious disease in Alaskan wolf populations (K.B. Beckmen, pers. comm.). *Neospora caninum* detected in this study may be a new parasite in this ecosystem with potentially serious consequences for wildlife. The remaining parasites are presumed present in local wildlife and can have a negative effect on the health of dogs and wildlife. More detailed, quantitative investigation is required to evaluate the role of dogs as potential sources of new, or amplifiers of existing, pathogens for wildlife.

Our results highlight important health issues associated with the interface between dogs, wildlife, and humans in remote northern communities. Disease associated with parasites in this study is often subclinical but can have serious effects on health and productivity of humans, dogs, and wildlife (e.g., *Giardia* spp.) (*14*). Although these parasites are relatively easy to control, there was no evidence that sporadic veterinary presence in Fort Chipewyan reduced parasitism. This finding emphasizes the need for a new approach to domestic animal healthcare in the north. Inaccessibility of communities, uncertain and changing roles of dogs, and current regulations in the veterinary profession restricting remote delivery of services hinder development

Table 1. Prevalence and median intensity (range) of parasite eggs or oocysts in feces or positive titers for *Toxoplasma* and *Neospora* in communities in northern Canada

	Fort Chipewyan				Fort Resolution			
	Puppies		Adults		Puppies		Adults	
Characteristic	M	F	M	F	M	F	M	F
No. dogs	6	1	32	20	5	7	37	21
Prevalence (%), median Intensity (range)*								
Alaria spp.†	0	0	0	0	0	0	14, 2 (1–134)	4, 7
Diphyllobothrium spp.†	17, 50	0	3, 2	0	20, 2	14, 1	11, 6 (1–603)	4, 6,429
Taeniid spp.†‡	0	0	6, 4 (2–5)	5, 55	0	0	11, 7 (1–770)	0
Cytoisospora spp.	0	0	0	5, 43	20, 30	0	0	4, 4
Sarcocystis spp.	0	0	0	15, 30 (3–53)	40, 2 (1–2)	14, 21	11, 13 (3–27)	13, 3 (1–255)
Toxascaris spp.†	0	0	0	5, 221	60, 251 (149–530)	29, 195 (93–297)	0	17, 138 (35–248)
Toxocara spp.†	33, 10,000 (610–20,000)	0	0	0	20, 6	14, 2	3, 11	9, 161 (1–321)
Uncinaria spp.†	0	0	47, 31 (1–333)	5, 63	20, 14	0	35, 40 (9– 251)	26, 27 (17–367)
No. dogs	4	1	30	13	3	7	23	15
Giardia spp.†	0	0	0	8	33	0	0	20
No. dogs	6	1	29	16	3	5	30	18
Toxoplasma gondii †§	50	100	41	50	100	80	60	56
Neospora caninum¶	0	0	3	6	0	0	7	0

*Range not reported if only 1 dog was positive. Intensity is the number of eggs or oocytes per gram of wet feces.
†Zoonotic parasites.
‡*Echinococcus multilocularis, E. granulosus*, or *Taenia* spp.
§23 dogs had titers of 25, 22 had titers of 50, 1 had a titer of 100, 1 had a titer of 200, and 1 had a titer of 400.
¶All 4 dogs had titers of 25.

of effective disease detection and preventative medicine programs. Innovative new methods for delivery of animal healthcare services are required. These methods should include long-term commitment to an integrated health approach, focusing on education, engagement, and development, and support of local capacity for delivery of basic animal health services. Ongoing communication and partnerships between animal and human health professionals will enhance the effectiveness of such initiatives.

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Table 2. Percentage of dogs with multiple parasite genera detected by fecal flotation in 2 communities in northern Canada

	Fort Chipewyan				Fort Resolution				Total			
	Puppies		Adults		Puppies		Adults		Puppies		Adults	
Characteristic	M	F	M	F	M	F	M	F	M	F	M	F
Sample size	6	1	32	20	5	7	37	21	11	8	69	41
No. parasite genera												
0	50	0	47	70	20	71	49	52	36	75	48	61
1	50	0	50	25	40	0	30	24	46	0	39	24
2	0	0	3	5	20	14	11	10	9	13	7	7
3	0	0	0	0	0	14	11	14	0	24	6	7
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	20	0	0	0	9	0	0	0
>2	0	0	3	5	40	29	22	24	18	25	13	15

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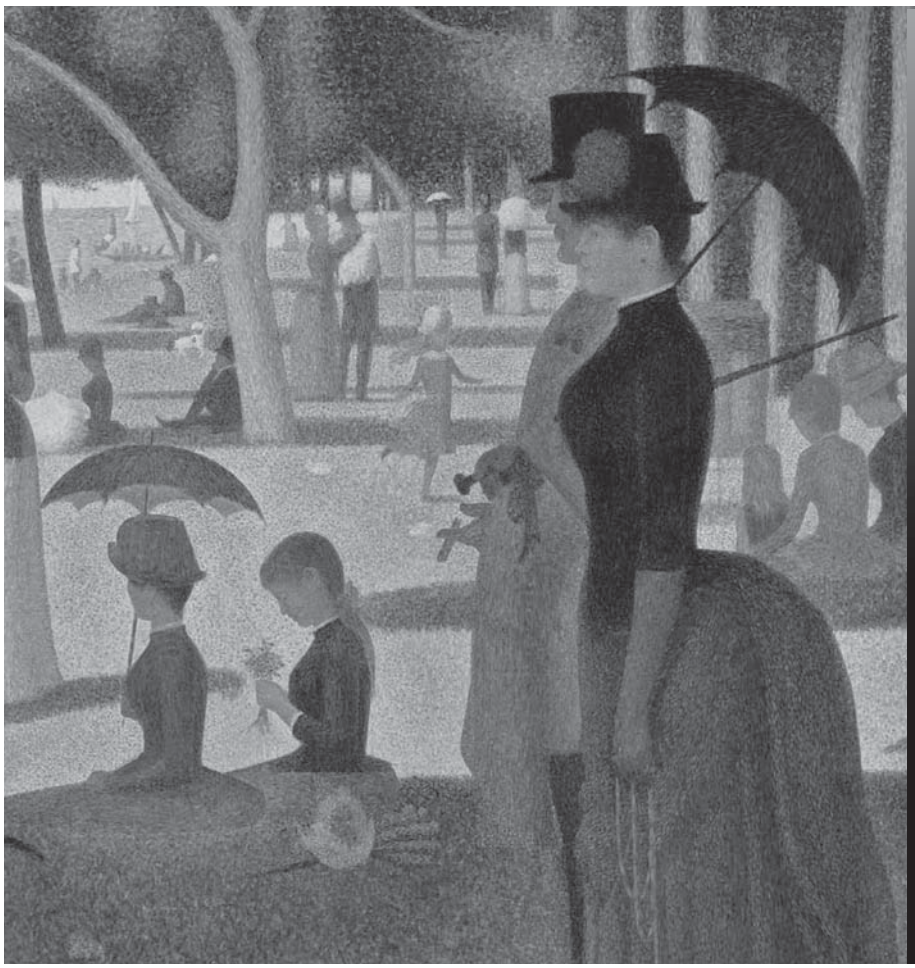

Human Ophthalmomyiasis Interna Caused by *Hypoderma tarandi,* Northern Canada

Philippe R.S. Lagacé-Wiens,* Ravi Dookeran,* Stuart Skinner,* Richard Leicht,* Douglas D. Colwell,† and Terry D. Galloway*

Human myiasis caused by bot flies of nonhuman animals is rare but may be increasing. The treatment of choice is laser photocoagulation or vitrectomy with larva removal and intraocular steroids. Ophthalmomyiasis caused by *Hypoderma* spp. should be recognized as a potentially reversible cause of vision loss.

Ophthalmomyiasis interna is invasion of the globe by larvae of any species of oestrid flies; ophthalmomyiasis externa involves only the external ocular structures (*1*). *Dermatobia hominis,* endemic to tropical or subtropical areas, and *Oestrus ovis* (sheep bot fly) cause most cases of ophthalmomyiasis (*2*). Both typically cause ophthalmomyiasis externa (*2*). Only 2 bot flies inhabit Nearctic circumpolar regions: the Caribou bot fly (*Hypoderma tarandi*) and the Caribou nasal bot fly (*Cephenemyia trompe*), a nonhuman pathogen (*2*). *H. tarandi* is a nonbiting fly whose obligate endoparasitic larvae typically affect caribou throughout their circumpolar range (*2*,*3*). From late June to early September. the fly lays eggs directly on the guard hairs of the caribou (*2*,*3*). Once deposited, eggs hatch into larvae that penetrate skin (*3*). They move subcutaneously to reach the animal's dorsal region, where they cut breathing holes and are encased within granulomatous cysts, termed warbles. There they develop for 9–11 months; in May or June of the following year, they leave the animal and pupate on the ground (*3*). Mated adult females are capable of long flights (≈900 km) in search of suitable hosts (*3*).

Infestations by *H. tarandi* in humans are rare but are likely underreported (*4*). Related species, *H. bovis* and *H. lineatum,* inhabit various regions of North America, Europe, and Asia and have also been implicated in human disease (*2*,*5*). A third species that affects cattle and yaks in China (*H. sinense*) is also responsible for human infestations (*2*). The pathophysiology of human ophthalmomyiasis by *Hypoderma* spp. is not known. Eyebrows and eyelashes have been suggested as possible targets for oviposition (*3*). Oviposition on human scalp hair has been achieved experimentally and could be the preferential site in humans (*3*). An alternative explanation is transfer of the larvae directly from the guard hairs of the caribou to the human eye or skin through close contact with animal pelts. The parasite does not appear to complete its life cycle in humans (*1*,*2*).

We present the first, to our knowledge, 2 published cases of ophthalmomyiasis interna caused by *H. tarandi* in Canada. Furthermore, we present the first published use of *Hypoderma* spp. serologic testing to assist in the diagnosis of myiasis in humans.

The Cases and Literature Review

The first patient was a 41-year-old woman from Rankin Inlet, Nunavut, Canada, who noticed floaters (objects in the field of vision that originate in the vitreous) in her right eye in August 2006. Initial funduscopic examination showed posterior vitreous detachment. Two weeks later, her vision was more impaired; repeat funduscopy showed panuveitis. Pretreatment blood count was within normal limits with no eosinophilia. Topical steroids were ineffective. At a third assessment, her visual acuity was 20/400; funduscopic examination showed an intraocular larva (Figure 1). The parasite appeared to recede behind the retina and could not be photocoagulated. A pars plana vitrectomy and intraocular laser treatment of the entry and exit sites were performed. Triamcinolone (0.4 mg) was administered for intraocular inflammation, and antibiotics were given as prophylaxis. The larva was not recovered. Postoperative magnetic resonance imaging demonstrated no parasite or abnormality. The larva was assumed to be that of *H. tarandi* because of its appearance (shape, size, and segments), the late summer timing, and the patient's residence in the subArctic. Serologic testing for *H. tarandi* by Western blot, as described by Baron and Colwell, was performed (*6*). Six weeks after symptom onset, serum was positive for immunoglobulin (Ig) G, IgE, and IgM to hypodermin C, a larval collagenase of *H. tarandi* (*7*). No seroreactivity to the hypodermins A and B of *H. lineatum* was observed. Because the larva was unrecoverable, the patient was treated with 1 oral dose (9 mg) of ivermectin (Merck & Co., Kirkland, Quebec, Canada) 1 week after vitrectomy. After 6 months, her visual acuity had improved to 20/30.

The second patient was an 11-year-old Inuit boy from Chesterfield Inlet, Nunavut, Canada, who was examined in October 1997 because of a painful right eye, scleral redness, and blurry vision. Examination found uveitis and glaucoma. Topical steroid therapy was begun, after which retinal detachment, hemorrhages, and a 4-mm larva in the subretinal space were noted. Blood work showed marginal eosinophilia; computed tomographic examination of the

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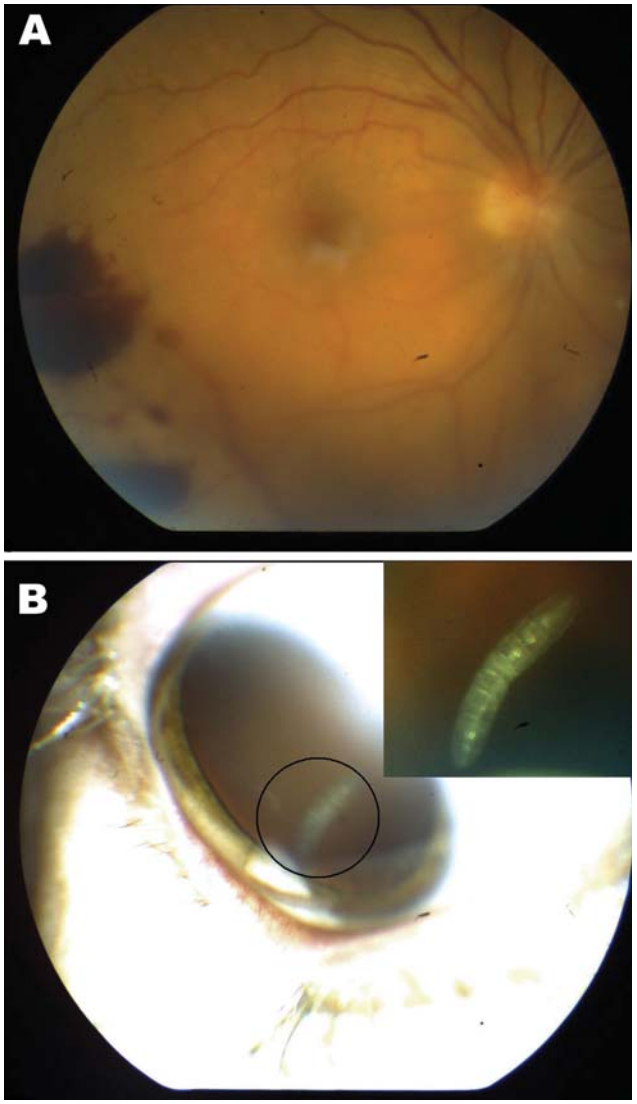

Figure 1. A) Retinal hemorrhages visible on funduscopic examination of right eye of a 41-year-old woman, Nunavut, Canada, with ophthalmomyiasis interna. B) Segmented 3-mm larva with a cylindrical body, no visible spines, and indistinguishable anterior and posterior ends in the vitreous cavity, corresponding to the first instar of *Hypoderma tarandi*.

brain and orbits showed no abnormalities. The larva was removed by pars plana vitrectomy and retinotomy. Examination of the larva by dissection microscopy showed a 4 × 1–mm larva. Scanning electron microscopy, performed according to Colwell et al. (*8*), confirmed the characteristic of the first instar of *Hypoderma* spp., i.e., mouth hooks, anterior sensory structure, spines, and spiracles (Figure 2). The presence of only 2 lateral spines per spiracle indicated *H. tarandi*. Western blot testing showed the presence of IgG only to hypodermin C. When asked about his exposure to caribou, the boy admitted to tracking, hunting, and skinning caribou. The patient's disease progressed despite therapy, and his eye was enucleated. Pathologic examination showed retinal detachment, eosinophilic choroiditis, and retinitis. Chronic nongranulomatous inflammation was noted in the ciliary body and iris. No additional larvae were found.

We searched the literature, using PubMed, for the terms "ophthalmomyiasis" with limits "human" and "English." We also reviewed references of selected publications. We reviewed only cases of ophthalmomyiasis interna caused by oestrid flies found in North America, which were confirmed by visible larvae (online Appendix Table, available from www.cdc.gov/EID/content/14/1/64-appT.htm). Demographics and clinical presentation of patients are in the Table. For statistical analysis, outcomes were separated into good (vision unchanged from baseline or better than 20/80), moderate (able to see shapes or fingers or vision worse than 20/80 but not blind), and severe (able only to see light or movement, completely blind or enucleated). Although moderate or severe vision loss appeared to occur more commonly with *Hypoderma* than *Cuterebra* infestations (53.3% vs 14.3%), this frequency was not statistically significant (p = 0.08). Photocoagulation appeared

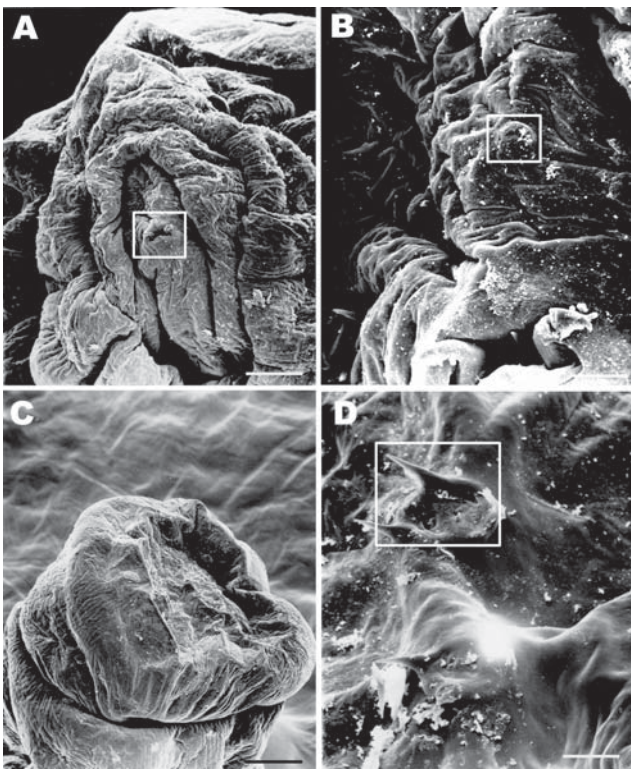

Figure 2. Scanning electron microscope images of the parasite from an 11-year-old Inuit boy, Nunavut, Canada. A) Anterior end of the maggot. The cephalic segment is evident; mouth and mouth hooks are present (boxed). Scale bar = 50 μm. B) The characteristic cephalic sensory array (boxed). Scale bar = 10 μm. C) Posterior segments of the maggot. Scale bar = 100 μm. D) Spiracular openings on the posterior segments of the maggot characteristic of first instar of *Hypoderma*. Scale bar = 10 μm.

Table. Demographics and clinical presentation of 32 patients with ophthalmomyiasis interna*

Characteristic	No. (%)
Symptom	
Red eye	21 (60)
Vision loss	24 (68)
Floaters	24 (68)
Eye pain	6 (17)
Scotomas	3 (9)
Sex	
Male	27 (77)
Female	7 (20)
Loss of vision	
Severe or total†	12 (34)
Moderate	2 (6)
Mild or none	19 (54)

*Patients identified during literature search and described in the online Appendix Table. Mean patient age was 25.2 y; median, 16 y.
†Includes blindness caused by enucleation.

to produce better outcomes (80% good outcomes) than no intervention (73.7% good outcomes) or surgical removal/vitrectomy (41.2% good outcomes); however, χ^2 analysis showed no statistically significant difference ($p = 0.121$). This observation may suggest that by the time the patient seeks treatment, damage to ocular structures has already occurred. Recent cases have been treated with photocoagulation or vitrectomy and intraocular steroid administration. Although no specific intervention is associated with better outcomes, given the difficulty in studying outcomes in this rare condition and the success of this therapy for other foreign bodies in the eye, this course of action is reasonable. Ivermectin as medical therapy for ophthalmomyiasis interna has not been reported, but it is effective as therapy and prophylaxis for bot fly infestation of livestock (*9*). Ivermectin as adjunctive therapy in select cases of ophthalmomyiasis may be of benefit, but evidence is lacking. Only 1 case of ophthalmomyiasis externa caused by *D. hominis* and treated with ivermectin has been reported (*10*).

Conclusions

Human myiasis caused by bot flies of nonhuman animals is rarely reported. However, diagnoses may increase as a result of increasing population in northern latitudes, encroachment into habitats with natural hosts like caribou, and better access to ophthalmologists. The treatment of choice seems to be laser photocoagulation or vitrectomy with larva removal and coadministration of intraocular steroids. In areas where *Hypoderma* spp. are common, healthcare providers should consider this condition and promptly refer patients to an ophthalmologist.

P.R.S.L.-W. received honoraria from Sanofi-Pasteur for providing educational sessions.

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Q Fever Update, Maritime Canada

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Since the 1990s, reports of Q fever in Nova Scotia, Canada, have declined. Passive surveillance for Q fever in Nova Scotia and its neighboring provinces in eastern Canada indicates that the clinical manifestation of Q fever in the Maritime provinces is pneumonia and that incidence of the disease may fluctuate.

The first cases of Q fever in Nova Scotia were recognized in 1979 during a study of atypical pneumonia (*1*). This observation led to a series of studies that showed that Q fever was common in Nova Scotia (50–60 cases per year in a population of ≈950,000) and that the epidemiology was unique; exposure to infected parturient cats or newborn kittens was the major risk factor for infection (*2*). At about the same time, cat-related outbreaks were noted in neighboring Prince Edward Island and New Brunswick (*2*). In the early 1990s, cases began to decline; but, to our knowledge, since 1999 Q fever in this area has not been systematically studied. We undertook the current study to determine whether Q fever was still occurring in Maritime Canada (the 3 provinces of New Brunswick, Nova Scotia, and Prince Edward Island in eastern Canada) and whether the decline in cases was real or an artifact of decreased surveillance.

The Study

The study began in December 2004 and terminated on February 17, 2007. A notice sent to all physicians in Maritime Canada described the study and asked the physicians to submit serum samples from patients with suspected Q fever (febrile illness or pneumonia after exposure to parturient cats or other animals; outbreaks of pneumonia in a family). All samples were sent to the Nova Scotia Public Health Laboratory for testing for antibodies to *Coxiella burnetii*. Physicians of patients with a positive test result were contacted, and they in turn contacted their patients to ask if they would participate in the study. This study was approved by the University of Alberta Research Ethics Review Board and the Capital Health Research Ethics Board.

During the study period, serum samples from 210 patients suspected of having *C. burnetii* infection were tested by using commercially available immunoglobulin (Ig) M and IgG ELISAs (PanBio, Brisbane, Queensland, Australia). Patients with positive IgM ELISA results were asked to participate in the study. Convalescent-phase samples were collected from those who agreed; further testing for IgG antibodies to phase I and phase II *C. burnetii* antigen was conducted by using an indirect immunofluorescence test as previously described (*3*).

Of the 210 patients, 35 had antibodies to *C. burnetii* and 13 met the criteria for acute Q fever (positive IgM ELISA result and a ≥4-fold rise in antibody to phase II antigen between the acute- and convalescent-phase samples). Phase I titers ≥512, suggestive of chronic Q fever, were found for 3 patients. The other 19 patients had serologic profiles suggestive of previous exposure to *C. burnetii*. Of the 13 patients who fit the case definition of acute infection, 11 agreed to participate in the study, 1 declined, and 1 moved to another country.

Of the 11 participating patients, 7 were from Nova Scotia, 2 were from New Brunswick, and 2 were from Prince Edward Island; 6 were male; and mean age was 54.6 years (Table). One case occurred in December 2004; 6 in 2005; 4 in 2006, and no cases in the first 6 weeks of 2007. Cases occurred in every month except August, September, October, and February.

All patients except patient 6 had risk factors for Q fever (Table). One patient (patient 8) was a sheep farmer who had recently had ≈60 lambs born on his farm, several of which were stillborn in the 2 weeks before the farmer became ill; 7 patients had ≥1 cat as a pet; and only 2 (patients 5 and 8) had no pets.

In terms of clinical signs, all 11 patients had sweats, fever, and myalgia; 9 had chills; 8 had a cough; 7 were short of breath; 5 each had nausea, diarrhea, sputum production, and confusion; 4 had chest pain, which was pleuritic for 2; and 2 had abdominal pain and vomiting. Of the 7 patients for whom chest radiographs were taken, 6 had acute opacities compatible with pneumonia. Patient 11 had diffuse bilateral pneumonia, which required him to be admitted to an intensive care unit to receive ventilatory support (Figure).

All 11 patients recovered. Only 4 received initial empiric therapy that would be considered effective against *C. burnetii,* e.g., doxycycline (n = 2), ciprofloxacin (n = 1), or levofloxacin (n = 1). Four other patients received azithromycin, which may have been effective but has suboptimal in vitro activity against *C. burnetii* (*4*).

Conclusions

Acute Q fever is still present in Maritime Canada; however, the number of cases has diminished considerably from the 1980s and early 1990s. Since 2004, only 4–5 cases have been reported each year. The passive design of our study may have underestimated the number of cases. However, in the 1980s, a number of Q fever outbreaks involved entire families. A typical scenario was exposure to the

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Table. Selected features of 11 patients with acute Q fever, Maritime Canada

Patient	Date of onset	Sex	Age, y	Hospitalized	Pneumonia	Risk factors
1	2005 Jan 10	F	42	No	Yes	Farmer*
2	2004 Nov 1	F	50	Yes	Yes	Farm visit
3	2005 July 18	F	67	Yes	ND†	Farm visit
4	2005 Apr 17	M	56	Yes	No	Farm visit, deer hunting
5	2006 May 24	M	44	Yes	Yes	Newborn calves
6	2006 June 30	F	51	No	Yes	None
7	2005 Dec 31	M	59	No	Yes	Newborn lambs
8	2006 May 22	M	57	No	Yes	Newborn lambs
9	2005 Mar 1	F	50	No	ND	Parturient cat and her kittens
10	2005 Mar 1	M	69	No	ND	Parturient cat and her kittens
11	2006 Jan 1	M	56	Yes‡	Yes	Newborn poodles, livestock auction

*Within 2 weeks of onset of symptoms, this patient had occupied a small space (automatic bank teller area) with 2 farmers who smelled of manure.
†ND, not determined because chest radiographs were not taken.
‡Required intubation.

parturient family cat and her newborn kittens, after which everyone in the family became ill (*2*). Some outbreaks involved poker players (*5*) or most of the employees of a factory (*6*). For our study, we carefully asked whether family members were ill; only 2 patients mentioned such illness, and for each, it was a spouse.

Pneumonia seems to still be the dominant form of acute Q fever in Maritime Canada. Of the 7 patients for whom chest radiographs were taken, 6 had pneumonia. Major differences in the manifestations of Q fever occur in different regions. In Maritime Canada and in the Basque region of Spain, the predominant manifestation is pneumonia (*7*,*8*); in Newfoundland and Australia, fever with no apparent localization of infection (*9*,*10*); in the Canary Islands, fever and hepatitis; and in southern France, hepatitis and pneumonia, although hepatitis is more frequent than pneumonia (*11*,*12*). The factors responsible for these disparate manifestations are not known. When isolates of *C. burnetii* from different geographic areas were typed by using multisequence typing, all 7 isolates from Nova Scotia were identical and shared this type with 2 isolates from France and 1 from the United States (*13*).

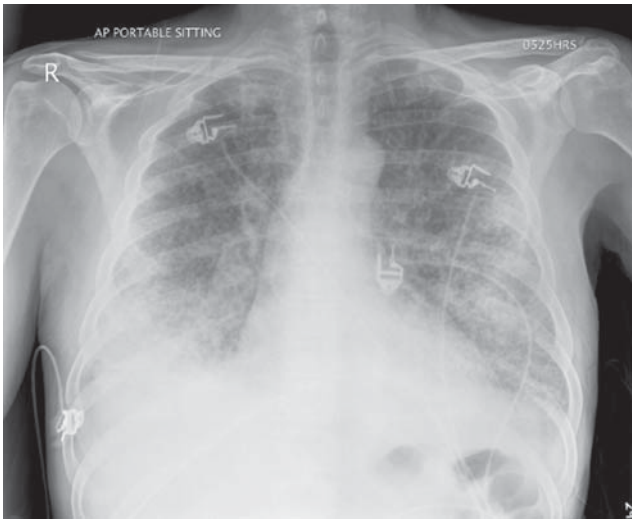

Figure. Chest radiograph of patient 11 at time of admission to hospital, before intubation, demonstrating extensive bilateral airspace disease.

The reservoirs for human infection with *C. burnetii* in Nova Scotia have likely spread from cats and dogs (*14*) to the more traditional reservoirs of sheep and cattle (*12*). Patient 8, a sheep farmer, had pneumonia that appeared on radiographs as a rounded opacity in the right middle lobe. Rounded opacities are very common in cat-associated cases of Q fever but may not be specific for cat-associated infection (*15*).

Our findings indicate that after *C. burnetii* is established in an area, it is likely to persist, although the incidence may fluctuate. Clinical manifestations, which in our study were limited to pneumonia, remain stable.

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Dissemination of Multidrug-Resistant Bacteria into the Arctic

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We show that *Escherichia coli* isolates originating from Arctic birds carry antimicrobial drug resistance determinants. This finding implies that dissemination of drug-resistant bacteria is worldwide. Resistance genes can be found even in a region where no selection pressure for resistance development exists.

Bacteria display a unique ability to adapt to changes in their environment and to develop mechanisms to protect themselves against toxic compounds. Their ability to develop resistance mechanisms to antimicrobial drugs has assumed catastrophic proportions, rendering more and more infections difficult or impossible to treat (*1*). Most reports suggest that the main force behind emergence of drug resistance is the use and misuse of antimicrobial drugs during the past few decades, but there is also evidence for the epidemic spread of drug-resistant bacteria as a contributing factor (*2*).

The Study

We investigated bacteria from a region considered to be one of the last outposts of wilderness, the Arctic, with the belief that in this region human influence on the ecology of antimicrobial resistance would be minimal. Antimicrobial drug resistance in *Escherichia coli* isolated on site from fecal or cloacal swabs of Arctic birds was studied.

During the Beringia expedition organized by the Swedish Polar Research Secretariat in 2005, fecal or cloacal samples were collected from 97 birds in 3 geographic regions: northeastern Siberia; Point Barrow, Alaska, USA (71º30′N, 156º78′W); and northern Greenland. Samples were transported in a swab-transport system to a laboratory on the expedition ship. Upon arrival, the samples were immediately cultured, and single colonies of enterobacteria were isolated on blood agar plates (*3*). One isolate of *E. coli* was selected from each sample and stored at –70°C for further analysis. Susceptibility to 17 antimicrobial drugs was determined by using disk diffusion with disks on Iso-Sensitest agar (Oxoid, Basingstoke, UK) in accordance with the recommendations of the Swedish Reference Group for Antibiotics (*4*). MIC determinations were performed by using the E-test (AB Biodisk, Solna, Sweden) on Iso-Sensitest agar. All bacteria were tested against the following antimicrobial drugs: ampicillin, cefadroxil, cefuroxime, cefpodoxime (to screen for *E. coli* with extended-spectrum β-lactamases), chloramphenicol, ciprofloxacin, fosfomycin-trometamol, gentamicin, imipenem, mecillinam, nalidixic acid, nitrofurantoin, streptomycin, sulfamethoxazole, tetracycline, trimethoprim, and tigecycline.

Clonal diversity of the 97 isolates was assessed by using a commercial typing system, the PhenePlate (PhP) system (PhPlate Microplate Techniques AB, Stockholm, Sweden). This system uses results of 11 biochemical reactions in a microplate to obtain a biochemical fingerprint of each tested isolate and has been used for typing of *E. coli* (*5*,*6*). Compared with traditional qualitative biochemical testing where the results are read once, PhP typing is based on several readings and thereby includes dynamics of the reaction in the analysis. PhP typing was performed as described with 2 modifications: 1 of the 11 reagents, melabionate, was replaced with raffinose, and *E. coli* reference strain ATCC 25922 was used as a positive control (*6*). Biochemical fingerprints obtained were compared with each other, and similarity was calculated by using PhPWIN4 software (*6*). PhP types with ≥2 isolates with the same biochemical fingerprint were designated as common types (CTs); types with 1 isolate, which represented rare or unique clones, were designated as single (Si) types.

E. coli isolates from Arctic birds carried antimicrobial drug resistance determinants; among 17 antimicrobial drugs tested, resistance to 14 was detected. Resistance was observed in 8 isolates, 4 of which displayed resistance to ≥4 drugs (Table), and occurred most often to ampicillin, sulfamethoxazole, trimethoprim, chloramphenicol, and tetracycline. Two resistant isolates displayed isolated fosfomycin resistance with MIC values of 256 mg/L and 1,024 mg/L. No resistance to gentamicin, imipenem, or tigecycline was observed.

PhP typing divided the 97 isolates into 11 CTs (CT1–CT11) and 34 Si types (online Appendix Table, available from www.cdc.goc/EID/content/14/1/70-appT.htm). The 3 most frequent CTs were CT3, CT2, and CT5, which contained 29, 9, and 5 isolates, respectively. Among the isolates from Lorino, Siberia (65º39′N, 172º15′W), (n = 13), Si types were most common (n = 5). Likewise, among isolates from Novo Chaplino, Siberia (64º70′N, 173º00′W) (n = 33), Si types (n = 19) predominated. In contrast, only

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Table. Antimicrobial drug resistance phenotypes in 8 *Escherichia coli* isolates from Arctic birds

Isolate	Avian host species	Geographic origin	PhenePlate type*	Drug resistance profile†
2	Western sandpiper	Lorino, Siberia	Si	Amp, Cpd, Cdr, Cxm
18	Vega/Glaucous gull	Novo Chaplino, Siberia	CT3	Amp, Cpd
26	Vega/Glaucous gull	Novo Chaplino, Siberia	Si	Amp, Sul, Chl, Tet, Tri, Nit, Str
35	Vega/Glaucous gull	Novo Chaplino, Siberia	Si	Fos
36	Vega/Glaucous gull	Novo Chaplino, Siberia	Si	Fos
75	Emperor/Brent goose	Kolyuchin, Siberia	CT5	Tet
94	Iceland/Glaucous gull	Thule, Greenland	CT5	Amp, Sul, Chl,Mec, Tet, Tri, Str
97	Iceland/Glaucous gull	Thule, Greenland	Si	Amp, Sul, Chl, Mec, Tri, Str, Nal, Cip

*Si, single type; CT, common type.
†Amp, ampicillin; Cpd, cefpodoxime; Cdr, cefadroxil; Cxm, cefuroxime; Sul, sulfamethoxazole; Chl, chloramphenicol; Tet, tetracycline; Tri, trimethoprim; Nit, nitrofurantoin; Str, streptomycin; Fos, fosfomycin; Mec, mecillinam; Nal, nalidixic acid; Cip, ciprofloxacin.

2 Si types were found among isolates from Kolyuchin, Siberia (67º04′N, 173º21′W) (n = 29). Most of these isolates (n = 18) were defined as CT3. Six of the 11 CTs were restricted to 1 sample site: CT1 at Lorino, CT6 at Novo Chaplino, CT7 at Kolyuchin, CT9 and CT10 at Wrangel Island (70º55′N, 179º29′W), and CT11 at Thule, Greenland (76º32′N, 68º44′W). Among the 8 drug-resistant isolates, 1 was CT3 and 2 were CT5; the other 5 were defined as Si types (Table).

Conclusions

Antimicrobial drug resistance in *E. coli* isolated from wild birds has been described (*7*,*8*). A high frequency of *E. coli* isolates from migratory Canada geese sampled on the eastern shore of Maryland in the United States were resistant to penicillin G, ampicillin, cephalothin, and sulfathiazole (*8*). Similarly, a high prevalence of antimicrobial drug–resistant *E. coli* isolated from black-headed gulls in the Czech Republic has been observed (*7*).

There are several explanations for antimicrobial drug resistance in the normal microbiota of Arctic birds. First, resistance may develop de novo through spontaneous mutation(s) (*9*). Second, resistance can be acquired by horizontal gene transfer from other microbes; many bacteria and fungi constitute natural sources of drug resistance genes and may serve as reservoirs in the environment (*10*). Third, bacteria with antimicrobial drug resistance could be imported into the region either by migratory birds or through human refuse (food, excretions) from fishermen, settlers, and prospectors in the area. The region around the Bering Strait constitutes the breeding ground of a large number of waterfowl, geese, and shorebirds. Although many of these species are largely confined to one side of the Bering Strait, other species pass through both continents during migration or occasionally wander across the strait (*11*). Moreover, bird species that spend the winter in up to 6 different continents can be found in this area (*11*). Thus, migratory birds that have acquired drug-resistant bacteria during wintering or stops at lower latitudes before migrating to the Arctic provide a potential explanation for introduction of drug resistance into this region. The fact that 1 isolate from a juvenile Western sandpiper sampled far from human settlements on the tundra had resistance to cefadroxil, cefuroxime, and cefpodoxime, a resistance pattern commonly seen in clinical isolates, supports the theory of introduction by migration and transfer of bacteria between birds.

To the best of our knowledge, the most remote and isolated environment investigated for drug-resistant bacteria is the Bolivian community of 130 Guaraní Indians, as described by Bartoloni et al. (*12*). This community is located at an altitude of ≈1,700 m and can only be reached by a 3-hour steep climb. Nevertheless, high carriage rates of drug-resistant commensal *E. coli* were found in this community, although exposure to antimicrobial drugs in the area had been limited. Similarly, antimicrobial drug–resistant bacteria in wild animals with little or no contact with human settings has been reported (*13*,*14*). These findings and those of our study suggest that commensal bacteria in humans and animals constitute hidden reservoirs of antimicrobial drug resistance (*15*). A possible explanation for the unexpectedly high carriage rate of drug-resistant *E. coli* in the Indian community in Bolivia is the importation of drug-resistant isolates by migratory birds.

We have shown that antimicrobial drug resistance genes are present in 1 of the most remote areas on Earth, the Arctic. Resistant as well as multiresistant isolates of *E. coli* were detected in the normal flora of Arctic birds. This finding highlights the unique nature of bacterial adaptation and the complexity of dissemination of antimicrobial drug resistance. To fully understand the extent of environmental and commensal reservoirs of resistance, studies of antimicrobial drug resistance in different habitats are warranted.

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Spatial Distribution of *Echinococcus multilocularis*, Svalbard, Norway

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In Svalbard, Norway, the only intermediate host for *Echinococcus multilocularis,* the sibling vole, has restricted spatial distribution. A survey of feces from the main host, the arctic fox, showed that only the area occupied by the intermediate host is associated with increased risk for human infection.

The cestode *Echinococcus multilocularis* is the causative agent of alveolar echinococcosis, a rare but potentially lethal human disease. In the Arctic, *E. multilocularis* depends on the arctic fox (*Vulpes lagopus,* formerly *Alopex lagopus*) or domestic dog (*Canis lupus familiaris*) as its definitive hosts, and human infections are caused by ingestion of infective eggs distributed with the feces of these hosts. A wide variety of small rodents, especially voles and lemmings of the subfamily *Arvicolinae,* can function as intermediate hosts (*1*,*2*). In 1999, *E. multilocularis* was first identified on the Arctic island Spitsbergen, in the Svalbard archipelago (*3*); since then, several human seropositive cases have been reported (*3*). These cases have caused health concerns for the public health of residents and tourists as well as for the small but flourishing tourist industry on the island.

Arctic foxes are common throughout Spitsbergen (*4*); they are most plentiful near seabird cliffs along the coast and less plentiful in the inland valleys (*5*,*6*). The only available intermediate host in Svalbard is the sibling vole (*Microtus levis,* formerly *Microtus rossiaemeridionalis*). The distribution of sibling voles on the island seems to be limited by availability of plants for food and is at present restricted to the heavily fertilized bird cliffs along the coastline in the Grumant area ([*3*], Figure). During years when vole densities were high in Grumant, voles may have spread out from the coast toward the east and west (Figure), but they have not yet established permanent populations outside the Grumant area (*3*). To evaluate how the restricted distribution of the intermediate host affects the spatial distribution of human risk for infection, we sampled fox feces at increasing distances from the core vole range (Grumant) and tested the feces for evidence of *E. multilocularis* infection by using specific *E. multilocularis* coproantigen ELISA. We also estimated the density of fox feces in Grumant and next to Longyearbyen by using line transect methods.

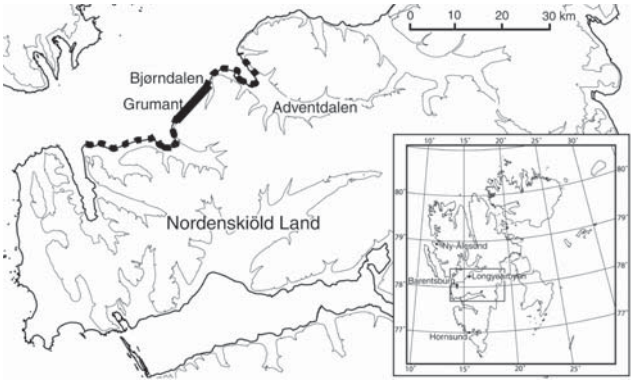

Figure. Main study area on the archipelago of Svalbard. Thick solid line, core area for sibling vole; broken line, area of distribution in peak vole year. Inset shows the 4 main settlements on the archipelago of Svalbard.

The Study

During July 2000 and 2004, feces were collected around arctic fox dens during the annual den surveys conducted by the Norwegian Polar Institute in Svalbard. Fox feces were also collected in the Grumant area in 2004. We grouped the feces into 4 distance categories (Table, Figure): A) Grumant, the core sibling vole range, Grumant to Lille Bjørndalen; B) Bjørndalen, the neighboring valley 2–6 km from Grumant; C) Nordenskiöld Land, the more distant areas surveyed on the Nordenskiöld Land peninsula, 6–40 km from Grumant; and D) Distant, the Hornsund area 130 km south of Grumant and the Ny-Ålesund area 110 km north of Grumant. The main Norwegian settlement, Longyearbyen, (population ≈2,100) is located 12 km east of Grumant; the Russian settlement, Barentsburg, (population ≈500), is located 24 km southwest of Grumant.

Fecal samples were bagged and stored at –80 degrees for >1 week before they were processed further. Specific *E. multilocularis* coproantigen detection was performed as described by Deplazes et al. (*7*) on the samples collected in 2000; the Chekit Echino-test (Dr. Bommeli AG, Liebefeld-Bern, Switzerland) was used on the samples from 2004. As recommended by Agresti and Coull (*8*), we used score confidence limits for the proportions. The score confidence limits used are given by

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$$\frac{\left(\hat{p}+\frac{z^2_{\alpha/2}}{2n} \pm z_{\alpha/2}\sqrt{\frac{\left[\hat{p}(1-\hat{p})+\frac{z^2_{\alpha/2}}{4n}\right]}{n}}\right)}{\frac{1+z^2_{\alpha/2}}{n}}$$

where $\hat{p}$ denotes the sample proportion, n denotes the sample size, and $z^2_{\alpha/2}$ denotes the $1 - \alpha/2$ quantile of the standard normal distribution (*8*); α was set to 0.05 to obtain 95% confidence limits.

We estimated the density of fox feces by using line-transect methods. Eight line transects were placed in Grumant (total length 118 m), and 10 transects of 20 m each (total length 200 m) were placed in Adventdalen, next to Longyearbyen and 15 km east of Grumant. The distance from the line to detected feces was noted; all feces detected up to 1.6 m on each side of the transect lines were included in the density estimation. The data were analyzed by using Distance 5.0 (*9*). In Adventdalen, no feces were found along the transect lines, and confidence limits for the estimated zero density of feces were calculated assuming the same detection function as in Grumant and a binomial distribution for the presence of feces along the 20-m transect lines.

The proportion of arctic fox feces that contained *E. multilocularis* showed a strong spatial pattern. The *E. multilocularis*–positive proportion within the core vole range Grumant was high, but from the nearby Bjørndalen and more distant areas on Nordenskiöld Land, no feces contained *E. multilocularis* (Table). This finding shows that the area of high risk for human infection overlaps exactly with the core distribution range of the intermediate host. The lack of *E. multilocularis*–positive fox feces in the adjacent valley, Bjørndalen, also suggests that foxes in this area do not include part of the Grumant area in their territories. Surprisingly, fecal samples collected in the Hornsund and Ny-Ålesund areas (1 each) in 2004 were *E. multilocularis* positive. These may be false-positive results, or the feces may have come from arctic foxes that were infected in the Grumant area before a long-distance dispersal event or perhaps from even more distant locations, e.g., Northern Russia, which has been speculated to be the initial source of infection to Svalbard (*3*).

The density of fox feces in Grumant was estimated to be 4.5 feces per 100 m^2 (95% confidence interval 1.7–12.0). No fox feces were detected along the 10 transects in Adventdalen, but the estimated confidence intervals suggest a density of 0–0.4 feces per 100 m^2. The density of fox feces is therefore likely to be at least 11 times higher in Grumant than in Adventdalen (4.5/0.4 = 11.25). This difference can be explained by differences in fox densities as a result of lower resource availability in the inland valley of Adventdalen compared with the high resource availability at large seabird cliffs in the Grumant area (*5*,*6*).

Conclusions

The combination of a high proportion of *E. multilocularis*–positive feces and a high density of arctic fox feces in the Grumant area suggests that this is an area of high risk for human infection. Infected foxes have the potential to spread infective *E. multilocularis* eggs far from this area; however, our data suggest that the risk for human infection drops to low levels at a very short distance from the Grumant area. A simple approach for reducing the risk for human infection on Spitsbergen is therefore to limit human exposure to this area, e.g., minimize the use of the Grumant area for recreational and tourist purposes. Further monitoring should focus on the possible vole colonization of new areas with high fox densities, i.e., areas under large seabird cliffs, which might increase the endemic range of infection. In summary, human alveolar echinococcosis risk in Svalbard is associated with the spatially restricted sibling vole population, and efficient risk management could be achieved by limiting recreational use of the vole habitat.

Acknowledgments

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Dr Fuglei is a scientist at the Norwegian Polar Institute. Her interests include terrestrial Arctic ecosystem processes.

Table. Results of coproantigen ELISA tests of arctic fox feces for *Echinococcus multilocularis**

		2000				2004			
Area	Distance, km†	n	No. positive	Proportion positive	95% CI	n	No. positive	Proportion positive	95% CI
Grumant	0	35	7	0.20	0.10–0.36	224	135	0.60	0.54–0.66
Bjørndalen	2–6	13	0	0	0–0.23	9	0	0	0–0.30
Nordenskiöld Land	6–40	91	0	0	0–0.04	74	0	0	0–0.05
Distant	110–130	0	0	0	NA	27	2	0.07	0.02–0.23

*Samples were collected in 2000 and 2004 from Grumant to Lille Bjørndalen (Grumant), from the neighboring valley Bjørndalen (Bjørndalen), from an extended area on Nordenskiöld Land peninsula (Nordenskiöld Land); and from the Ny–Ålesund and Hornsund area (Distant). CI, confidence interval.
†Approximate distance from the Grumant area.

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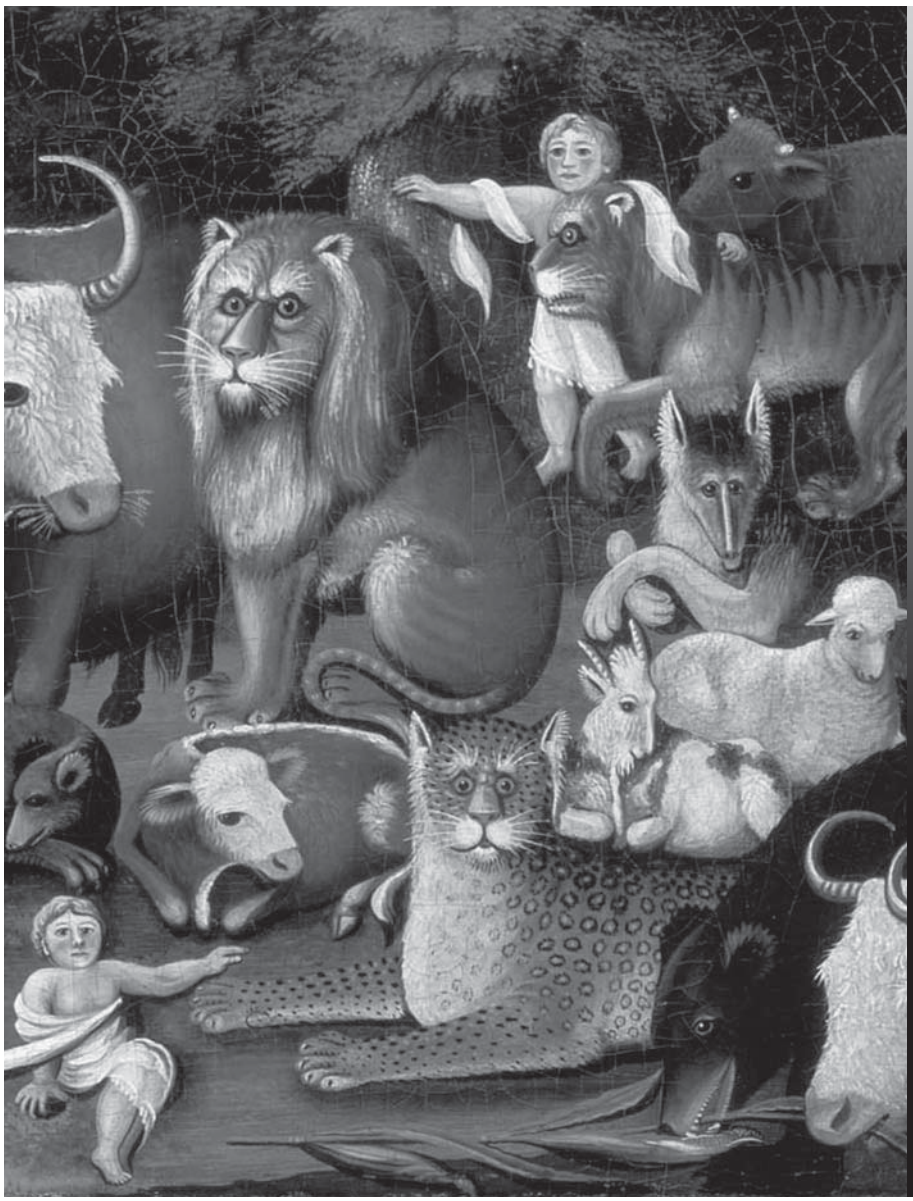

Population-based Survey of Invasive Bacterial Diseases, Greenland, 1995–2004

Annette Meyer,* Karin Ladefoged,† Peter Poulsen,† and Anders Koch*

Invasive bacterial disease occurs frequently among native populations in the Arctic. Although a variety of bacteria are involved in invasive bacterial disease in Greenland, *Streptococcus pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, and other staphylococci are responsible for most cases (69%); incidence varies according to region and ethnicity.

The incidence of invasive disease caused by *Streptococcus pneumoniae* and several other bacteria is markedly higher in the Inuit populations of Alaska, Canada, and Greenland than in the non-Inuit populations of the same areas (*1*–*3*). The clinical extent of invasive disease caused by *S. pneumoniae* is severe; sequelae and case-fatality rates are almost 4× higher in native than in nonnative populations (*1*,*4*). As living conditions in Arctic populations in many ways are comparable, an international, cooperative, population-based surveillance for invasive diseases was established in 1999 (*5*). The International Circumpolar Surveillance (ICS) network registers invasive disease caused by *S. pneumoniae*, *Haemophilus influenzae*, *Neisseria meningitidis*, group A streptococcus, and group B streptococcus in Alaska, Canada, Greenland, Iceland, northern Norway, Finland, and Sweden (*5*).

In Greenland, case data for this network are reported from the treating doctors to the chief medical officer of Greenland, who in turn reports to the ICS. However, the full picture of invasive bacterial disease in Greenland and the relative extent of disease caused by the 5 bacteria on which the ICS focuses have been unknown. Using laboratory data for a 10-year period in Greenland, we determined microbiologic causes of all invasive bacterial disease cases, microbial-specific trends in incidence over time, and variation in incidence by ethnicity and place of residency.

The Study

Greenland is inhabited by 56,000 persons. One fourth of the population lives in the capital, Nuuk, while the rest live in 16 towns and several settlements (the districts) scattered around the island. Most persons (86%) are native Greenlanders; the rest are Caucasians, mostly Danes.

The laboratory at Queen Ingrids Hospital in Nuuk is the only microbiologic laboratory in Greenland. From laboratory files, we identified all bacterial isolates from invasive disease cases reported from January 1, 1995, though December 31, 2004. Demographic information about patients was obtained from the Civil Registration System of Greenland (*6*). Patients were categorized as Greenlanders if born in Greenland. Population data for incidence calculations were obtained from Statistics Greenland (*7*). The study was approved by the Commission for Scientific Research in Greenland and reported to the Danish Data Protection Agency.

The laboratory used standard microbiologic methods. Until 1999, positive blood cultures were detected by using manual readings of blood culture bottles from Statens Serum Institut, Copenhagen, Denmark; beginning in 1999, the Bactec/Alert system (Organon Teknika, Turnhout, Belgium) was used. In this system, 28–30 mL of blood is injected directly into aerobic and anaerobic blood culture bottles. Positive samples are characterized by Gram stain and cultured on blood agar supplemented with 5% defibrinated horse blood. Antigen/antibody tests are carried out for *S. pneumoniae*, *N. meningitidis*, and *H. influenzae* (the last 2 only on cerebrospinal fluid). Serotyping and group typing of *S. pneumoniae and H. influenzae* (Quellung method) and *N. meningitidis* (latex agglutination method) are performed at Statens Serum Institut.

In total, 281 unique bacterial isolates from 254 episodes of invasive disease among 242 patients were identified: 72% of isolates in blood, 18% in cerebrospinal fluid, and 10% in other samples. Of the 254 episodes, 47% occurred among patients from the districts and 53% in patients from Nuuk. In 53% of episodes among patients from the districts, the first microbiologic sample was taken after patient transfer to Nuuk. There were no differences in age, sex, ethnicity, underlying conditions, or mortality rates between district and Nuuk patients, but Greenlandic patients were younger (median 46 years, 25%–75% quartiles 17–59 years) than Danish patients (median 57 years, 25%–75% quartiles 44–62 years) ($p = 0.07$).

Twenty different bacterial species or groups were identified (22 isolates could not be characterized by species); the most numerous were *S. pneumoniae,* staphylococci, and *E. coli* (Table). For most bacteria, the invasive disease incidence varied by age in a U-shaped fashion (Figure 1). Most *N. meningitidis* cases (73%) occurred in children <10 years of age, the 4 cases of *H. influenzae* group B infection occurred in children <1 year of age, and the youngest case-patient with GBS was 12 years of age.

Incidence of invasive bacterial disease did not differ

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according to patient sex but did differ markedly according to ethnicity and region. The age-adjusted rate ratio was 1.8 (95% confidence interval [CI] 1.1–2.9) for Greenlanders compared with Danes and 3.5 (95% CI 2.7–4.4) for persons living in Nuuk compared with persons living in the districts, a rate ratio observed for almost all bacterial species.

The overall incidence of invasive bacterial isolates increased during the study period from 17.9/100,000 in

Table. Characteristics of 281 bacterial isolates and 242 case-patients from 254 episodes of invasive bacterial infection, Greenland, 1995–2004

Bacterial class	No. isolates	Median age, y (range)	Overall incidence*	Incidence* by sex		Incidence* by ethnicity (place of birth)		Incidence* by region (place of living)	
				M (n = 129)	F (n = 125)	Greenland (n = 235)	Denmark/ other (n = 18)	Nuuk (n = 134)	Districts (n = 120)
Streptococcus spp.									
S. pneumoniae†	92	46 (0–76)	16.4	15.7	17.2	17.6	8.0	39.9	8.7
Group A streptococci	5	58 (37–76)	0.9	1.3	0.4	1.0	0	0.7	0.9
Group B streptococci	5	43 (12–70)	0.9	1.0	0.8	1.0	0	2.9	0.2
Other‡	10	38 (6–67)	1.8	2.7	0.8	1.8	1.6	5.1	0.7
Staphylococcus spp.									
S. aureus§	35	50 (0–90)	6.2	4.7	8.0	6.5	4.8	13.8	3.8
Other¶	22	41 (0–70)	3.9	4.7	3.1	4.1	3.2	7.3	2.8
Enterococcus faecalis	8	45 (0–72)	1.4	2.0	0.8	1.4	1.6	2.9	0.9
Neisseria meningitidis#	15	5 (0–54)	2.7	2.3	3.1	3.0	0	5.8	1.6
Moraxella catarrhalis	2	18 (0–36)	0.4	0	0.8	0.4	0	0.7	0.2
Haemophilus influenzae									
Type b	4	0 (0–0)	0.7	0.7	0.8	0.8	0	0.7	0.7
Non-b	10	22 (0–71)	1.8	1.3	2.3	2.0	0	2.9	1.4
Pseudomonas aeruginosa	2	45 (36–55)	0.4	0.7	0	0.4	0	1.5	0
Enterobacteriaceae spp.									
Escherichia coli	44	58 (0–84)	7.8	7.7	8.0	7.9	8.0	14.5	5.7
Klebsiella pneumoniae	6	67 (22–74)	1.1	1.0	1.1	1.0	1.6	2.9	0.5
Salmonella spp.	5	42 (12–51)	0.9	1.0	0.8	0.6	3.2	2.2	0.5
Enterobacter cloacae	1	72	0.2	0.3	0	0.2	0	0	0.2
Other									
Gram-positive cocci**	5	46 (4–65)	0.9	0.7	1.1	1.0	0	1.5	0.7
Gram-positive rods††	7	58 (13–72)	1.2	1.3	1.1	1.4	0	2.2	0.9
Gram-negative rods‡‡	3	54 (13–72)	0.5	0	1.1	0.6	0	0.7	0.5
Total	281	47 (0–90)	50.0	49.0	51.1	52.9	32.1	108.1	31.1

*Per 100,000 population.
†Most numerous serotypes were 12F (9) and 22F (5); 41 isolates not were serotyped. Serotype distribution for 1996–2002 described in detail in (*1*).
‡Other hemolytic and nonhemolytic streptococci.
§None of the *S. aureus* isolates were methicillin-resistant strains.
¶*S. epidermidis* (6) and other non–*S. aureus* staphylococci.
#Two isolates in group B, 4 in group C, and 9 not grouped.
**S. mitis* (1) and unspecified cocci.
††*Bacillus* sp. (1), corynebacterium (1), *Clostridium perfringens* (2), *Listeria monocytogenes* (1), and *Propionebacterium* sp. (1).
‡‡*Bacteroides* sp. (1) and unspecified rods.

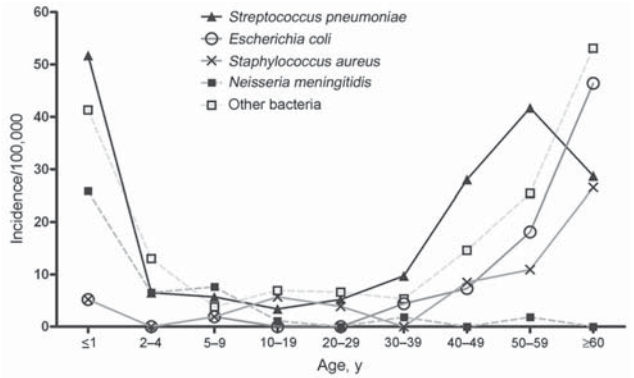

Figure 1. Incidence by age, invasive bacterial disease, Greenland, 1995–2004.

1995 to 79/100,000 in 2004; the most marked increase occurred in 1997–1998, when incidence almost doubled. The increase occurred mainly in blood culture samples; cerebrospinal fluid samples remained constant over time. The increase in isolates from blood cultures occurred equally in Nuuk and in the districts and for most bacteria, although the incidence of *N. meningitidis* remained constant (Figure 2). *H. influenzae* group B isolates were not identified after 1998 (childhood vaccination was introduced in 1997); the 5 GAS isolates first appeared in 2001–2004.

Conclusions

A variety of bacteria were found to be associated with invasive disease in Greenland. *S. pneumoniae* was the most commonly involved, confirming the importance of this pathogen in native populations in the Arctic (*1*–*3*). However, the second and third most frequent invasive bacteria were *E. coli* and *S. aureus*, a finding which, to our knowledge, had not been described before in Greenland. In contrast, the 5 bacteria included in the Circumpolar Surveillance System accounted for only 47% of microorganisms (*5*). Surveillance of these 5 bacteria is relevant, though, as they are all serious, potentially fatal diseases of infants or children and are associated with some form of preventive action (either vaccines or prophylaxis). *S. aureus* and *E. coli* are not amenable to simple specific public health actions, so surveillance for these infections is less useful. Evaluation of outcome and risk factors for *S. aureus* and *E. coli* in Arctic areas may lead to potential preventive or clinical care options.

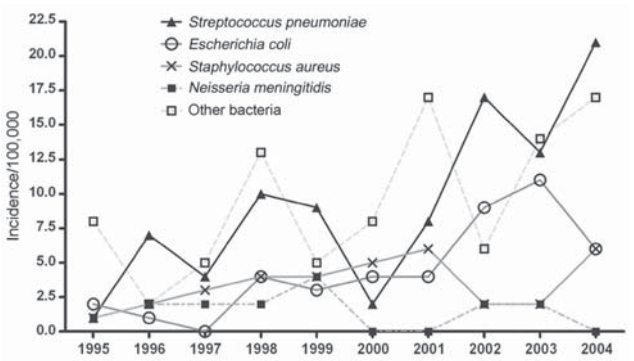

Figure 2. Incidence by year, invasive bacterial disease, Greenland, 1995–2004.

Worldwide, microbiologic causes of bacteremia have shifted from gram-negative organisms being the most common in the 1970s to gram-positive organisms with coagulase-negative staphylococci, *S. aureus*, and enterococci being the most frequent in the last part of the 20th century (*8*). Likewise, we found gram-positive bacteria in 67% of invasive disease cases and gram-negative bacteria (mostly Enterobacteriaceae) in 33% and increasing incidence of both gram-positive and gram-negative bacteria over the study period.

The overall incidence of invasive bacterial disease was 3.5-fold higher among Nuuk patients than among district patients. Most likely this finding represents sampling bias; microorganisms sent from the districts are less likely to survive than samples taken in Nuuk, and district doctors may be less likely to submit samples than Nuuk doctors, given the longer time to receive test results. However, we cannot confirm this explanation because of lack of information on the total number of submitted samples. Furthermore, the 39% higher average income in Nuuk compared with the rest of Greenland makes a Nuuk/district invasive disease incidence rate ratio of 3.5:1 less likely (*7*). The difference suggests that the true invasive disease rates in Greenland may be much higher than those found in this study or reported to ICS. A clear recommendation from this study is to develop a more accessible, rapid, and reliable diagnostic system for districts outside of Nuuk.

The lower age-adjusted invasive bacterial disease incidence in Danes than Greenlanders may be explained by the "healthy worker effect." Danes in general come to Greenland to work and belong to higher social classes and live in better housing conditions than the general population of Greenland, factors that may reduce the risk for infection (*9*). Hence, although genetic factors may account for some of the difference, the difference is probably confounded by environmental and social factors.

The increase in invasive bacterial isolates during the study period most likely represents changes in submission or detection practices. The increase occurred almost exclusively in blood cultures, and a new blood culture detection system was introduced in 1999. Similar findings have been observed in Europe (*10*). These data may lead to improved surveillance of invasive bacterial disease in Greenland.

Acknowledgments

We thank Thomas Hjuler and Nina Nielsen for assistance in designing the study.

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Molecular Epidemiology of Dengue Virus Strains from Finnish Travelers

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We characterized 11 dengue virus (DENV) isolates obtained from Finnish travelers during 2000–2005 using monoclonal antibodies and phylogenetic analysis. The analysis of DENV isolated from travelers contributes to the global picture of strain distribution and circulation. The isolates included all serotypes, including a DENV-2 isolate from Ghana.

Dengue viruses (DENV 1–4) are mosquito-borne members of the family Flaviviridae, genus *Flavivirus*. Dengue is regarded as the most significant arboviral disease in the world. Disease incidence and prevalence are rising in dengue-endemic areas, and travelers are increasingly affected. The disease can vary from asymptomatic to febrile disease, classic dengue fever, or complications such as dengue hemorrhagic fever or dengue shock syndrome. Several virus- and host-specific factors have been suggested to correlate with severe disease outcomes, which are mostly associated with secondary infections (*1*). These outcomes are not common in European travelers, and deaths are rare (*2*). In recent years, the number of annually diagnosed cases has increased in Finland from an average of 10 to >20 in 2006 (Huhtamo et al., unpub. data). In the present study, samples collected during 1999–2005 were studied by virus isolation. Virus isolates were not obtained from year 1999 samples; all isolates obtained from these samples were from the years 2000–2005.

The Study

Patients returning from dengue-endemic areas with fever and other symptoms compatible with dengue were treated mainly at university hospitals in Finland. Because of clinical suspicion, serum samples were tested for antibodies to DENV at Helsinki University Central Hospital Laboratory. The diagnosis was based on detection of immunoglobulin (Ig) M in the acute- or convalescent-phase sample or on a 4-fold IgG titer rise in paired serum specimens in an in-house IgG immunofluorescence assay (IFA), and IgM-enzyme immunoassay (Focus Technologies, Cypress, CA, USA). For this study, serum specimens from all patients were aliquoted and stored at –70ºC.

From patients with dengue diagnosis, acute-phase serum specimens with IgG titers ≤320 (IFA) were chosen for virus isolation (n = 40). Virus isolations were done simultaneously in 2 cell lines: in Vero E6 cells (ATCC CRL-1586) grown in minimal essential medium at 37°C and 5% CO_2, and in C6/36 *Aedes albopictus* cells (ATCC CRL-1660) grown in Leibowitch L-15 medium at room temperature. Cells in 25-cm^2 flasks were incubated with 50 μL of patient serum for 1 hour and observed for 24 days for cytopathic effects (CPEs). When CPEs were evident, cells were harvested for IFA, and RNA was extracted from supernatants for reverse transcriptase–PCR (RT-PCR). In the absence of CPEs, cells were subcultured after 7 days into 75-cm^2 culture flasks and studied by IFA on days 7 and 24.

In IFA, the cells were stained with a DENV-positive serum and DENV–type-specific monoclonal antibodies (MAbs) (*3*). RNA was extracted from IFA- or CPE-positive culture supernatants with a Viral RNA Mini Kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions. RT-PCR targeting the capsid–premembrane (C-preM) region was performed using DENV-specific primers (*4*), Expand reverse transcriptase (Roche, Basel, Switzerland) and Taq DNA polymerase (Fermentas, Glen Burnie, MD, USA).

A total of 11 DENV strains were isolated from different geographic locations, including the 4 serotypes (DENV-1, n = 4; DENV-2, n = 2; DENV-3, n = 3; DENV-4, n = 2; Table). The serum samples yielding virus isolates were drawn within 1 week after onset of symptoms, which included fever, headache, muscular pain, rash, and nausea. Most of these samples were positive for antibodies to DENV (IgM positive, n = 8; IgG positive, n = 5).

Isolates were either strains that grew in both of the tested cell lines (n = 6) or strains that grew only in C6/36 cells (n = 5). Two of the DENV-3 isolates (2 and 7) were detectable considerably earlier in Vero E6 than in C6/36 cells. DENV-1 isolates showed 2 distinct growth patterns; isolates 4 and 8 grew only in C6/36 cells, and isolates 3 and 11 grew in both tested cell lines (Table).

All isolates were successfully serotyped with the RT-PCR of Lanciotti et al. (*4*), in agreement with results of the MAb IFA. However, isolate 3 (DENV-1) had particular properties in type-specific MAb IFA, depending on the cell type because it showed positive results in infected C6/36 cells and negative results in infected VE6 cells.

First-round RT-PCR amplicons were purified by using ExoSAP-IT (US Biochemicals, Cleveland, OH, USA),

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Table. Dengue virus isolates from Finnish travelers, 2000–2005*

Virus serotype	Patient travel history	Year	Isolate/ case no.	Strain designation (GenBank accession no.)	IFA screening of infected cells†				Isolation serum antibody status			Patient sex/ age, y
					Vero E6		C6/36					
					dpi	Pos	dpi	Pos	dpo	IgM	IgG	
DENV-1	Thailand	2002	3	F9.D1.02 (EU005250)	7	+++	7	++	5	–	<10	F/23
DENV-1	Malaysia/ Thailand	2002	4	F12.D1.02 (EU005249)	–	–	24	+	7	+	20	F/43
DENV-1	Thailand	2005	8	F31.D1.05 (EU005248)	–	–	24	+	7	+	<10	F/56
DENV-1	India	2005	11	F37.D1.05 (EU005247)	5	+++	5	+++	3	NA	NA	M/31
DENV-2	Sri Lanka	2003	6	F18.D2.03 (EU005252)	–	–	24	+	5	+	320	M/54
DENV-2	Ghana	2005	9	F32.D2.05 (C-preM, EU005251; E, EU005258)	7	+++	7	+	2	+	<10	F/22
DENV-3	Cuba	2002	2	F7.D3.02 (EU005253)	7	+	24	+	6	+	20	M/55
DENV-3	Brazil	2003	5	F13.D3.03 (EU005254)	–	–	24	+	5	+	<20	M/26
DENV-3	Sri Lanka	2004	7	F24.D3.04 (EU005255)	4	+++	24	+	2	–	20	F/39
DENV-4	Sri Lanka	2000	1	F2.D4.00 (EU005256)	10	+++	10	+	4	+	<10	F/42
DENV-4	Indonesia	2005	10	F34.D4.05 (EU005257)	–	–	24	+	5	+	20	M/37

*IFA, immunofluoresence assay; dpi, day postinfection; Pos, proportion of positive cells; dpo, day post-onset; Ig, immunoglobulin; DENV, dengue virus; NA, not available; c-preM, capsid–premembrane; E, envelope.
† +, ++, and +++ indicate the relative amount of positive cells observed in IFA using fluorescence microscope: individual positive cells observed <10% (+), 10–50% of the cells positive (++), 50%–100% of the cells positive (+++). –, not detected.

and directly sequenced. When necessary, the envelope gene was amplified using previously described primers (*5*) and sequenced. Nucleotide sequences of the isolates were aligned with published DENV sequences from GenBank (online Appendix Table, available from www.cdc.gov/EID/content/14/1/80-appT.htm) using ClustalW (www.ebi.ac.uk/tools/clustalw). Phylogenetic analysis was performed by the neighbor-joining method with a Kimura 2-parameter model using MEGA3 software version 3.1 (*6*).

Phylogenetic analyses (Figure 1) showed that isolates 3, 4, and 8 (DENV-1) clustered with Asiatic DENV-1 strains of genotype I (*7*), which corresponded with the patients' travel history. Isolate 11 (DENV-1) from India clustered with a genotype III strain isolated a year earlier from the Seychelles. Isolate 6 (DENV-2), obtained from Sri Lanka in 2003, clustered with a strain isolated in the same year from India. Unlike the other isolates, isolate 9 (DENV-2), obtained in Ghana in 2005, did not group with any of the representative strains of the C-preM region, for which no African sequences were available in GenBank. The additionally studied envelope gene sequence grouped with previous African isolates of the cosmopolitan genotype (*8*) (Figure 2).

The DENV-3 isolates represented genotype III (*9*) (Figure 1). Isolate 2 from Cuba clustered with strains from Martinique in agreement with previous data on Cuban strains (*10*). Isolate 7 (DENV-3), obtained in Sri Lanka in 2004, clustered with strains from Singapore, Sri Lanka, and Taiwan. Isolate 5 was identical in sequence to a strain isolated 1 year earlier from a patient in Brazil who died (*11*). DENV-4 isolates represented 2 different genotypes; isolate 1 from Sri Lanka clustered with genotype I strains, and isolate 10 from Indonesia clustered with genotype II (*12*).

Conclusions

Studies on imported DENV have provided interesting insights to the global picture of circulating strains (*13*,*14*), and also have led to the discovery of novel DENV strains and lineages (*15*,*16*). In this study, we characterized 11 strains of DENV isolated from Finnish travelers in 2000–2005 and provided new information about strains circulating in India, Sri Lanka, and Ghana.

Previous studies have shown that DENV isolation is possible when antibody levels are low (*17*). However, in this study, most samples yielding virus isolates were antibody positive. The patients had primary infections, except for 1 patient, who had an IgG titer of 320 in the acute phase, which is suggestive of a secondary infection. This was the only patient with any bleeding symptoms, i.e., prolonged bleeding from the venopuncture site.

Virus isolates from Finnish travelers were heterogeneous. All patients had dengue fever, including the patient

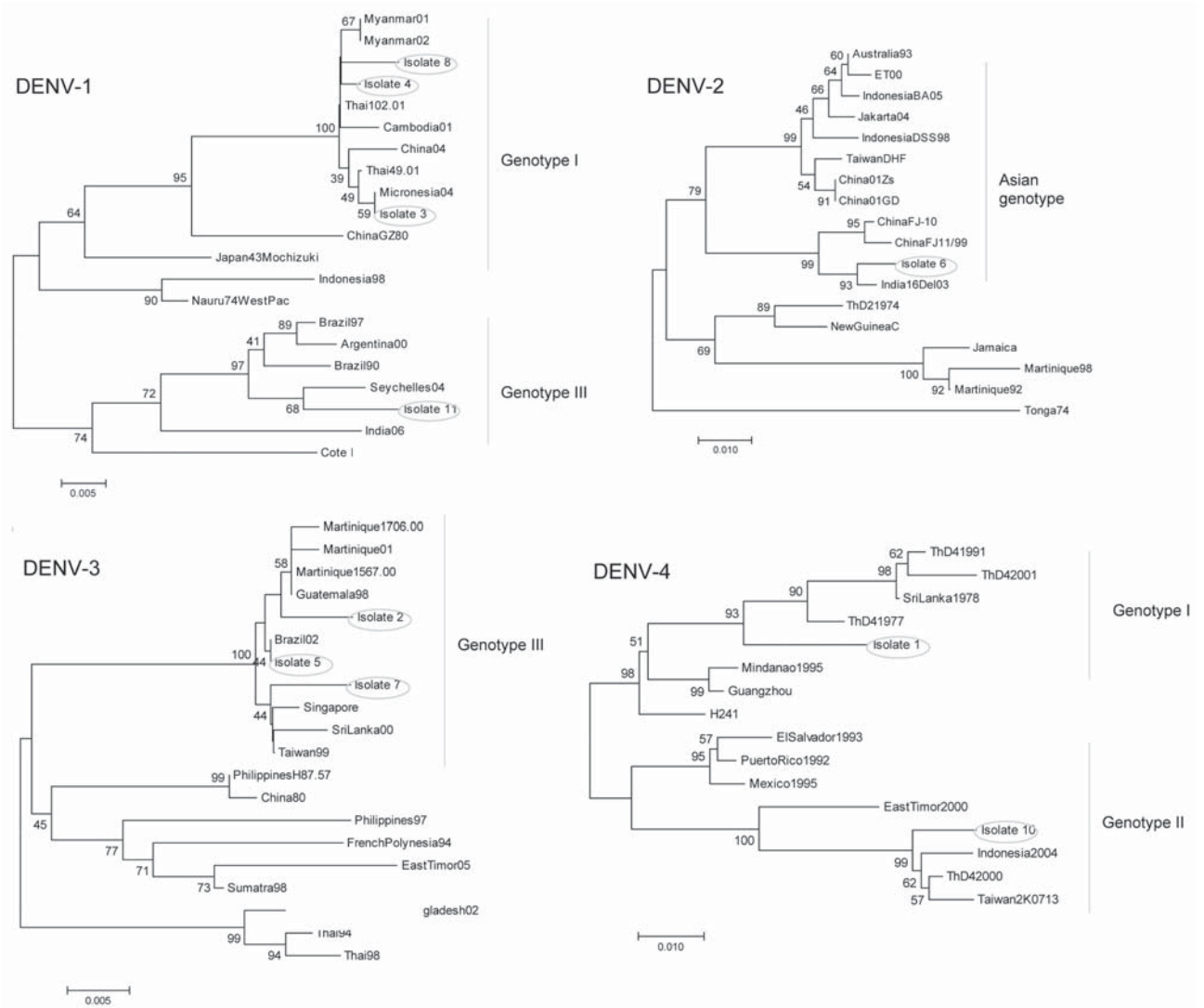

Figure 1. Neighbor-joining phylogenetic trees of the 4 dengue virus (DENV) serotypes based on the 454-bp capsid–premembrane (C-preM) region sequences obtained from first-round amplicons (*6*). Isolates described in this study are in circled. Bars represent nucleotide substitutions/site.

whose isolate was identical in sequence to a strain isolated from a patient who had died. Since the disease outcomes of the patients were uneventful, no associations could be made between the infective virus serotype or strain and disease severity.

Both mammalian and mosquito cells were used in virus isolation, which enabled the detection of other flaviviruses that may have caused seropositivity through cross-reaction. All DENV isolates grew in C6/36 mosquito cells; however, use of 2 cell lines showed variation in the growth patterns of the isolates in different cell types. We observed that some DENV-3 strains were detectable earlier in mammalian Vero E6 cells than in C6/36 cells, which suggested a different capability to infect these cells. This property could not be associated with pathogenicity in this study; thus, the biologic relevance of this phenomenon is unknown.

The DENV type-specific MAb IFA showed that one of the DENV-1 isolates (isolate 3) had distinct antigenic properties when cultured in mammalian or mosquito cells. Whether this strain represents MAb-escape properties requires further studies.

The phylogenetic grouping of the isolates was consistent with the travel history of the patients in most cases. However, isolate 11 (DENV-1) from India clustered with a genotype III strain isolated a year earlier from the Seychelles, which suggested strain transfer between these countries.

Phylogenetic analysis of isolate 9 (Ghana 2005) showed that it could be grouped with other African isolates

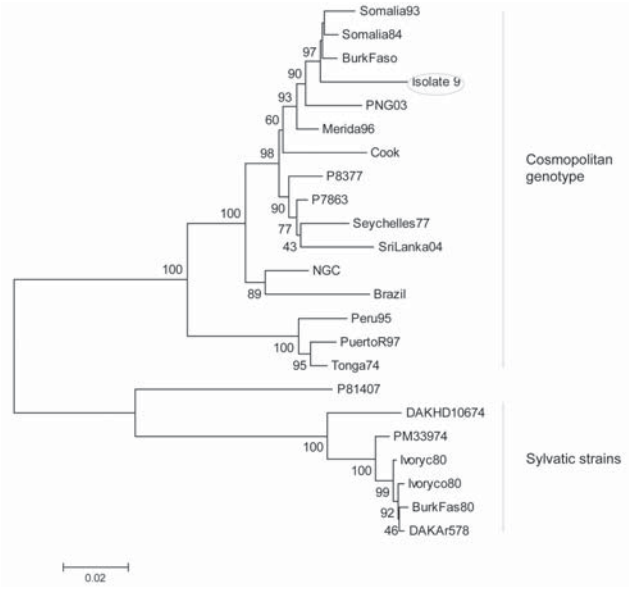

Figure 2. Neighbor-joining phylogenetic tree of dengue virus type 2 (DENV-2) based on the envelope gene sequence (1,485 bp). Isolate 9 from Ghana is circled. Bar represents nucleotide substitutions/site.

of the cosmopolitan genotype (Figure 2). To our knowledge, this is the first DENV-2 strain characterized from Ghana (the geographically nearest isolate is from Burkina Faso in 1983). This grouping demonstrates sustained circulation of DENV-2 strains in Africa for decades.

The 11 DENV isolates represent a random sample from different geographic locations. Three strains were isolated from travelers returning from Sri Lanka, first in 2000 (DENV-4), followed by isolates in 2003 (DENV-2) and 2004 (DENV-3). These strains demonstrate extensive DENV serotype cocirculation.

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Wild Bird Influenza Survey, Canada, 2005

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Of 4,268 wild ducks sampled in Canada in 2005, real-time reverse transcriptase–PCR detected influenza A matrix protein (M1) gene sequence in 37% and H5 gene sequence in 5%. Mallards accounted for 61% of samples, 73% of M1-positive ducks, and 90% of H5-positive ducks. Ducks hatched in 2005 accounted for 80% of the sample.

To provide baseline information about the strains and distribution of influenza viruses in Canadian wild ducks and to respond to the emergence of highly pathogenic avian influenza (HPAI) type H5N1 in Asia, Europe, and Africa, Canada's Interagency Wild Bird Influenza Survey was initiated in July 2005. The goals of the survey were to identify avian influenza viruses in wild ducks in Canada and to detect HPAI strains early (*1*). We report the results of real-time reverse transcriptase–PCR (RRT-PCR) analysis.

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The Study

Single cloacal swabs were collected from apparently healthy ducks at 56 sites within 6 geographic regions: British Columbia, Alberta, Manitoba, Ontario, Québec, and the Atlantic provinces (New Brunswick, Nova Scotia, Prince Edward Island, and Newfoundland and Labrador) (Figure). The target number of samples for each region was 800: 500 from mallard ducks (*Anas platyrhynchos*) and 300 from other duck species. Ducks were trapped and handled as part of annual duck banding carried out by the Canadian Wildlife Service and its associates. They were either caught in baited traps or netted from air boats. In all regions, duck banders were asked to preferentially sample birds hatched in 2005 to maximize virus detection within the sample (*2–4*).

Swabs were immediately placed in virus transport medium, refrigerated for up to 3 days, and then frozen at ≤–20°C until tested. The transport medium comprised Hanks balanced salt solution supplemented with 10% glycerol, 200 U/mL penicillin, 200 μg/mL streptomycin, 100 U/mL polymyxin B sulfate, 250 μg/mL gentamicin, and 50 U/mL nystatin.

All samples were tested at regional veterinary diagnostic laboratories within Canada's Influenza Virus Laboratory Network by RRT-PCR, which targets a conserved region of the M1 gene within influenza A segment 7. If the M1 gene sequence was detected, RRT-PCR for H5 and H7 hemagglutinin gene segments was performed. All laboratories followed uniform procedures (*5*) with positive and negative controls, and quality assurance was provided by the National Centre for Foreign Animal Disease (Canadian Food Inspection Agency).

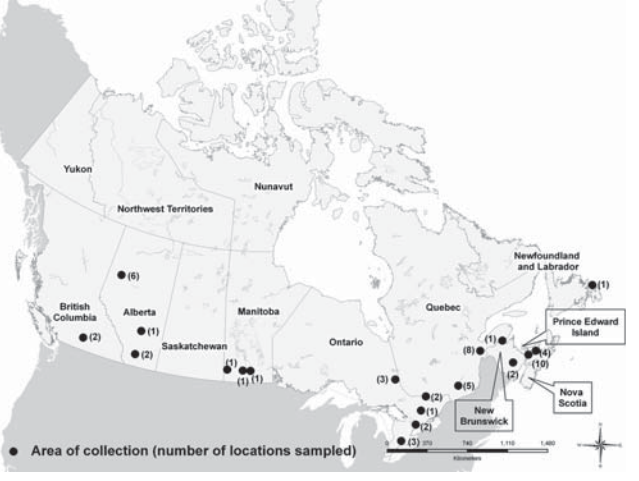

Figure. Live bird survey sample site locations.

All field and laboratory data were entered directly into a national database developed and maintained by the Canadian Cooperative Wildlife Health Centre. Duplicate records and any sample records missing RRT-PCR results or field collection data (species, age, sex, location) were removed from this analysis. Data were exported to Microsoft Excel 2003 (Microsoft Corp., Redmond, WA, USA) and prepared for analysis. Statistical analysis was performed with EpiInfo version 3.3.2 (Centers for Disease Control and Prevention, Atlanta, GA, USA) and SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). Maps were generated in ArcGIS 9.0–ArcMap version 9.1 (ESRI, Redlands, CA, USA).

To assess differences in M1 and H5 virus detection by age class and species, main effects logistic regression models were constructed by using a manual stepwise procedure. Age class, species, sampling area, and sex were included in the model. Age class was categorized into 2005 hatch-year birds and birds from another hatch year. Species was categorized into mallard (mallard and mallard–American black duck [*A. rubripes*] hybrids), other dabbling duck species, diving ducks, and other tribes (Table 1). All variables were assessed for potential confounding; variables that changed estimates by >20% were left in the main effects models as confounders (*6*).

Conclusions

A total of 4,268 valid sample records were available for this analysis; 37% (1,572) of ducks were M1-positive and 5% (208) were H5-positive by RRT-PCR. No samples tested positive for the H7 gene sequence. Sampled areas varied considerably in the proportion of M1-positive samples, ranging from 63% (348/556) in southern Quebec to 9% (22/254) in southern Alberta (Table 2). Three percent (138) of samples were collected in July, 83% in August (3,539), 11% (454) in September, 1% (59) in October, none in November, and 2% (73) in December. Of all samples, 80% (3,401/4,268) were collected from ducks hatched in 2005. Hatch-year ducks accounted for 88% (1,388/1,572) of M1-positive and 99% (205/208) of H5-positive samples. Of all ducks sampled, 90% (3,824/4,268) were dabbling ducks (tribe Anatini) (Table 1). Mallards (including mallard–American black duck hybrids) accounted for 61% (2,600/4,268) of all ducks sampled, 73% (1,148/1,572) of all M1-positive samples, and 90% (187/208) of all H5-positive samples. The duck species with the highest proportion of M1-positive samples was the wood duck (*Aix sponsa*) (68%, 71/104).

Age, area sampled, and species were included in the final logistic regression model for M1 RRT-PCR test results. Age, sex, and species were included in the final model for H5 RRT-PCR results. Mallards were more likely to be M1-positive than other dabbling ducks or diving ducks but not more likely to be M1-positive than other duck tribes (Cairinini and Oxyurini). Mallards were more likely to be H5-positive than other dabbling ducks, diving ducks, or other duck tribes. Hatch-year ducks were 1.7 times more likely to be M1-positive and 13 times more likely to be H5-positive than older ducks. Male ducks were 1.4 times more likely to test positive by H5 RRT-PCR than female ducks.

These data provided a snapshot of the frequency and distribution of influenza A viruses in wild ducks across southern Canada in 2005. This unique nationwide snapshot showed wide variation in detection of M1 and H5 gene sequences among 6 regions that were broadly representative of the northern terminus of duck migration corridors in

Table 1. Duck species sampled in 2005 and RRT-PCR results, Canada*

Common name	Taxonomic name	No. sampled	No. M1-positive (%)	No. H5-positive (%)
Mallard†	*Anas platyrhynchos*	2,600	1148 (44)	187 (7)
American black duck†	*A. rubripes*	293	99 (34)	2 (1)
American wigeon†	*A. americana*	101	33 (33)	0
Blue-winged teal†	*A. discors*	431	105 (24)	2
Cinnamon teal†	*A. cyanoptera*	4	1 (25)	0
Gadwall†	*A. strepera*	36	1 (3)	0
Green-winged teal†	*A. crecca*	224	52 (23)	5 (2)
Northern pintail†	*A. acuta*	131	26 (20)	4 (3)
Northern shoveler†	*A. clypeata*	4	0	0
Wood duck‡	*Aix sponsa*	104	71 (68)	2 (2)
Common goldeneye§	*Bucephala clangula*	18	2 (11)	0
Canvasback§	*Aythya valisineria*	19	0	0
Hooded merganser§	*Lophodytes cucullatus*	26	8 (31)	0
Lesser scaup§	*Aythya affinis*	1	0	0
Redhead§	*Aythya americana*	223	18 (8)	6 (3)
Ring-necked duck§	*Aythya collaris*	51	8 (16)	0
Ruddy duck¶	*Oxyura jamaicensis*	2	0	0

*RRT-PCR, real-time reverse transcriptase–PCR.
†Dabbling duck (Tribe Anatini).
‡Perching duck (Tribe Cairinini).
§Diving duck (Tribe Aythini and Tribe Mergini).
¶Ruddy duck (Tribe Oxyurini).

Table 2. Sample sizes and RRT-PCR results for each sampling area included in the 2005 wild duck survey for influenza A viruses, Canada*

Region/area (no. sites sampled)	No. valid samples	No. M1 RRT-PCR positive (%)	No. H5 RRT-PCR positive (%)†
British Columbia interior (2)	640	351 (55)	161 (25)
Alberta			
Northern (6)	260	30 (12)	0
Central (1)	262	25 (10)	0
Southern (2)	254	22 (9)	0
Manitoba			
Western (1)	175	21 (12)	0
Central (1)	174	25 (10)	3 (2)
Eastern (1)	175	48 (27)	1 (1)
Ontario			
Southwestern (3)	269	135 (50)	3 (1)
Southcentral (2)	23	11 (49)	1 (4)
Central (1)	48	24 (50)	0
Eastern (2)	144	103 (72)	3 (2)
Northern (3)	284	66 (23)	2 (1)
Québec			
Southern (5)	556	348 (63)	28 (5)
Eastern (8)	221	32 (14)	0
Atlantic provinces‡			
Northern New Brunswick (1)	15	6 (40)	0
Central New Brunswick (2)	20	9 (20)	0
New Brunswick–Nova Scotia border (10)	646	290 (45)	6 (1)
Prince Edward Island (4)	21	13 (62)	0
Newfoundland and Labrador (1)	73	8 (11)	0
Total	4,268§	1,572 (37)	208 (5)

*RRT-PCR, real-time reverse transcriptase–PCR.
†All M1-positive samples were subsequently tested for H5.
‡New Brunswick, Nova Scotia, Prince Edward Island, and Newfoundland and Labrador.
§The sampling area of 8 ducks was not recorded (3 from Alberta, 1 from Ontario, 4 from Québec).

North America (*7*). Previous studies have focused on smaller subregions of Canada sampled once or across multiple years (*4*,*8*–*10*). Direct comparisons with previous studies should be made cautiously. Considerable variation exists across studies in the age and species of birds sampled and seasonality of sampling. All of these variables may affect reported prevalence of infection with influenza A viruses. Also, most previously published results relied on the cultivation of viable virus as the detection method rather than on RRT-PCR applied directly to cloacal swabs.

Because samples for this survey were secured from preestablished duck-banding operations, and young healthy ducks, particularly mallards, were targeted to maximize virus recovery (*1*,*11*,*12*), these data may not represent true infection prevalence in the sampled duck populations. Despite these biases, avian influenza viruses were common in wild ducks across Canada in the summer and fall of 2005. Infection rates detected by RRT-PCR among the 6 regions of Canada were similar in scale and degree of variation to rates reported from the Alberta region, which were measured over 8 consecutive years from 1976 through 1983 (*4*).

Acknowledgments

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This survey was funded by the Canadian Food Inspection Agency, the Public Health Agency of Canada, Environment Canada, and the provinces of Alberta, British Columbia, Manitoba, New Brunswick, Newfoundland and Labrador, Nova Scotia, Ontario, Prince Edward Island, and Québec. National consensus, survey protocols and coordination, information technology, fiscal management, and data analysis were provided by the Canadian Cooperative Wildlife Health Centre.

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Influenza Virus Samples, International Law, and Global Health Diplomacy

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Indonesia's decision to withhold samples of avian influenza virus A (H5N1) from the World Health Organization for much of 2007 caused a crisis in global health. The World Health Assembly produced a resolution to try to address the crisis at its May 2007 meeting. I examine how the parties to this controversy used international law in framing and negotiating the dispute. Specifically, I analyze Indonesia's use of the international legal principle of sovereignty and its appeal to rules on the protection of biological and genetic resources found in the Convention on Biological Diversity. In addition, I consider how the International Health Regulations 2005 applied to the controversy. The incident involving Indonesia's actions with virus samples illustrates both the importance and the limitations of international law in global health diplomacy.

On May 23, 2007, the World Health Assembly (WHA) adopted a resolution on sharing influenza viruses and promoting access to vaccines in connection with pandemic influenza preparedness (*1*). This resolution constituted the latest development in a controversy sparked by Indonesia's decision to withhold influenza A (H5N1) samples from the World Health Organization (WHO) (*2*). The negotiations that produced WHA's resolution involved complex international legal questions, which stimulated different answers from the parties involved. This article reviews this controversy and analyzes key international legal issues it generated.

Indonesia's Decision to Withhold Influenza A Virus (H5N1) Samples

This controversy began toward the end of 2006, when Indonesia decided not to share influenza A virus (H5N1) samples with WHO for risk assessment (e.g., surveillance) or risk management (e.g., vaccine development) purposes. Indonesia's decision reportedly stemmed from its reaction to an Australian company's development of an avian influenza vaccine derived from a virus strain that Indonesia provided to WHO (*3*). WHO's acknowledgment that patents had been sought on modified versions of influenza (H5N1) samples shared through the Global Influenza Surveillance Network (GISN) without the consent of the countries that supplied the samples reinforced Indonesia's discontent. Indonesia argued that this incident exposed inequities in the global influenza surveillance system. Developing countries provided information and virus samples to the WHO-operated system; pharmaceutical companies in industrialized countries then obtained free access to such samples, exploited them, and patented the resulting products, which the developing countries could not afford. Avian influenza's spread and fears about pandemic influenza heightened this perceived inequity; experts argued that developing countries would have little access to vaccine for pandemic influenza without major changes in global vaccine production (*4*,*5*).

Indonesia's action alarmed the global health community. Indonesia has been hit hard by avian influenza (*6*), so its cooperation in tracking the influenza virus (H5N1) was critical. Without access to Indonesia's influenza strains, global surveillance was jeopardized, as was the refinement of diagnostic reagents and the development of intervention strategies, which depend on the information surveillance provides.

Regaining access to Indonesia's samples motivated WHO to try to find a solution to the problem that Indonesia highlighted. In essence, Indonesia was making sample sharing for risk assessment dependent on action taken by WHO and industrialized countries to increase Indonesia's access to influenza vaccines derived from samples it pro-

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vided. Restarting sample sharing and improving vaccine access proved difficult and contentious. Before the WHA meeting in May 2007, negotiations between Indonesia and WHO did not produce agreement. For example, neither the Joint Statement issued by Indonesia and WHO in February 2007 (*7*) nor subsequent attempts to end the impasse succeeded (*8*). Independent efforts to increase vaccines access, such as the agreement of the United States and Japan in March 2007 to provide $18 million to 6 developing countries (Brazil, India, Indonesia, Mexico, Thailand, and Vietnam) to facilitate the building of vaccine-manufacturing capacity and of a vaccine stockpile (*9*), did not alter the stand-off.

The World Health Assembly's Resolution

Agreement at WHA was reached only through last-minute negotiations, which again illustrates the difficulties raised by Indonesia's strategy to gain better access to influenza vaccines. The WHA resolution sets out a series of actions to achieve both "the timely sharing of viruses and specimens" in GISN and the promotion of "transparent, fair and equitable sharing of the benefits arising from the generation of information, diagnostics, medicines, vaccines and other technologies" (*1*). Most of the resolution consists of requests by WHO member states for the director-general to undertake activities designed to achieve fair and equitable sharing of benefits derived from influenza surveillance activities, especially access to vaccines (Table).

Particularly important are the requests for the director-general to convene a) a working group to review, and propose reforms for, the sharing of influenza viruses and their use within and outside GISN; and b) an intergovernmental working group to consider progress being made toward the resolution's goals, especially fair and equitable access to influenza vaccine for developing countries. These requests ensure that the linkage between virus sample sharing and equitable access to influenza vaccine remains prominent on the global health agenda for the foreseeable future.

The resolution reflects the current structure of global influenza governance (*10*). International sharing of influenza virus samples has occurred for decades within GISN (*11*). Although WHO and partners, such as the United Nations Children's Fund (UNICEF) and GAVI Alliance, have increased developing-country access to childhood vaccines, mechanisms for increasing these countries' access to influenza vaccines are weaker. Fears about avian influenza's spread and the emergence of pandemic influenza highlighted the weakness of international efforts to increase vaccine availability in developing countries. The resolution attempts to build a multilateral process to address the lack of fair and equitable access for developing countries to pharmacologic benefits derived from the sharing of influenza virus samples. The resolution expresses a desire to craft a more equitable system of global influenza governance, the substantive elements of which remain to be negotiated.

Political Dynamics of Influenza Virus Samples and Sovereignty over Biological Resources

The need to improve influenza vaccine access was recognized before this controversy (*4*,*5*), but Indonesia's willingness to leverage control over virus samples to provoke more multilateral responses to the access problem changed the political dynamics of this issue. As typically happens when countries or international organizations challenge the status quo, the parties in this controversy framed their posi-

Table. Summary of actions that World Heath Organization member states requested of director-general

- To identify and propose, in consultation with member states, frameworks and mechanisms that aim to ensure fair and equitable sharing of benefits among all member states, taking strongly into consideration the specific needs of developing countries
- To establish, in consultation with member states, an international stockpile of vaccines for (H5N1) or other influenza viruses of pandemic potential
- To formulate mechanisms and guidelines, in consultation with member states, aimed at ensuring fair and equitable distribution of pandemic influenza vaccines at affordable prices in the event of a pandemic to ensure timely availability of such vaccines to member states in need
- To mobilize financial, technical, and other appropriate support from member states, vaccine manufacturers, development banks, charitable organizations, private donors, and others to implement mechanisms and increase the equitable sharing of benefits as described in the resolution
- To convene an interdisciplinary working group to revise the terms of reference of WHO Collaborating Centers, H5 Reference Laboratories, and national influenza centers, devise oversight mechanisms, formulate draft standard terms and conditions for sharing viruses between originating countries and WHO Collaborating Centers, between the latter and third parties, and to review all relevant documents for sharing influenza viruses and sequencing data, based on mutual trust, transparency, and overriding principles
- To assure a member of the interdisciplinary working group consisting of 4 member states from each of the 6 WHO regions, taking into account balanced representation between industrialized and developing countries and including both experts and policymakers
- To convene an intergovernmental meeting to consider the reports by the director-general and by the interdisciplinary working group, which shall be open to all member states and regional economic organizations
- To commission an expert report on the patent issues related to influenza viruses and their genes, and report to the intergovernmental meeting
- To continue work with member states on the potential for conversion of existing biological facilities, such as those for the production of veterinary vaccines, so as to meet the standards for development and production of human vaccines, thereby increasing the availability of pandemic vaccines, and to enable them to receive vaccine seed strains
- To report on progress on implementation of the resolution to the World Health Assembly

tions by using international law. This section analyzes how the stakeholders used international law to shape the debate. This incident illustrated the importance and limitations of international law in global health diplomacy.

By withholding samples, Indonesia asserted sovereignty over them because they originated within its territory. Despite controversies surrounding it, the principle of sovereignty remains a central tenet of international law (*12*). Traditionally, sovereignty holds that a state has authority and control over the people, resources, and activities within its territory (*12*). International law supplements sovereignty with the rule prohibiting states from intervening in each other's domestic affairs (*12*). Limits on sovereignty arise when the state agrees to follow rules of international law found in treaties or customary international law.

In essence, Indonesia claimed that the samples are its sovereign property and do not constitute resources that other countries or the international community can access and use without Indonesia's consent. This claim cut against the ethos and practice of sample sharing under which GISN had operated. This ethos and practice are based on accessing and analyzing influenza virus samples to produce accurate surveillance data, which inform development of interventions (e.g., vaccines).

Indonesia did not equate this ethos with an international legal obligation to engage in sharing that limited its sovereign rights over the samples. From a legal perspective, Indonesia's arguments were plausible. GISN was not organized under treaty law, so no countries had treaty obligations to share samples. In addition, international law on infectious diseases applicable to Indonesia when this controversy began contained no obligations to share samples with WHO. The most relevant international legal rules, the International Health Regulations (IHRs) adopted by WHO in 1969 (IHR 1969), did not include influenza as a disease subject to the Regulations, nor did IHR 1969 require sharing of biological samples for the diseases covered (*13*).

Whether sharing obligations arose under customary international law when this controversy arose is also doubtful. To rise to the level of customary law, evidence must exist that states generally and consistently follow a practice out of a sense of legal obligation (*12*). GISN has, however, functioned without much, if any, reference to international law, making it difficult to establish that countries shared samples with WHO because they felt legally obligated to do so.

Sovereignty Claims and the Application of Convention on Biological Diversity

In addition to exploiting basic principles of international law, Indonesia exploited precedents in other areas to bolster its sovereignty claims over the samples. Specifically, Indonesia borrowed from the international law developed to address biological diversity. The Convention on Biological Diversity (CBD) recognizes that countries have sovereign control of biological resources found within their territories (*14*). CBD defines biological resources to include "genetic resources, organisms or parts thereof, populations, or any other biotic component of ecosystems with actual or potential use or value for humanity" (article 2). Genetic resources are defined to mean "genetic material of actual or potential value"; genetic material means "any material of plant, animal, microbial or other origin containing functional units of heredity" (article 2). CBD further states that "the authority to determine access to genetic resources rests with the national governments and is subject to national legislation" (article 15.1). In addition, "access to genetic resources shall be subject to prior informed consent of the Contracting Party providing such resources" (article 15.5). Any access granted "shall be on mutually agreed terms" (article 15.4).

Indonesia's claims that it controlled access to samples collected in its territory, that no use of such samples by other parties could occur without its prior informed consent, and that any use of such samples should produce benefits for Indonesia reflect the approach taken in CBD. Evidence that Indonesia framed the controversy by using these principles can be found in WHA's 2007 resolution, which states that the Assembly "[r]ecogniz[es] the sovereign right of States over their biological resources" (preamble).

However, equating influenza virus samples with biological resources addressed by CBD raises questions that undermine Indonesia's use of CBD. To begin, interpreting CBD to apply to pathogenic viruses may be contrary to CBD's purpose. CBD was created, in part, to help developing countries rich in biological diversity control access to this diversity to conserve and manage it for sustainable development. Developing countries were concerned that corporate entities from industrialized countries were accessing their biological diversity and creating profitable products without the populations of these developing countries benefiting. Critics called this practice biopiracy (*15,16*).

Thus, the biological and genetic materials of primary CBD concern are indigenous resources in which governments, communities, and persons have invested time, effort, and resources to protect, cultivate, understand, and use. CBD provides that "States have sovereign rights over *their own biological resources*" (preamble [emphasis added]). In short, companies in the industrialized world were unjustly enriching themselves by profiting from previous efforts made in the developing country.

The avian influenza viruses affecting Indonesia are not the kind of biological and genetic resources that CBD sought to protect and regulate through the principles of sovereignty, prior informed consent, and mutual benefits from access and exploitation. These viruses invaded Indonesia; their presence and spread owes nothing to the investment,

nurturing, and utilization of the Indonesian government or people. Rather than seeking to conserve this virus, the strategy is to contain and ultimately eradicate it. Applying CBD's principles to influenza virus samples seems inappropriate given the difference between CBD's object and purpose and the threat posed by influenza viruses.

State practice under CBD supports the conclusion that CBD does not apply to avian influenza virus. States parties to CBD have addressed avian influenza, not as a biological resource subject to CBD but as a threat to biological diversity. CBD discussions of avian influenza have considered its potential impact on wildlife, and the CBD process emphasized that surveillance is critical for combating avian influenza's threat to biological diversity. Surveillance suffers without sharing information and samples of avian influenza viruses (*17*). Rather than protecting biological diversity, as mandated by CBD, Indonesia's withholding virus samples from global surveillance efforts jeopardizes biological diversity in addition to population health.

Using CBD as a template in the context of influenza virus samples may be questionable on other grounds (*18*). The definitions of biological resources and genetic resources emphasize that the resources in question should be of actual or potential use or value for humanity. When these definitions are read in conjunction with CBD's principles, this potential use or value for humanity is understood to derive from the protection, conservation, and sustainable use of the resources in question. CBD uses the principle of sovereignty as a regulatory instrument to achieve these goals. The use or value for humanity of influenza viruses comes from their widespread sharing for surveillance and vaccine development purposes because of the global threat such viruses pose. In this context, the principle of sovereignty central to the CBD approach is not a useful basis for facilitating timely and comprehensive sharing that global health governance requires.

Virus Sharing and the Application of IHR 2005

One reason Indonesia stressed the CBD is that it provided a way to finesse the implications of the revised IHRs adopted by WHA in May 2005 (IHR 2005) (*19*), which provide that "[t]he provisions of the IHR shall not affect the rights and obligations of any State Party deriving from other international agreements" (article 57.1). Appeal to this rule begs the question raised by the first sentence of article 57.1, which states that "the IHR and other relevant international agreements should be interpreted so as to be compatible." Thus, interpreting IHR 2005 became important in the controversy over influenza virus (H5N1) sharing. IHR 2005 is a treaty, "an international agreement concluded between States in written form and governed by international law" (*20*). This controversy represented an early test for how IHR 2005 would be interpreted and applied.

IHR 2005's use proved complex for technical and substantive reasons. Technically, IHR 2005 had no binding force under international law until it officially entered into force on June 15, 2007. Thus, IHR 2005 created no international legal obligations for Indonesia with respect to the withholding of samples in the period before the Regulations entered into force. However, IHR 2005's imminent entry into force made its substantive provisions relevant to the negotiations over Indonesia's position on virus sharing.

Under international law, a state must refrain from acts that would defeat a treaty's object and purpose when the state has expressed its consent to be bound by the treaty, pending the treaty's entry into force (*20*). Indonesia had expressed its consent to be bound by IHR 2005 because it did not reject IHR 2005, or submit reservations to it, by the December 2006 deadline to do so. Thus, whether Indonesia's decision to withhold samples constituted an act that would defeat the object and purpose of the IHR 2005 became a relevant question. Criticisms that Indonesia's action fundamentally jeopardized global health security—the very object of IHR 2005 (*21*)—demonstrate that Indonesia could be considered in violation of its duty to not defeat the object and purpose of IHR 2005 before its entry into force.

This argument is supported by the claim that had IHR 2005 actually been in force, Indonesia would have violated its obligation to share samples. WHO Director-General Margaret Chan argued at the WHA meeting in May 2007 "that countries that did not share avian influenza virus would fail the IHR" (*22*). Addressing the credibility of these legal claims requires interpreting what IHR 2005 mandates States Parties to disclose and share with WHO. At least 2 differing interpretations exist. The first interpretation argues that IHR 2005 requires States Parties to share relevant biological samples as part of the duty to provide WHO with accurate and detailed public health information about all events that might constitute a public health emergency of international concern (PHEIC). Given that the spread of highly pathogenic influenza viruses is considered a PHEIC, the IHR 2005 mandates that States Parties provide WHO with samples for surveillance purposes without preconditions or expectations of benefits in return.

Supporting this interpretation is a WHA resolution adopted in May 2006, which called upon WHO member states "to comply immediately, on a voluntary basis, with provisions of the IHR 2005 considered relevant to the risk posed by avian influenza and pandemic influenza" (para. 1) (*23*). This resolution urged WHO member states "to disseminate to WHO collaborating centres information and relevant biological materials related to highly pathogenic avian influenza and other novel influenza strains in a timely and consistent manner" (para. 4[5]). The encouragement to share biological materials with WHO could be considered authoritative guidance from WHO's highest policymaking

body about the scope of the obligation to share public health information with WHO with respect to all events that might constitute a PHEIC.

This interpretation was succinctly stated by the US delegation to WHA: "All nations have a responsibility under the revised IHRs to share data and virus samples on a timely basis and without preconditions. The United States wishes to be clear that our view is that withholding influenza viruses from GISN greatly threatens global public health and will violate the legal obligations we have all agreed to undertake through our adherence to IHRs" (*24*).

Even though IHR 2005 never expressly requires the sharing of biological samples, a good faith interpretation of IHR 2005 in light of its object and purpose acknowledges a duty to share such samples for surveillance purposes. An opposite interpretation could lead to a manifestly absurd or unreasonable result, which treaty interpretation principles do not support. This interpretation of IHR 2005 also is compatible with CBD because IHR 2005 requires sample sharing for risk assessment purposes, not risk management activities. Thus, the sharing mandate in IHR 2005 does not preclude WHO and its member states from crafting arrangements to improve access to benefits, such as vaccines, derived from samples shared for surveillance purposes.

The second interpretation comes to the opposite conclusion. This position asserts that, under principles of treaty interpretation, IHR 2005 does not require States Parties to share biological samples with WHO. The first principle of treaty interpretation is that a treaty shall be interpreted in good faith in accordance with the ordinary meaning to be given to the terms of the treaty in their context and in light of its object and purpose (*20*). IHR 2005 requires States Parties to provide WHO with "public health information" about events that may constitute a PHEIC (article 6). IHR 2005 does not define what "public health information" means, so its meaning has to be discerned through treaty interpretation principles. The second interpretation holds that the ordinary meaning of "information" encompasses knowledge and facts (*25*) but does not include biological samples.

The second interpretation maintains that IHR 2005, its negotiations, and the WHA resolutions of 2006 and 2007 support it. Nowhere does IHR 2005 contain any express requirement to share samples of biological materials. The only provision that refers to biological substances provides that: "States Parties shall, subject to national law and taking into account relevant international guidelines, facilitate the transport, entry, exit, processing and disposal of biological substances and diagnostic specimens, reagents and other diagnostic materials for verification and public health response purposes under these Regulations" (article 46). The use of "biological substances" here suggests that the negotiators considered this concept separate from "public health information."

The provision that contains the duty to communicate public health information to WHO about a reported event also contains a list of things that fall within this obligation: case definitions, laboratory results, source and type of risk, number of cases and deaths, conditions affecting the spread of disease, and the health measures used (article 6.2). This list refers to things that would fall within the ordinary meaning of "information" and contains nothing that could be considered biological samples, substances, or specimens. The absence of express reference to biological samples is particularly telling in light of the fact that WHO and its member states were, at the time IHR 2005 was being negotiated, aware of concerns about the failure of countries to share samples of pathogens of global concern (e.g., the severe acute respiratory syndrome virus, the influenza [H5N1] virus) for surveillance and other purposes.

Similarly, an earlier negotiating text included the following provision: "In the context of a suspected intentional release of a biological, chemical or radionuclear agent, States shall immediately provide to WHO all relevant public health information, materials and samples, for verification and response purposes" (*26*). Here again, the negotiators used "public health information" and "samples" as distinct terms. Further, this provision does not appear in IHR 2005. Even if it had so appeared, it would have underscored that sharing samples was only required in connection with suspected intentional use of a biological, chemical, or radionuclear agent, which does not include the natural emergence of avian or pandemic influenza.

WHA resolutions of 2006 and 2007 also support this interpretation. The 2006 resolution on early compliance with IHR 2005 with respect to influenza threats urges WHO member states to disseminate to WHO "information *and* relevant biological materials" (*23*) (emphasis added), which further demonstrates that WHO member states consider public health information and biological materials different, not equivalent, terms. WHA's 2007 resolution uses the same language in recalling the 2006 resolution's urging of WHO member states to disseminate information *and* relevant biological materials (*1*). This interpretation is also compatible with CBD because it leaves the decision whether to share biological samples in the hands of the state party in which the samples originate.

Beyond Differing Treaty Interpretations and the WHA Resolution

Stepping back from the differing treaty interpretations, Indonesia's actions exposed ambiguity in a critical aspect of IHR 2005 on the eve of its entry into force. The WHA's 2007 resolution did not resolve this controversy because, on this question, its provisions provide no clear answer. The resolution reaffirms the obligations of States Parties under IHR 2005 and the sovereign right of states over their

biological resources, a key principle in CBD. The bargain that underpins the resolution has, however, established the utility of countries' withholding samples to force WHO and industrialized countries to address neglected aspects of global influenza governance. Dueling treaty interpretations may matter less than the old legal adage that possession of property in dispute is nine-tenths of the law. When possession is cloaked in the principle of sovereignty, those who require access to the property have to come to terms with the need to bargain for it.

Conceptually, the WHA's 2007 resolution seeks to achieve equitable use of influenza virus samples. Such equitable use encompasses timely sharing of samples for global surveillance and more effort to ensure that developing countries share in the benefits of knowledge and technologies derived from the samples, especially influenza vaccines. Equitable use has not occurred because sharing influenza virus samples proves easier than producing equitable access to technologies derived from the knowledge produced by surveillance. The resolution itself obviously does not produce equitable use, but it establishes a WHO-based process for moving global health diplomacy in this direction. The resolution is a general blueprint for building new global governance mechanisms on equitable use of influenza samples. This blueprint is, however, technically limited to influenza virus sharing and vaccine development, and its creation raises questions about governance of the sharing of samples of other pathogens of global concern and of benefits derived from such samples.

WHO and its member states had started the process described in the resolution by, among other things, meeting in Singapore in July 2007 and scheduling another intergovernmental session in November 2007. The meeting in Singapore did not produce consensus, and Indonesia continued to withhold the samples (*27*). In reporting on the Singapore meeting, Branswell observed that many feared the talks would follow Indonesia's lead and produce "a system where countries would exercise sovereign rights over viruses or bacteria found within their borders, seeking quid pro quos from vaccine makers or assessing the potential for gain before co-operating with global health authorities to squelch new disease threats like SARS." (*28*) Media reported in September 2007 that Indonesia had shared some virus samples with WHO related to 2 fatal influenza (H5N1) cases in Bali (*29*), but this action did not mean that Indonesia had abandoned or repudiated the position it had staked out on virus sharing and access to vaccine. Thus, as of this writing, the fundamental issues at the heart of this controversy, including the international legal questions analyzed in this article, had not been resolved.

Whether the process sketched in WHA's resolution produces an effective multilateral regime for equitable use remains to be seen. The process itself is not legally binding because WHA resolutions do not have the force of international law (*30*). The agreement to create this process will perpetuate legal disagreements about sovereignty, CBD, IHR 2005, and other legal issues (e.g., intellectual property rights) because neither side currently has an interest in having the legal questions definitively answered. Instead, constructive legal ambiguity informs the political willingness of countries to shoulder the equitable use responsibilities the WHA resolution envisions.

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Pandemic Influenza and Pregnant Women

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Planning for a future influenza pandemic should include considerations specific to pregnant women. First, pregnant women are at increased risk for influenza-associated illness and death. The effects on the fetus of maternal influenza infection, associated fever, and agents used for prophylaxis and treatment should be taken into account. Pregnant women might be reluctant to comply with public health recommendations during a pandemic because of concerns regarding effects of vaccines or medications on the fetus. Guidelines regarding nonpharmaceutical interventions (e.g., voluntary quarantine) also might present special challenges because of conflicting recommendations about routine prenatal care and delivery. Finally, healthcare facilities need to develop plans to minimize exposure of pregnant women to ill persons, while ensuring that women receive necessary care.

Influenza pandemics occur when a new influenza type A virus to which the population has no immunity emerges, spreads efficiently between humans, and results in worldwide outbreaks of severe disease. Pandemics occur infrequently but can be devastating in terms of the effects on illness and mortality. Most influenza experts consider influenza pandemics inevitable (*1*). The emergence of avian influenza A virus (H5N1) as a cause of severe human infections has increased concerns about an impending pandemic. Although human disease caused by influenza (H5N1) is a rare event (*2*), the virus has become endemic among bird populations in some areas of Asia and has continued to spread geographically and to broaden its host range. Concerns that the virus might acquire the ability to efficiently spread between humans have led public health authorities to accelerate preparations for pandemic influenza.

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A key component of pandemic preparedness (*3*) involves addressing the specific needs of vulnerable populations, including pregnant women. Pregnant women are at high risk for severe complications of influenza during interpandemic periods (*4*) and previous pandemics (*5–8*). In addition, some studies suggest an increased risk for adverse outcomes among infants born to mothers infected with influenza during pregnancy (*9–12*). Special considerations for pregnant women should be addressed in all 3 categories of public health response to pandemic influenza—nonpharmaceutical interventions, antiviral medications, and vaccines. Many articles have discussed issues regarding pandemic influenza in the general population, but limited attention has been given to the effects on the pregnant woman and her fetus. This article focuses on issues regarding pregnant women that should be considered by public health and medical professionals as they prepare for a future influenza pandemic.

Pandemic versus Seasonal Influenza

Influenza viruses that infect humans are classified into 3 principal types (A, B, and C), of which types A and B are important causes of human disease. Types A and B are associated with seasonal epidemics; only type A viruses have caused pandemics. Influenza A viruses are further classified on the basis of 2 surface proteins, hemagglutinin (H) and neuraminidase (N). Minor mutations that result in subtle changes in these proteins (antigenic drift) occur continuously. Because these mutations produce viruses that can be sufficiently different antigenically from previous influenza viruses, influenza vaccines must be updated annually. More dramatic changes in the surface proteins of influenza viruses, through mutation of nonhuman (e.g., avian or swine) viruses or reassortment of human and nonhuman viruses, result in the creation of novel human subtypes (termed an-

tigenic shift). When novel subtypes that can be efficiently transmitted among humans emerge within a population that lacks immunity, an influenza pandemic can occur (*3*).

Avian species are an important reservoir for influenza virus, but avian influenza viruses do not typically infect humans. However, in 1997, human exposure to ill birds infected with avian influenza A (H5N1) led to a severe outbreak in Hong Kong Special Administrative Region, People's Republic of China.The H5N1 virus subtype reemerged in early 2003, and since that time, the virus has caused poultry and wild bird illnesses in >50 countries. In addition, 321 confirmed human cases of influenza (H5N1) in 12 countries have been reported to the World Health Organization, 194 of which have resulted in death (*2*). Thus far, influenza (H5N1) transmission has been predominantly bird-to-human, but the ongoing avian disease and occasional human disease have raised concerns about the possibility that this virus could gain the capacity for efficient human-to-human transmission and possibly lead to an influenza pandemic (*3*). Although influenza (H5N1) represents the greatest current threat for a pandemic virus, global public health authorities recommend increased vigilance for any novel influenza virus infections in humans as a cornerstone of pandemic preparedness.

Effects of Influenza on Pregnant Women

Pregnancy has been a risk factor for increased illness and death for both pandemic and seasonal influenza. The increased risk is believed to be related to several physiologic changes that occur during pregnancy. Because of mechanical and hormonal alterations that occur during pregnancy, several changes also occur to the cardiovascular and respiratory systems, including increased heart rate, stroke volume, oxygen consumption, and decreased lung capacity (*13*). Relevant immunologic alterations also occur during pregnancy, with a shift away from cell-mediated immunity toward humoral immunity. This shift can render pregnant women more susceptible to, or more severely affected by, certain viral pathogens, including influenza (*14*).

Although appropriate nonpregnant control groups were generally not available, mortality rates among pregnant women in the pandemics of 1918 and 1957 appeared to be abnormally high (*5*,*7*). Among 1,350 reported cases of influenza among pregnant women during the pandemic of 1918, the proportion of deaths was reported to be 27% (*5*). Similarly, among a small case series of 86 pregnant women hospitalized in Chicago for influenza in 1918, 45% died (*6*). Among pregnancy-associated deaths in Minnesota during the 1957 pandemic, influenza was the leading cause of death, accounting for nearly 20% of deaths associated with pregnancy during the pandemic period; half of women of reproductive age who died were pregnant (*7*).

Pregnant women have also been shown to be at increased risk for influenza complications during interpandemic periods (*15*). In a large study of >4,300 women of reproductive age during 19 interpandemic influenza seasons, pregnant women were compared with postpartum women (a group considered to be most similar to pregnant women demographically and with regard to their health) and were found to be significantly more likely to be hospitalized for a cardiopulmonary event during the influenza season (*4*). The risk for hospitalization increased as pregnancy progressed, with women at term nearly 5 times more likely to be hospitalized than postpartum women (*4*). Similarly, during 3 influenza seasons in the late 1970s, rates of medical visits for acute respiratory disease were more than twice as high among pregnant women than nonpregnant women (*16*). At particularly high risk during the influenza season are pregnant women with underlying medical conditions for which influenza vaccination is recommended, such as asthma (*17*). On the basis of these data, pregnant women should be considered a population for which special considerations for prevention and treatment for influenza need to be made.

Effects of Influenza on the Fetus

Although certain infections are well recognized to increase the risk for adverse pregnancy outcomes, the effects of maternal influenza infection on the fetus are not well understood. Viremia is believed to occur infrequently in influenza (*18*), and placental transmission of the virus also appears to be rare (*19*). However, even in the absence of fetal viral infection, animal studies suggest that adverse effects can still occur. Prenatal influenza infection in the mouse has been associated with histopathologic changes in the brain (*20*) and behavioral alterations (*21*) in offspring. Although influenza virus RNA has not been detected in the fetal brain, these changes suggest that fetal effects could be secondary to the maternal inflammatory response, rather than the result of a direct viral effect (*22*).

Adverse pregnancy outcomes have been reported following previous influenza pandemics. During the influenza pandemic of 1918, remarkably high rates of spontaneous abortion and preterm birth were reported (*5*,*6*), especially among women with pneumonia (for example, in 1 study, >50% of pregnancies in which the pregnant woman had influenza and accompanying pneumonia were not carried successfully to term) (*5*). During the Asian influenza pandemic of 1957, studies suggested a possible increase in defects of the central nervous system (*10–12*) and several other adverse outcomes, including birth defects, spontaneous pregnancy loss, fetal death, and preterm delivery (*8*). Studies of the effects of seasonal influenza infection on the fetus have been contradictory. A small increased risk for

birth defects in general and for specific birth defects have been observed in some but not all studies (*9*). Using data from a recent case-control study, investigators showed that mothers of infants with any type of birth defect were slightly more likely to report influenza during early pregnancy than mothers of control infants (adjusted odds ratio 1.4; 95% confidence intervals 1.3–1.6), with statistically significant associations for cleft lip with or without cleft palate, and neural tube and congenital heart defects. Verification of maternal report of influenza illness with prospectively collected clinical data was possible for similar numbers of case and control infants (*9*), which suggests that recall bias was unlikely to explain the association. The risk associated with influenza was reduced for women who received treatment with antifever medications and for those who had taken folic acid before and during early pregnancy (*9*).

Associations between maternal influenza infection after both pandemic and seasonal influenza and outcomes observed long after birth have been reported. Associations between maternal influenza infection and childhood leukemia (*23*), schizophrenia (*24*), and Parkinson disease (*25*) have been suggested by some studies. Even if the influenza virus does not have a direct effect on the fetus, fever that often accompanies influenza infection could have adverse effects. Both animal and human epidemiologic studies suggest that hyperthermia is associated with an increased risk for adverse outcomes (*26*), especially neural tube defects (*27*). Factors that might attenuate this risk include shorter fever duration (*28*), use of fever-reducing medications (*28–30*), and use of folic acid–containing supplements (*29*,*31*).

More study is needed to better understand the fetal risks of maternal influenza infection. However, data from previous pandemics, although limited, suggest that pregnancy loss and preterm delivery could be important issues during a future influenza pandemic. Information on seasonal influenza indicates that influenza infection or its accompanying hyperthermia might also increase the risk for certain birth defects. Data on these potential risks to the fetus, combined with available information on risks for influenza infection on maternal health, provide ample support for considering pregnant women a high-risk population in an influenza pandemic.

Pandemic Influenza Response for Pregnant Women

Nonpharmaceutical Interventions

A main component of the public health response to pandemic influenza will be nonpharmaceutical interventions to mitigate disease rates and severity and the societal impact of the pandemic beyond health outcomes. The Centers for Disease Control and Prevention recently released guidance for nonpharmaceutical interventions during a pandemic that focuses on isolation of ill persons, voluntary quarantine of households with ill persons, and social distancing techniques (e.g., avoiding crowded settings, closing schools and child care centers) to limit exposure to ill persons (*32*). However, these recommendations present special challenges for pregnant women. For example, pregnant women will need guidance on how to protect themselves from becoming infected (e.g., use of protective devices) if they are quarantined with or directly providing care for ill persons. Responsibilities of pregnant women as members of the workforce and as caregivers of their children and other family members may further complicate their adherence to public health recommendations. In addition, because healthy pregnant women will continue to require both outpatient prenatal care and inpatient delivery services during a pandemic, they might be more likely to be exposed to clinical settings where ill persons are receiving care. Given the potential risk to women in clinical settings, guidance will need to be developed regarding whether some routine prenatal care visits could be omitted. Healthcare facilities need to develop plans to ensure that pregnant women receive necessary care, but with minimal exposure to ill persons or their contacts. In addition, plans for care and delivery of pregnant women with confirmed influenza or recent exposure must ensure that these women receive appropriate care without unduly exposing other healthy pregnant women and their infants to illness. An appropriate strategy to address these issues might include designating a location and staff for care of pregnant women and their newborns, separate from those used by patients with influenza. Another strategy could include developing an algorithm that triages pregnant patients on the basis of pregnancy stage and symptoms to ensure that pregnant women most in need of attention receive care, but avoid the risk of influenza exposure when that risk might be greater than the benefit of care. Consideration of these strategies as part of overall community pandemic planning activities will be essential.

Experience from the international outbreak of severe acute respiratory syndrome (SARS) (*33*) can shed light on how to approach these complicated issues. In Toronto, obstetric services were moved into a newly designed facility that had entrances, elevators, and air-handling systems that were separate from the rest of the hospital. Hospital staff, patients, and visitors were screened at the hospital entrance for SARS symptoms and to ensure that they had not visited a SARS-affected area. Staff members wore N95 respirator masks, face shields or eye protection, gowns, and non-latex gloves, and employed frequent hand washing with ethanol-based gels. Patients were limited to 1 visitor during labor and delivery, and no visitors were allowed on postpartum wards. All patients and visitors wore N95 respiratory masks. The length of postpartum stay was

decreased and, after discharge, women were instructed to stay at home under quarantine for 10 days; a nurse visited them on their third day postpartum. Healthcare workers were asked to observe work quarantine; they were encouraged to go directly to work and home minimizing contact with the public (*34*). In Hong Kong, obstetric services were transferred to a hospital separate from hospitals in which SARS cases were managed. Women were discharged sooner after delivery, and all obstetric services considered nonessential (e.g., routine ultrasonography and prenatal diagnosis) were temporarily suspended (*33*).

Antiviral Medication Use by Pregnant Women

During a pandemic, 2 pharmaceutical options—antiviral medications and vaccination—will be available to reduce the expected illness and death. Given that a vaccine is unlikely to be available for a substantial portion of the population at the beginning of a pandemic, antiviral medications are expected to play an important role in the response to pandemic influenza, both for postexposure prophylaxis and for influenza treatment. Two antiviral medications are currently recommended for treatment and prophylaxis of influenza in humans (*15*). These medications, both neuraminidase inhibitors, are available in oral (oseltamivir [Tamiflu]) and inhaled (zanamivir [Relenza]) forms, and make up the bulk of stockpiled anti-influenza medications. Two additional anti-influenza medications, the M2 ion channel blockers rimantidine and amantadine, are currently not recommended for use because of high rates of resistance among circulating human influenza A viruses and some avian influenza viruses.

As is the case with >90% of medications introduced in recent years (*35*), insufficient information on oseltamivir and zanamivir is available to assess potential risks to the fetus. This is reflected by their category C use-in-pregnancy rating from the US Food and Drug Administration (i.e., insufficient information available to assess their potential risk and benefit during pregnancy) (*35*). Animal studies have shown no evidence of increased risk for adverse effects for either medication (*36*,*37*), but animal data do not always predict the effects on human pregnancies (*35*). Human data are very limited. Among 61 pregnant women exposed to oseltamivir in the post-marketing period, most pregnancies had a normal outcome (*36*). Single cases of trisomy 21 and anencephaly were reported among these exposed pregnancies, but these cases were not believed to be causally related to oseltamivir exposure. Three pregnancies were inadvertently exposed to zanamivir during the clinical trials, with one ending in spontaneous abortion, one in elective termination, and one in an outcome with no apparent adverse effects (*37*). The bioavailability (the proportion of active drug that reaches the systemic circulation) of zanamivir is lower (12%–17%) than that of oseltamivir (≈80%) (*38*), leading some to suggest that it might be preferred during pregnancy (*3*).

Many pregnant women will require treatment with other medications, such as antibiotics for secondary bacterial pneumonia and antipyretic medications for fever control. Healthcare providers need access to information on these medications and their safe use during pregnancy so that effects on the fetus can be taken into account.

Another issue to consider is that even in the case of serious exposure or illness, pregnant women might fail to comply with recommendations for use of antiviral medications because of concern for the health of the fetus. This emphasizes the importance of communication of the risks and benefits of medications and the serious nature of untreated influenza. As with all communications related to pandemic influenza, messages must be culturally and linguistically appropriate and conducted at an appropriate level of literacy to ensure such efforts are inclusive, given the diversity of the population of pregnant women.

Further research to understand the effects of anti-influenza medications on the pregnant woman and her fetus is essential to guide treatment recommendations during a future pandemic. Although exposures to these medications are likely to be rare in the pre-pandemic period, collection of data on these exposures in a pregnancy registry, as has been used to collect data on other rare exposures (*35*), could provide important data to guide pandemic recommendations. In the absence of additional information, healthcare providers will need to consider the type of exposure, risk for serious illness or death, and trimester of pregnancy when weighing the risks and benefits of these medications to the woman and her fetus. Guidelines for the pandemic scenario, similar to those developed for management of sporadic avian influenza (H5N1) infection (*39*), could assist healthcare providers in weighing these risks and benefits.

Use of Pandemic Influenza Vaccine among Pregnant Women

Once available, a vaccine will be a vital component of the public health response to pandemic influenza. Given their increased risk for illness and death during pandemic influenza, pregnant women should be considered a high priority for receipt of influenza vaccine. Several studies have demonstrated no adverse fetal effects when women received inactivated vaccine during pregnancy (*15*,*40*). Both the Advisory Committee on Immunization Practices and the American College of Obstetricians and Gynecologists recommend annual vaccination with trivalent inactivated influenza vaccine for women who will be pregnant during the influenza season (October–mid May) to prevent seasonal influenza (*15*,*40*). (Live, attenuated, influenza virus vaccine, available as an intranasal spray, is not approved

for use during pregnancy, given the theoretical risk associated with use of live vaccine during pregnancy.) Inactivated influenza vaccine is recommended in all 3 trimesters of pregnancy (*15*,*40*). Despite these recommendations, compliance has been low (*15*), probably because of concerns among women and their healthcare providers regarding the safety of vaccination during pregnancy. Development of culturally and linguistically appropriate messages will be necessary to ensure that pregnant women receive information regarding care required for their health and that of their fetus in the event of a future pandemic. Professionals who develop these messages need to be aware that some women will have limited access to healthcare services. Innovative strategies will be needed to ensure that these messages reach them.

Incorporating Issues of Pregnant Women into Preparedness Exercises

Pandemic influenza planning that specifically addresses the concerns of pregnant women is critical because special issues need to be considered for this high-risk group. Pandemic influenza preparedness exercises should include scenarios in which issues specific to pregnant women require attention. In the event of an influenza pandemic, identification and close monitoring of pregnant women will be important, given their increased risk for influenza-associated illness and death. Intake procedures for women seeking prophylaxis and treatment for pandemic influenza need to incorporate questions about the possibility of pregnancy. By including scenarios involving pregnant women in pandemic influenza preparedness exercises, public health professionals will have the opportunity to weigh the risks and benefits of anti-influenza medications in the context of a specific pandemic scenario. Their inclusion in preparedness exercises will help to identify gaps in our current capacity to provide optimal care for this high-risk population.

Conclusions

Because of their risk for severe disease and death and the potential for risk for the fetus, pregnant women should be considered to be high-risk in the event of an influenza pandemic. Research into the effects of maternal influenza and its treatment on the pregnant woman and her fetus is sorely needed. Based on the limited information available, pregnant women who become ill with influenza should be treated aggressively with antifever therapy, and should adhere to standard recommendations for folic acid consumption (*9*). Given the limited data currently available, plans for prophylaxis and treatment for pandemic influenza will need to include reassessment of risks of influenza and risks and benefits of treatment strategies as a pandemic evolves. In addition to incorporating considerations specific to pregnant women into pandemic influenza planning efforts, strategies to communicate this guidance to pregnant women and their healthcare providers must be planned, developed, and tested. Only through consideration of all these issues, from research and planning to communications and intervention, will the health and well-being of pregnant women be ensured in a future influenza pandemic.

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Human Metapneumovirus Infections in Children

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Human metapneumovirus (hMPV) is an important cause of lower respiratory tract infections in hospitalized children, but the age-related incidence and effect of hMPV in unselected children in the community have not been evaluated. We studied a cohort of 1,338 children <13 years of age throughout 1 respiratory season in Finland during 2000–2001. We examined children and obtained a nasal swab for viral detection at any sign of respiratory infection. hMPV was detected in 47 (3.5%) of the 1,338 children. The age-related incidence of hMPV infection was highest (7.6%) in children <2 years of age, in whom hMPV accounted for 1.7% of all infections during the season. During the epidemic peak, hMPV caused 7.1% of all respiratory infections in the cohort. Acute otitis media developed in 61% of hMPV-infected children <3 years of age. Our findings demonstrate that the effect of hMPV in the community is greatest in children <2 years of age.

Human metapneumovirus (hMPV) was isolated in 2001 by van den Hoogen et al. in previously virus-negative nasopharyngeal aspirates from children with respiratory tract infections (*1*). Since then, hMPV has been identified wordwide (*2–9*). In temperate regions, hMPV circulates mainly during the winter (*6*,*7*,*10–12*). Clinical symptoms of hMPV infection resemble those caused by respiratory syncytial virus and range from mild upper respiratory tract infections to wheezing and severe lower respiratory tract illnesses that require hospitalization (*4*,*5*,*10–14*). Although hMPV infections have been diagnosed in all age groups, the virus likely has its greatest effect in children (*13*,*14*).

Several studies have demonstrated that hMPV accounts for a major proportion of hospitalizations for lower respiratory tract infections in infants and young children (*10*,*13*,*15*,*16*). The most frequent diagnoses in hospitalized children are bronchiolitis and pneumonia, but occasionally hMPV may also cause severe illnesses that require treatment at intensive care units (*17*,*18*).

Clinical features of hMPV infection in hospitalized children and the role of hMPV as a cause of hospitalization have been well described. However, most children infected with hMPV are treated as outpatients. Although hMPV has been found in substantial numbers of selected outpatient children (*12*,*19–21*), to our knowledge, no population-based studies of the incidence and clinical effect of hMPV on unselected children of different ages have been conducted. We determined the incidence, clinical features, and total effect of hMPV infection in a large, prospective, cohort study of respiratory infections in children in Finland.

Methods

Study Participants and Study Protocol

This prospective study was conducted in Turku, Finland, from October 9, 2000, through May 20, 2001. The participating children were recruited before the start of the respiratory season in daycare centers, family daycare, and schools in our area (*22*). All children <13 years of age were eligible for participation; no exclusion criteria were used. Of 1,458 children initially enrolled, 1,338 were closely monitored throughout the entire follow-up period. The baseline characteristics of participating children are shown in Table 1.

Parents were asked to bring their children to the study clinic for examination by a study physician whenever fever or signs of respiratory tract infection appeared. The study clinic was open every day, and all visits were free. In addition to full clinical examination, chest or sinus radiographs

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Table 1. Baseline characteristics of 1,338 children at beginning of follow-up, Finland, 2000–2001

Variable	No. children (%)
Age, y	
<1	30 (2.2)
1–<2	219 (16.4)
2–<4	362 (27.1)
4–<6	247 (18.5)
6–<9	232 (17.3)
9–12	248 (18.5)
Sex	
Male	692 (51.7)
Female	646 (48.3)
Child care	
Home or family daycare	261 (19.5)
Daycare center	658 (49.2)
School	419 (31.3)
Diagnosis of asthma	72 (5.4)
Previous wheezing	253 (18.9)
Exposure to tobacco smoke	512 (39.1)*

*Information available for 1,310 children.

were obtained if pneumonia or sinusitis was suspected on the basis of clinical symptoms. Acute otitis media (AOM) was diagnosed by pneumatic otoscopy, aided by routine use of tympanometry and spectral-gradient acoustic reflectometry. Children without any complications at the first visit were reexamined after 5–7 days, or whenever the parents deemed it necessary.

The parents were provided with a symptom diary in which they recorded daily the child's symptoms and absences from daycare or school and the parents' absences from work because of the child's illness. Only actual days lost were recorded. Thus, days of illness occurring on free weekends or other days off were not recorded as causing absenteeism.

Viral Sampling

At each visit for a new respiratory tract infection, a nasal swab was obtained from a depth of 2–3 cm in the nostril by using a sterile cotton swab that was then inserted into a vial containing viral transport medium (*23*). The specimens were kept in a refrigerator and transported daily to the laboratory at the Department of Virology, University of Turku, where they were subjected to viral culture for influenza viruses, parainfluenza viruses, respiratory syncytial virus, and adenovirus, and PCR assays for rhinoviruses and enteroviruses. The specimens were then frozen at −70°C until thawed for the purposes of this study.

Detection of hMPV

RNA was extracted from nasal swab specimens by using a High Pure Viral Nucleic Acid Kit (Roche, Basel, Switzerland) according to the manufacturer's protocol. hMPV was identified in specimens by using reverse transcription–PCR (RT-PCR). Briefly, hMPV was amplified in a 1-step RT-PCR. The RT-PCR mixture contained RT-PCR buffer (50 mmol/L Tris-HCl, pH 8.4, 50 mmol/L NaCl, 4 mmol/L $MgCl_2$), 0.5 mmol/L $MgCl_2$, 2 mmol/L dithiothreitol, 0.6 mmol/L deoxynucleoside triphosphates, 1 μmol/L of each primer, 20 U Maloney murine leukemia virus reverse transcriptase (Promega, Madison, WI, USA), 4 U Recombinant RNAsin ribonuclease inhibitor (Promega), and 1 U DyNAzyme (Finnzymes, Espoo, Finland). The total volume of the reaction mixture was 50 μL and contained 5 μL of extracted RNA. The RT-PCR for hMPV RNA was conducted at 42°C for 45 min, then at 95°C for 7 min. cDNA amplification consisted of 40 cycles (denaturation at 95°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 1 min) and final extension at 72°C for 10 min. Primers for hMPV were from the L gene (*10*), and the forward primer was biotinylated.

Biotinylated hMPV RT-PCR products were detected by using a liquid hybridization assay. In this assay, 10 μL of each RT-PCR product was mixed with 50 μL of DELFIA assay buffer (PerkinElmer Finland Oy, Turku, Finland), added to 3 parallel microtitration wells coated with streptavidin (PerkinElmer Finland Oy), and incubated at room temperature for 30 min. Wells were washed at room temperature with Tris-NaCl buffer containing 0.5% Tween 20, denatured for 5 min with 150 μL denaturation buffer (25 mmol/L NaOH and 5 mmol/L EDTA), hybridized with Eu-labeled hMPV-probe (5′-CTG TTA ATA TCC CAC ACC AG-3′) at 40°C for 2 hours. The hybridization was performed with 100 μL of hybridization solution, in which 2 ng Eu-labeled hMPV probe per well was diluted with DELFIA assay buffer that included 0.85 mmol/L NaCl and 0.1% Tween 20. Unspecific hybridizations were removed with hot (50°C) Tris-NaCl buffer containing 0.5% Tween 20. To enhance fluorescence, 200 μL DELFIA Enhancement solution (PerkinElmer Finland Oy) was added per well and incubated for 10 min with shaking. Fluorescence was measured by using a Victor 1420 Multilabel Counter (PerkinElmer Finland Oy).

Definitions

AOM was diagnosed by signs of inflammation of the tympanic membrane, the presence of middle ear effusion, and ≥1 signs of acute infection. The diagnosis of pneumonia was based on radiologic confirmation of the condition. Both complications were associated with hMPV infection if they were diagnosed ≤14 days after the clinical visit at which the hMPV-positive specimen was obtained.

Ethics

The study protocol was reviewed and approved by the Ethics Committee of Turku University Hospital. Written informed consent was obtained from the parents of all participating children.

Results

hMPV Outbreak

The first case of hMPV infection in the study cohort was identified during the week of December 4, 2000, and the last case was identified during the week of April 23, 2001 (Figure 1). hMPV infections were diagnosed weekly over 14 consecutive weeks from January 8, 2001, through April 15, 2001. Overall, hMPV infection was diagnosed in 47 children (26 boys and 21 girls). The median age of the children was 3.0 years; 81% were <5 years of age.

Incidence Rates of hMPV Infection in Different Age Groups

The incidence of hMPV infection was highest (7.6%) in children <2 years of age at the start of the respiratory season (Table 2). Of a subset of 30 children <1 year of age, 3 (10.0%) acquired hMPV infection during follow-up. The incidence rates of hMPV decreased gradually with age. The overall incidence of hMPV infection in the study cohort of 1,338 children was 3.5% (95% confidence interval [CI] 2.5%–4.5%).

Relative Effect of hMPV among All Respiratory Infections

The relative proportion of hMPV infections among all respiratory infections was greatest in children <2 years of age, in whom hMPV accounted for 1.7% of all respiratory infections during the winter season (Table 3). During the 14-week period of major hMPV circulation, hMPV accounted for 4.2% of all infections in children <2 years of age. The relative effect of hMPV decreased with age. In the entire cohort of 1,338 children, hMPV accounted for 1.3% (95% CI 0.9%–1.7%) of all respiratory infections during the whole respiratory season and 2.7% (95% CI 1.9%–3.5%) of all infections during the 14-week period of continuous hMPV circulation.

The proportion of hMPV infections among all respiratory infections during each week of the study is shown in Figure 2. The relative effect of hMPV was highest during the week of February 19, during which the virus accounted for 7.1% of all respiratory infections in the study cohort.

Co-infections with hMPV

Another virus was detected concomitantly in 8 (17%) of 47 hMPV-infected children. Three children had enterovirus, 2 had rhinovirus, and 1 each had influenza virus, parainfluenza virus, and a nontypeable picornavirus together with hMPV.

Clinical Characteristics of hMPV Infection

The clinical findings of hMPV infection were analyzed in 39 children in whom hMPV was the only virus detected (Table 4): 97% had a cough, 90% had rhinitis, and 72% had a fever. The median duration of symptomatic illness was 8 days. AOM was the most frequently diagnosed complication; it occurred in 61% of children <3 years of age. Wheezing was observed in 10% of the hMPV-infected children and laryngitis in 8%. A total of 38% of the children were treated with antimicrobial drugs. None of the children was referred to a hospital.

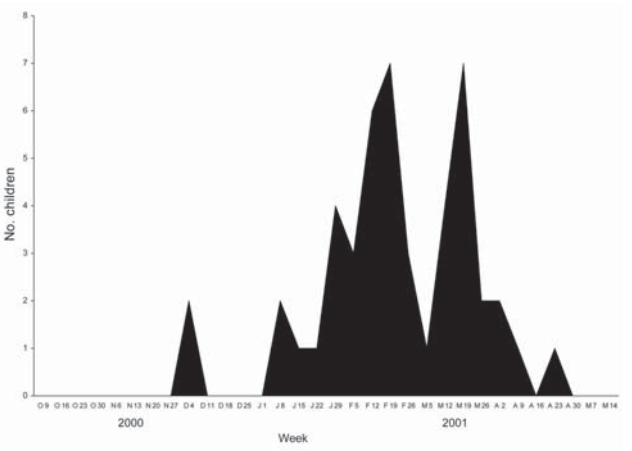

Figure 1. Number of children with human metapneumovirus infections during each week of the study period, Finland, 2000–2001.

Socioeconomic Effect of hMPV

Overall, 54% of hMPV-infected children were absent from daycare or school for ≥1 day (Table 4). The mean duration of their absence was 3.3 days. In 38% of cases, a parent missed ≥1 day of work because of the child's hMPV illness. The average duration of parental work loss was 2.9 days.

Persistence of hMPV RNA in Nasal Swabs after Acute Illness

In 27 (57%) of the 47 children with hMPV infection, a follow-up nasal swab was obtained during a subsequent respiratory infection. The median interval between the initial

Table 2. Incidence of human metapneumovirus (hMPV) infections in children during the respiratory season, Finland, 2000–2001*

Age, y†	No. children	No. children with hMPV	Rate of hMPV/1,000 children (95% CI)
<2	249	19	76 (43–109)
2–<4	362	14	39 (19–59)
4–<6	247	9	36 (13–60)
6–<9	232	3	13 (0–27)
9–12	248	2	8 (0–19)
Total	1,338	47	35 (25–45)

*CI, confidence interval.
†Age at beginning of follow-up.

Table 3. Proportions of human metapneumovirus (hMPV) infections in children, Finland, 2000–2001

	Whole respiratory season		Major hMPV outbreak†	
Age, y*	Total no. specimens	No. hMPV-positive specimens (%)	Total no. specimens	No. hMPV-positive specimens (%)
<2	1,107	19 (1.7)	430	18 (4.2)
2–<4	1,156	14 (1.2)	518	12 (2.3)
4–<6	631	9 (1.4)	309	9 (2.9)
6–<9	420	3 (0.7)	194	3 (1.5)
9 –12	307	2 (0.7)	151	2 (1.3)
All children	3,621	47 (1.3)	1,602	44 (2.7)

*Age at beginning of follow-up.
†Period of 14 consecutive weeks during which hMPV infections were detected every week in the children.

hMPV-positive specimen and the subsequent sample was 42 days (range 7–82 days). hMPV could not be detected in any of the subsequent samples after hMPV illness.

Discussion

This prospective cohort study provides new and detailed information about the effects of hMPV infections in unselected children. Close clinical follow-up of a large cohort of children throughout an entire respiratory season enabled us to determine the incidence and relative importance of hMPV among all respiratory viruses in children in different age groups. A particular strength of our study was that we obtained samples for viral detection during every episode of respiratory illness seen at the study clinic, regardless of the severity of the symptoms or the presence or absence of fever, thereby avoiding any bias caused by sampling only a selected population of children with respiratory infections. Furthermore, symptoms of the children and the socioeconomic effect of their illnesses were recorded daily throughout the study period.

Our findings demonstrate that the effect of hMPV in the community is greatest in the youngest children. The incidence of hMPV infection in children <2 years of age was approximately twice that of children 2–5 years of age and 10× higher than the incidence among children ≥9 years of age. This finding is consistent with those of serologic studies that demonstrated that most children contract hMPV by 5 years of age (*1*,*24*,*25*). The effect of hMPV on the youngest infants is likely even greater than what was observed in our study. In our cohort, the incidence of hMPV infection was highest in children <1 year of age, but the small number of children in this age group limits our drawing any firm conclusions about this finding.

In addition to the absolute incidence rates of hMPV being highest in children <2 years of age, the relative effect of hMPV among all respiratory viruses was greatest in this age group. During the period of continuous circulation of hMPV, this virus accounted for >4% of all respiratory infections in children <2 years of age. On an annual level, hMPV accounted for 1%–2% of all respiratory infections in our cohort. This estimate agrees with the results of a recent 20-year study in which the prevalence of hMPV ranged from 1% to 5% of all upper respiratory infections in a given year in children <5 years of age (*12*). In our cohort, the overall effect of hMPV was substantially smaller than that of influenza viruses, which accounted for ≈7% of all respiratory infections during the same winter season (*26*). However, the substantial effect of hMPV during local outbreaks is demonstrated by our finding that during the peak of the epidemic, hMPV was responsible for 7% of all respiratory infections in the children even though influenza virus was circulating in the community at the same time (*26*).

Most children with hMPV had cough, rhinitis, and fever. In contrast with previous reports of a high prevalence of wheezing in hospitalized children (*4*,*5*,*7*,*10*,*13*), only 10% of the children seen in primary care had wheezing, and none of these children was referred to a hospital. This is understandable because patients with more severe illnesses usually end up in hospitals, but these results also indicate that most hMPV infections in children are relatively mild and clinically indistinguishable from other viral infections. Of importance, however, is the high rate of AOM as a complication of hMPV infection. Together with similar reports by other investigators (*12*,*13*,*27*), hMPV has a particularly strong ability to predispose a child to AOM. This underscores the clinical similarity between hMPV and respiratory syncytial virus, which is also a major viral cause of AOM in children (*28*–*30*).

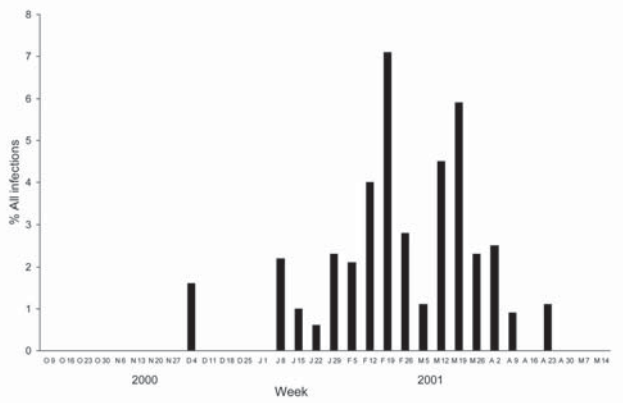

Figure 2. Weekly proportions of human metapneumovirus infections among all respiratory infections in the study children, Finland, 2000–2001.

Table 4. Clinical characteristics and socioeconomic effects of human metapneumovirus (hMPV) infections in children, Finland, 2000–2001*

Variable	Age group, y†			All children (n = 39)
	1–2 (n = 18)	3–4 (n = 15)	5–9 (n = 6)	
Signs and symptoms				
Cough	18 (100)	15 (100)	5 (83)	38 (97)
Rhinitis	17 (94)	13 (87)	5 (83)	35 (90)
Fever >37.5°C	13 (72)	13 (87)	2 (33)	28 (72)
Vomiting	1 (6)	1 (7)	0	2 (5)
Wheezing	2 (11)	1 (7)	1 (17)	4 (10)
Laryngitis	1 (6)	2 (13)	0	3 (8)
Median duration of illness, d	9.5	8	7	8
Complications				
Acute otitis media	11 (61)	4 (27)	1 (17)	16 (41)
Pneumonia	1 (6)	0	0	1 (3)
Antimicrobial drug treatment	11 (61)	3 (20)	1 (17)	15 (38)
Absenteeism				
Child´s absence from daycare or school for ≥1 d	10 (56)	8 (53)	3 (50)	21 (54)
Mean duration of child's absence, d‡	3.7	2.9	3.0	3.3
Parental absence from work for ≥1 d	9 (50)	4 (27)	2 (33)	15 (38)
Mean duration of parental absence, d‡	2.9	2.5	4.0	2.9

*Values are no. children (%) unless otherwise stated.
†Age at diagnosis of hMPV infection.
‡Calculated for children or parents who were absent for at least 1 d.

In previous studies, hMPV has been infrequently detected in asymptomatic children (*10*,*13*). We could not detect hMPV in any samples obtained during a subsequent respiratory infection after hMPV illness. These findings imply that hMPV in the nasal mucosa is short-lived and corroborate the view that detection of hMPV RNA in respiratory secretions is strongly indicative of a causal role for the virus in the illness.

The variation in prevalence of hMPV from season to season has been demonstrated (*12*,*13*). Therefore, our results obtained during 1 winter season are not directly generalizable to all other winters. However, on the basis of a 2-year hospital-based study in our area, circulation of hMPV was substantially greater during the winter of 2000–2001 than during the following winter (*31*). Thus, our season of follow-up likely did not represent a season with exceptionally low hMPV activity. We obtained nasal swabs instead of nasopharyngeal aspirates for reasons of compliance with repeated sampling. The sensitivity of nasal swabs for detection of various respiratory viruses is ≈90% compared with nasopharyngeal aspirates (*23*). Therefore, some cases of hMPV may not have been diagnosed. Furthermore, because we did not use serologic analysis, we have no data on the incidence of hMPV infections that were either asymptomatic or so mild that the parents did not bring the children to the study clinic. However, the clinical importance of such subclinical infections is negligible.

In conclusion, our prospective population-based study provides conclusive evidence for the effect of hMPV infections in children. The effect of hMPV is greatest in children <2 years of age. hMPV also appears to be one of the major viruses predisposing children to AOM. Although on an annual level hMPV accounts only for a small proportion of all respiratory infections in children, its relative role among all respiratory viruses is substantial during local hMPV outbreaks.

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High Genetic Diversity of Measles Virus, World Health Organization European Region, 2005–2006

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During 2005–2006, nine measles virus (MV) genotypes were identified throughout the World Health Organization European Region. All major epidemics were associated with genotypes D4, D6, and B3. Other genotypes (B2, D5, D8, D9, G2, and H1) were only found in limited numbers of cases after importation from other continents. The genetic diversity of endemic D6 strains was low; genotypes C2 and D7, circulating in Europe until recent years, were no longer identified. The transmission chains of several indigenous MV strains may thus have been interrupted by enhanced vaccination. However, multiple importations from Africa and Asia and virus introduction into highly mobile and unvaccinated communities caused a massive spread of D4 and B3 strains throughout much of the region. Thus, despite the reduction of endemic MV circulation, importation of MV from other continents caused prolonged circulation and large outbreaks after their introduction into unvaccinated and highly mobile communities.

The World Health Organization (WHO) has a goal of eliminating measles in the WHO European Region by 2010. The region extends from the Atlantic to the Pacific, including all western and eastern European countries and the former Soviet Republics (online Appendix Table, available from www.cdc.gov/EID/content/14/1/107-appT.htm). After the separation of Serbia and Montenegro in 2006, the number of countries in the WHO European Region increased from 52 to 53. Within a well-performing case-based nationwide surveillance system, countries with a goal of elimination are expected to reach <1 confirmed measles case per million population per year. To reach this goal countries are expected to achieve measles vaccination coverage of at least 95% with the first dose and at least 80% with the second (*1*). From 1995 through 2005, the number of countries in the WHO European Region that reported >95% coverage with a first dose of measles-containing vaccine increased from 18 (35.3%) of 51 to 31 (59.6%) of 52. In 2005 and 2006, however, at least 40% and 55%, respectively, of the member states had a measles incidence that was above the elimination threshold (online Appendix Table). A total of 36,426 and 55,578 measles cases, includ-

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ing 14 and 9 fatal cases, were reported in 2005 and 2006, respectively (online Appendix Table). Thus, measles continues to affect a large number of persons, despite enhanced vaccination strategies.

The pattern of measles virus (MV) genotypes, in combination with epidemiologic investigation, contributes to understanding measles transmission and helps distinguish between continuous circulation and importation and limited transmission of the viruses in a certain region (*2*,*3*). Genotype C2 has been continuously detected in the European Region since the early 1970s and is therefore considered to be the indigenous genotype of Europe (*4*,*5*). D6 viruses have been regularly reported from different countries of the European Region since the early 1990s (*4*–*12*), and genotype D6–associated outbreaks or sporadic cases on other continents were mostly due to MV importations from Europe (*13*–*15*) (Figure 1). These observations thus provide overwhelming evidence for the endemic circulation of genotype D6 in Europe at least during the past 15 years. Measles cases in the European Region associated with other genotypes were mostly due to virus importation from other continents

Methods

Laboratory case confirmation and MV genotyping have increased significantly since the establishment of a global laboratory network for measles and rubella in 2000 (*16*). During 2005–2006, MV genotypes were identified in 25 of the 53 member states of the WHO European Region (online Appendix Table). Most epidemics and sustained transmissions were associated with genotypes D6, D4, and B3 viruses (online Appendix Figure 1, available from www.cdc.gov/EID/content/14/1/107-appG1.htm). All strains were genotyped by sequencing the 450 nt that code for the C-terminus of the MV N protein (hypervariable region [HVR]), as recommended by WHO (*17*). Sequencing was performed in different laboratories of the WHO laboratory network for measles and rubella.

Results

Genotype D6

During 2005–2006, genotype D6 viruses were reported from 17 of the 53 countries in the WHO European Region. The overall diversity between these viruses was relatively low (Figure 2), with a maximum genetic distance of 7 nt (1.6%) in the HVR of the N gene. Two main variants, D6–2000 and D6–2005, differing by a single point mutation, accounted for most of genotype D6–associated cases and outbreaks.

The D6–2000 variant was predominant in the Russian Federation throughout 2005 and early 2006 (Figure 2) and was also detected in neighboring Kazakhstan (2006) and Uzbekistan (2006). Moreover, the D6–2000 strain caused outbreaks in Germany (March 2005–July 2005) (*18*) and Greece (September 2005–May 2006) (*19*) and was found in sporadic cases in Denmark (2005), Israel (2005, after importation from Georgia), and Switzerland (2006) (Figure 2). Because the same variant was reported throughout 2000–2005 from different European countries (Figure 1), the cases in 2005 and 2006 were most probably due to endemic transmission of MV in the European Region.

The D6–2005 variant caused the large outbreak in the Ukraine, which had >45,000 cases reported between the last quarter of 2005 and October 2006 (*20*) and was first identi-

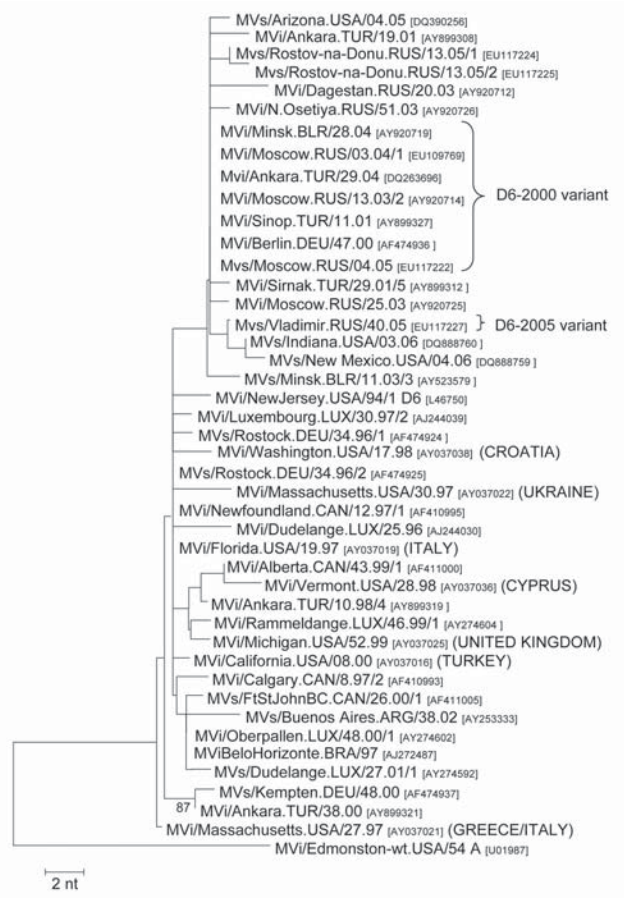

Figure 1. Phylogenetic tree showing representative genotype D6 strains identified in Europe before 2005, strains exported from Europe to other continents (source of exportation is shown in brackets), and the D6 variants that were dominant in Europe during 2005 (D6–2000) and 2006 (D6–2005). The phylogenetic tree was calculated on the basis of the 450 nt that code for the C-terminus of the MV N protein, by using MEGA 3.1 software and the neighbor-joining method (500 bootstraps). Genetic distances are represented as numbers of nucleotide differences between strains. Measles virus strains were named according to World Health Organization nomenclature: MVi/City of isolation.Country/epidemiologic week.year of isolation(/isolate number). Sequences obtained from RNA extracted from isolates (MVi) or clinical material (MVs) were distinguished. GenBank accession nos. are also shown for each strain.

fied during the last quarter of 2005 in the Russian Federation (MVs/Vladimir.RUS/40.05, Figure 2). The same virus was also found in Azerbaijan during the first quarter of 2006. Of the 32 viruses collected during the Ukraine outbreak, all but 6 were identical to the D6–2005 strain. The other 6 viruses correspond to 3 other variants that differ from the main variant by only single mutations.

Several other measles outbreaks and sporadic cases in 2006 were epidemiologically linked to the Ukraine. Multiple importations of MV from the Ukraine caused different small outbreaks and sporadic cases in Belarus from January through September 2006 (*21*). Among the 47 Belarusian viruses that were sequenced, 37 were identical to the main variant from the Ukraine. The other 10 isolates differed by no more than a single nucleotide from D6 viruses from the Ukraine or the Russian Federation collected during the same period (Figure 2). In Russia in 2006, the main variant from the Ukraine had largely replaced the earlier D6 strains. More than 94% of all genotype D6 strains (n = 88) obtained in the Russian Federation from the last quarter of 2005 and all of 2006 were D6–2005 variants. Although the D6–2005 variant was first reported from the Russian Federation, it may also have been imported from other countries that did not report MV genotypes despite significant numbers of measles cases (online Appendix Table).

The D6–2005 virus was also retrieved from a small outbreak in Tallinn (Estonia) during March 2006; the index patient was infected in the Ukraine (MVs/Tallinn.EST/12.06). Similarly, 2 persons infected with the same strain traveled from the Ukraine to Latvia and Bulgaria in April and July 2006, but the virus did not spread. In Spain, the same variant was also obtained from 2 sporadic cases, (MVs/Madrid.SPA/18.06/1 and MVs/Valencia.SPA/17.06), which were epidemiologically linked to the Ukraine, as well as from a small outbreak occurring in La Rioja (MVs/Logrono.SPA/02.06/1) from December 2005 through January 2006 (*22*).

In Germany, the D6–2005 variant caused 2 sporadic cases in Kiel and Stuttgart as well as a major outbreak in North Rhine Westphalia (MVs/Moenchengladbach.DEU/10.06 (*23*,*24*) and a small outbreak in Berlin. Although the index cases of the 2 outbreaks were not found, sequence identity with the main Ukrainian variant suggests that they were directly or indirectly linked to the outbreak in the Ukraine. In the United Kingdom (UK, March–July 2006) another outbreak associated with D6–2005 (MVs/Redhill.GBR/10.06) was epidemiologically linked to Italy, although genotype D6 had not been directly detected in this country.

In addition to the main genotype D6 variants, D6–2000 and D6–2005, somewhat different viruses were found in Greece, Germany, Spain, and Luxembourg. Besides the D6–2000 virus, a second genotype D6 variant (MVs/Attica.GRC/09/06/2), with a 2-nt difference, was identified in Athens during February 2006. The same virus was also obtained from a sporadic case in Munich (Germany) 10 weeks later and caused an outbreak in the Canary Islands (Spain) after importation from Germany (Figure 2). Another genotype D6 virus identified in a sporadic case from Luxembourg in April 2006 showed a 4-nt difference from the most closely related D6 strain. In absence of an epidemiologic link, the origin of these viruses could not be determined.

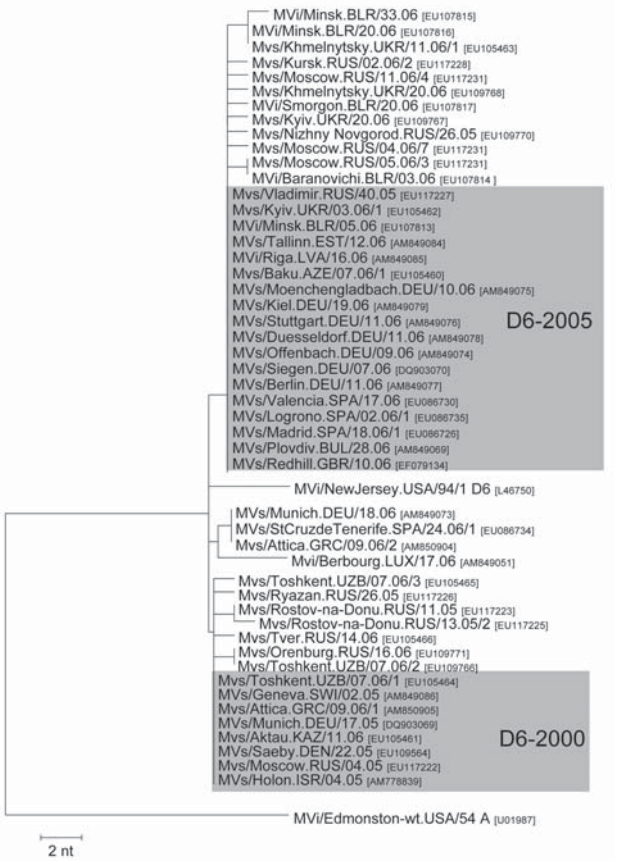

Figure 2. Two main variants, D6–2000 and D6–2005, of D6 identified in Europe during 2005–2006. Tree calculation and measles virus nomenclature are as delineated in Figure 1.

Genotype D4

In contrast to the relative genetic homogeneity of D6 viruses, at least 4 distinct genetic groups of genotype D4 were identified in Europe during 2005–2006 (Figure 3). The maximum genetic distance was 24 nt (5.3%), and the minimum distance between 2 groups was 11 nt (2.4%).

Group 1

A large outbreak in Romania, which included >8,000 cases and lasted from December 2004 until early 2007, started among unvaccinated members of the Roma and Sinti communities before spreading to the general popu-

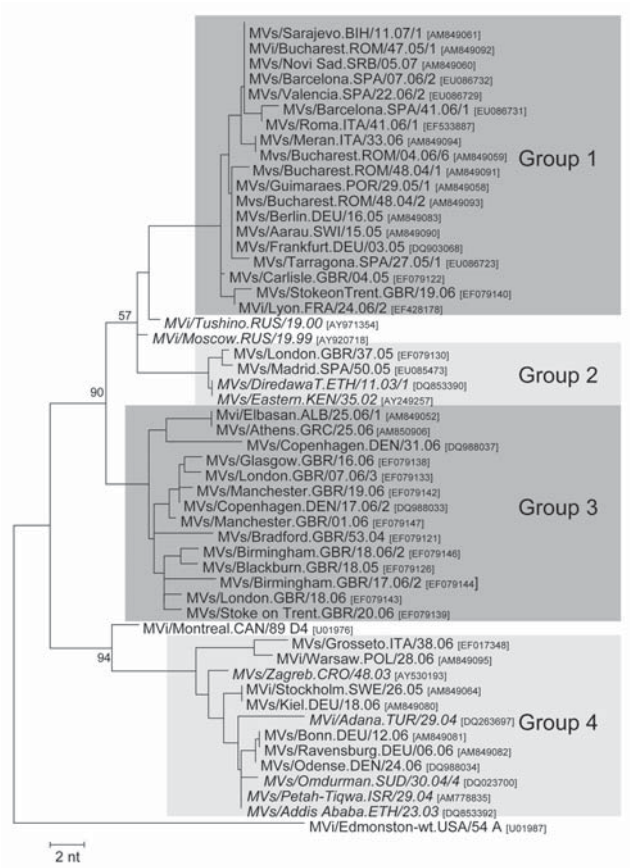

Figure 3. Four different genetic groups of genotype D4 identified in Europe during 2005–2006 and their closest relatives (in *italics*) identified on other continents. Tree calculation and measles virus nomenclature are as delineated in Figure 1.

lation. Viruses differing by ≤2 nt were identified during different phases of this outbreak (Figure 3). All group 1 viruses identified in other countries differed from the most closely related Romanian virus by a maximum of 2 nt. Viruses identical to MVs/Bucharest.ROM/48.04/2 were reported from an outbreak of >220 cases in Germany (Hesse, January–April 2005; MVs/Frankfurt.DEU/03.05), which was epidemiologically linked to Romania (*18*), and from small outbreaks in Berlin (Germany), Guimares (Portugal), and an isolated case in Aarau (Switzerland) during 2005 (Figure 3). The MVs/Bucharest.ROM/47.05 variant was found in 2 different small outbreaks in Spain during weeks 7 and 22 of 2006 (MVs/Barcelona.SPA/07.06/2 and MVs/Valencia.SPA/22.06/2), the first case of which could be directly epidemiologically linked to Romania. The same variant was also found in Roma populations in Serbia as well as in Bosnia and Herzegovina during the first quarter of 2007. Another small outbreak in Cataluña (Spain) was also caused by a virus imported from Romania (MVs/Tarragona.SPA/27.05/1), with a single mutation compared with MVs/Bucharest.ROM/48.04/2. Finally, the virus that caused an outbreak of >200 cases in Barcelona (MVs/Barcelona.SPA/41.06/1; August 2006–February 2007) (*25*) was imported from Italy and had differed by 1 nt from those from an epidemic in the region of Lazio (Italy, June–November 2006), which were also taken by Roma populations to Sardinia in August 2006. A closely related genotype D4 variant, identical to MVs/Bucharest.ROM/04.06/6, caused a small outbreak during the same period in South Tyrol (Italy, Figure 3) (*26*).

The source of the Romanian viruses could not be identified. However, the homogeneity of D4 viruses identified during the Romanian outbreak, as well as the large number of measles-susceptible persons, suggests that the virus has spread within the country only after importation from another country.

Group 2

MV variants of a second group were found in the UK and Spain (Figure 3). MVs/Madrid.SPA/50.05 had been imported from the UK into Spain but had 1 point mutation compared with MVs/London.GBR/37.05. These viruses had 1 or 2 mutations compared with D4 viruses detected earlier in Kenya (2002) and Ethiopia (2003), which suggests an East African origin. The putative epidemiologic link to Somalia for MVs/London.GBR/37.05 also supports this hypothesis.

Group 3

Group 3 includes mainly viruses identified in different outbreaks and sporadic cases in the UK throughout 2005–2006, as well as in Greece, Albania, and Denmark (Figure 3). Although the source could not be identified for all group 3 viruses, the overall genetic diversity in this group (7 nt, 1.6%) suggests multiple origins. Confirmed epidemiologic links to Pakistan for MVs/London.GBR/7.06/3, MVs/Birmingham.GBR/18.06/2, MVs/Manchester.GBR/01.06, and the Danish strains suggest that most of them were imported from Central Asia, where genotype D4 is still endemic (*27*). The genotype D4 virus, which caused a small outbreak in Albania during June 2006, was imported from Greece by Roma populations.

Group 4

In Germany and Denmark (February and April 2006), closely related viruses of this group were found in different outbreaks and sporadic cases, which could not be epidemiologically linked to each other (Figure 3). The Danish virus (MVs/Odense.DEN/24.06) was imported from Lebanon, which is perhaps consistent with the isolation of similar D4 strains in Israel 2004 (1-nt difference). Conversely, closely related strains (with a 1- to 2-nt difference) were also seen in Ethiopia (2003) and the Sudan (2004), which

suggests that the corresponding strains from Germany may also have been imported from East Africa. Another D4 variant of group 4 caused an outbreak in Tuscany (Italy, January–May 2006; MVs/Grosseto.ITA/38.06) after importation from India (*26*). This virus differed by only 3 nt from the D4 strains obtained from an outbreak in Poland that occurred from January through May 2006.

Genotype B3

During 2005–2006, B3 strains were reported from 8 European countries, in association with outbreaks of various sizes. The maximum genetic distance between the corresponding viruses was 13 nt (2.9%) (Figure 4).

A sporadic case in the Netherlands (MVs/Gouda.NET/47.05) resulted from contact with a measles patient from Kenya at the airport in Newark, New Jersey, USA (*28*). The same virus was identified during an outbreak in Germany (January–April 2006) and the UK (June 2006) (Figure 4). Although it was not clear whether these outbreaks were due to independent importations from Africa or to MV transmission within Europe, the very high genetic diversity of MV in Africa would suggest the latter. Three other B3 variants were identified in the UK during May/June 2005 and May 2006. Although the sources are unknown, their genetic distance of 3–6 nt suggests independent importations from sub-Saharan Africa. The most closely related virus from sub-Saharan Africa (MVs/Ibadan.NGA/05.04) was identical to MVs/London.GBR/19.05, which was the cause of a major outbreak in the UK. On the other hand, 2 B3 viruses with a single mutation in comparison with MVs/London.GBR/19.05 from the UK caused 2 independent outbreaks in Spain (Madrid and Las Palmas, 2006).

Another B3 variant was detected in Denmark, Sweden, and Spain in 2006 (Figure 4). The corresponding measles cases in Denmark and Sweden were most probably epidemiologically linked (*29*), but the source of importation into Europe could not be identified. The closest non-European virus (MVs/Ibadan.NGA/01.04), which differed by 1 nt, was from Nigeria (2004).

Identical B3 strains were identified during the last quarter of 2006 in Albania and Italy (MVs/Roma.ITA/43.06/2 and MVi/Shkodra.ALB/44.06). Two considerably different B3 strains were found within a period of 3 weeks in Caen, France, in 2005. The latter clustered with viruses from either Cameroon and Equatorial Guinea or Democratic Republic of the Congo, respectively, indicating that they resulted from 2 independent introductions (Figure 4). Finally, another B3 variant was obtained from a sporadic case in Switzerland in 2006.

Other Genotypes

In addition to D6, D4, and B3, some other genotypes (B2, D5, D8, D9, G3, H1) were found in Europe during 2005–2006 (online Appendix Figure 2, available from www.cdc.gov/EID/content/14/1/107-appG2.htm). Most of them were associated with sporadic cases or small outbreaks after importation of MV from other continents. The corresponding strains and confirmed sources of importation are shown in online Appendix Figure 2.

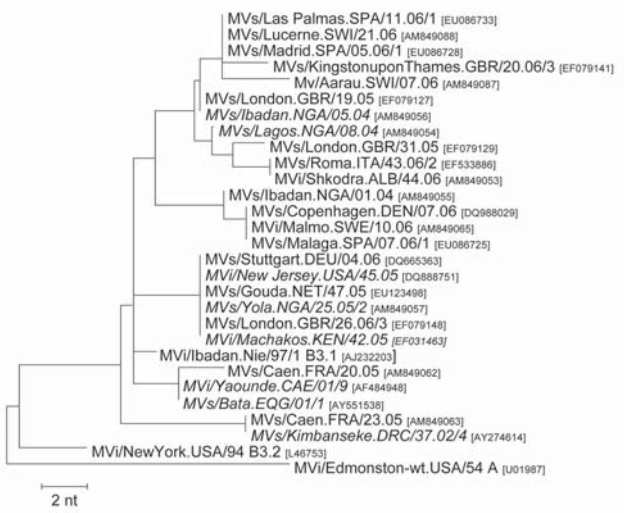

Figure 4. Genotype B3 variants identified in Europe during 2005–2006 and some closely related strains identified on other continents (in *italics*). Tree calculation and measles virus nomenclature are as delineated in Figure 1.

Discussion

All 53 member states of the WHO European Region except 1 have implemented a routine vaccination program against measles, resulting in an overall regional coverage of 94% for the first dose of measles-containing vaccine. In 2006, 45% of the member states had reached an incidence of <1/1,000,000 population (online Appendix Table), the threshold to declare measles elimination. To achieve regional elimination of measles by 2010, enhanced vaccination strategies are required, especially in countries with a persistently high measles incidence.

Molecular epidemiology, an essential component of measles surveillance, helps to distinguish between endemic transmission and importation of the virus. It enables identification of regions with continued MV circulation, which reflect suboptimal herd immunity. During 2005–2006, 9 of the 17 active MV genotypes (*27*) were found in the WHO European Region. The major epidemics were caused by different variants of genotypes D4, D6, and B3. The largest outbreaks occurred in the Ukraine (genotype D6, >45,000 as of June 2007), Romania (D4, >8,000 cases as of June 2007), Germany (North Rhine Westphalia, D6, ≈1,700 cases), and the Russian Federation (D6, >1,100 cases). Epidemics involving 100–500 cases were reported from the

UK (B3, originated in a traveler community), Spain (B3, D4), Germany (D4, D6, B3), Italy (D4), Belarus (D6), and Greece (D6, D4). Smaller epidemics and sporadic cases were caused by either the above-mentioned genotypes or others (B2, D5, D8, D9, H1, G3).

Since the early 1990s, genotype D6 has been continuously detected in Europe (*4*–*12*). During 2005–2006, D6 strains were reported from 17 European countries and were mostly related to the large outbreak in the Ukraine (online Appendix Table). The overall genetic diversity of the recent D6 strains was, however, much lower than that found during the 1990s and early 2000s, and recent D6 strains were most similar to those found during 2000–2004 mainly in the Russian Federation and Turkey (Figure 1). The low genetic diversity of D6 strains suggests that enhanced vaccination has largely reduced the cocirculation of highly diverse D6 variants in Europe. Moreover, genotypes C2 and D7, which were frequently identified in Europe until 2004 (*4*–*8*,*10*–*12*), were no longer found during the past 2 years, despite greatly improved virologic surveillance. Genotype C2 had been continuously identified in the European Region since the early 1970s, and genotype D7 had partially replaced genotypes C2 and D6 in Germany during 2000–2004 (*6*). Thus, the transmission of several indigenous MV strains from Europe has probably been interrupted by enhanced control programs.

In contrast, the prevalence of imported genotype D4 and B3 strains considerably increased during 2005–2006. Genotype D4 strains have a vast geographic distribution, and measles outbreaks associated with this genotype have been reported from all continents (*5*). D4 viruses are still endemic on the Indian subcontinent as well as in East and South Africa (*5*,*27*). Although some outbreaks and sporadic cases associated with genotype D4 had been reported from Europe, the corresponding viruses were mostly imported from other continents and differed considerably from the more recent strains (*6*,*11*,*30*). In 2005–2006, genotype D4 was found in 16 of the 27 countries in which MV has been genotyped; most of the corresponding viruses were related to the outbreak in Romania. Roma and Sinti, communities which had mostly low vaccination levels, were involved in the transmission of the Romanian D4 viruses to at least 10 other countries in Europe. The remaining genotype D4–associated cases from Europe (2005–2006) were often epidemiologically linked to the Indian Subcontinent, East Africa, and the Middle East. The high genetic diversity of the corresponding MV variants is characteristic of multiple importation events. The increased frequency of genotype D4 detection during recent years can be explained by the interruption of transmission of most of the indigenous strains, as well the introduction of genotype D4 strains into highly mobile and hard-to-reach populations with low vaccination levels.

Genotype B3 is the endemic genotype in sub-Saharan Africa; a large diversity of genotype B3 viruses continues to circulate in many countries of West and Central Africa (*31*–*37*). Before 2005, genotype B3 viruses had only been reported sporadically from Europe (*6*,*11*). The genetic diversity of the multiple B3 variants detected in Europe during 2005–2006 suggests that each one was directly or indirectly imported from sub-Saharan Africa. All of them belonged to subgroup B3.1 of genotype B3, a finding that suggests a high prevalence of B3.1 strains in Africa during 2005–2006.

Several other imported genotypes (B2, D5, D8, D9, G2, and H1), which usually do not occur in Europe, were also sporadically detected during 2005–2006. Sequence analysis confirmed or identified suspected sources of importation for most of these.

While some countries reported outbreaks caused by a single strain, other countries experienced epidemics that were caused by multiple importations of unrelated strains. As an example, the isolation of different genetic variants (D4, B3, D6–2000, D6–2005) from the 4 major epidemics in Germany (2005–2006) shows that these cases were not epidemiologically linked. The identification of multiple genotypes in Spain, Switzerland, and the UK also demonstrates multiple origins of the different outbreaks and sporadic cases. Cocirculation of different genotypes during what seemed to be 1 outbreak has been identified in Greece, Italy, and Germany. In Italy (Lazio region, 2006) and Greece (2006), viruses obtained toward the end of an epidemic (genotype B3 and D4) belonged to genotypes different from those found in the early phase (D4 and D6). In Germany very different variants of genotype D4 were found in distinct parts of the country simultaneous with the epidemic in North Rhine Westphalia. In Denmark (2006), the detection of genotypes B3 and D5 and 2 D4 variants occurred in close geographic and temporal proximity (*38*). The identification of genotype D4 in Poland did not corroborate the putative epidemiologic link between the outbreaks in Poland and the Ukraine (genotype D6) (*39*). The presence of both genotypes D4 and D6 in Poland can, however, not be excluded because no viruses were characterized from the early phase of what seemed to be 1 epidemic. The corresponding examples from Germany, Italy, Greece, Denmark, and Poland thus highlight the importance of MV genotyping during different phases of a presumably single protracted outbreak. On the other hand, MV genotyping alone may not always be sufficient to distinguish linked and unlinked outbreaks caused by multiple importations. Epidemiologic investigations in Belarus showed multiple importations from the Ukraine that caused different, unrelated outbreaks and sporadic cases in which highly similar or identical D6 variants were found.

Conclusion

In conclusion, the absence of previously circulating genotypes (C2 and D7) and the low genetic diversity of D6 strains during 2005–2006 suggest an interruption of several MV transmission chains in the WHO European Region attributable to enhanced vaccination. However, the virus continues to be imported from other continents where measles remains highly endemic. Moreover, prolonged circulation and spread of imported strains, mostly after introduction into unvaccinated and highly mobile communities, continue to cause a high incidence of measles disease in Europe. Some countries did not report measles genotypes despite large numbers of cases (online Appendix Table). As a consequence, to achieve measles elimination by 2010, measles surveillance and control need to be further optimized, and specific emphasis must be given to the vaccination of hard-to-reach populations.

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Dr Kremer is technical supervisor of the WHO Regional Reference Laboratory for Measles and Rubella in Luxembourg. His main research interest is related to genetic and phenotypic diversity of measles virus.

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Cryptosporidiosis and Filtration of Water from Loch Lomond, Scotland

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Previous evidence has suggested an association between consumption of unfiltered water from Loch Lomond, Scotland, and cryptosporidiosis. Before November 1999, this water had been only microstrained and disinfected with chlorine; however, since that time, physical treatment of the water (coagulation, rapid gravity filtration) has been added. To determine risk factors, including drinking water, for cryptosporidiosis, we analyzed data on laboratory-confirmed cases of cryptosporidiosis collected from 1997 through 2003. We identified an association between the incidence of cryptosporidiosis and unfiltered drinking water supplied to the home. The association supports the view that adding a filtration system to minimally treated water can substantially reduce the number of confirmed cryptosporidiosis cases.

Cryptosporidiosis is caused by ≥1 species or genotypes of the genus *Cryptosporidium*; the most important human pathogens are *C. hominis* and *C. parvum*. *C. hominis* infection is mainly restricted to humans; *C. parvum* infects a variety of mammals (especially neonatal cattle and sheep) as well as humans. Cryptosporidiosis is associated with bloating, abdominal pain, nausea, and diarrhea, which can be profuse and prolonged. Although the illness is normally self-limiting, a recent study showed that it can lead to serious health sequelae (*1*). Moreover, a more severe form of the disease, which may be prolonged or even fatal, may develop in persons with poor immune systems (*2*).

Infection is frequently disseminated by person-to-person transmission, by animals, and indirectly through the environment (particularly by water). Persons at increased risk for *Cryptosporidium* infection include household and family contacts, sex partners of infected patients, users of communal swimming pools, and travelers to disease-endemic regions. Most instances of person-to-person transmission occur directly by the fecal-oral route or indirectly by fomites. Zoonotic transmission from cattle and sheep to humans is a recognized mode of infection for this pathogen; these animals are an important reservoir of human disease (*3*).

Drinking water contaminated with *Cryptosporidium* oocysts is an internationally recognized risk factor for human illness (*4–6*); contamination can arise from a variety of sources (*7*) including oocysts from infected humans, livestock, and feral animals present in the catchment. Oocysts remain infectious in the environment and water for prolonged periods and are resistant to most disinfectants used to treat drinking water. Thus, inadequate treatment of drinking water can permit infectious oocysts to be transmitted to susceptible consumers of that water (*8–10*).

The need to control microbiologic contaminants in potable waters is dictated by the quality of the source water and requires an integrated multiple-barrier approach. The ability of treatment processes to remove oocysts ultimately depends on the abundance of oocysts in the raw water source and the nature of the treatment processes. For example, the introduction of rapid gravity filtration has been shown to result in ≈3 $\log_{10}$ removal of *Cryptosporidium* oocysts (*1*).

In Scotland, 600–900 laboratory-confirmed cases of cryptosporidiosis are reported to Health Protection Scotland (HPS) each year. Within the past 7 years, 2 large outbreaks of water-borne cryptosporidiosis have occurred in Scotland: 90 confirmed cases in Glasgow in 2000 associated with unfiltered Loch Katrine water (*11*) and 140 cases in Aberdeen in 2002 associated with suboptimal filtration of River Dee water. Outbreaks have also occurred in central Scotland; circumstantial evidence suggested an association

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with minimally treated water from Loch Lomond, particularly in 1998 (*12*,*13*).

Loch Lomond supplies water to ≈34% of the population of central Scotland. Before November 1999, this water was only microstrained (only particles ≥23 μm were filtered out) and disinfected with chlorine, and the risk of transmitting *Cryptosporidium* spp. oocysts (4–6 μm) to consumers of this water was relatively high. In November 1999, enhanced physical treatment (coagulation and rapid gravity filtration), designed in part to reduce the number of oocysts, was introduced. During this time, no substantial changes were made to water treatment for other sources supplying central Scotland. This treatment of the Loch Lomond supply was expected to reduce the oocyst load in the final supply.

To determine the validity of a preliminary study about cryptosporidiosis and water from Loch Lomond (*14*) and to maximize the robustness of the results, we collected data from the entire area supplied with drinking water from Loch Lomond. Our hypothesis was that if a proportion of cases of illness were attributable to drinking unfiltered Loch Lomond water, then illness incidence should be reduced after the introduction of enhanced water treatment. The null hypothesis was that no association would be noted between the enhanced treatment of the Loch Lomond supply and incidence of cryptosporidiosis.

Methods

The design was a retrospective cohort study of microbiologically confirmed cases of cryptosporidiosis among residents of central Scotland. The period of study (1997–2003) was chosen to enable the detection of any epidemiologic trends in cases and in exposure to risk factors. To calculate the odds of becoming ill if the home water was supplied by Loch Lomond water or another source, we analyzed information on all laboratory-confirmed cases in each of the 5 areas of the National Health Service for Scotland (NHS) Board (Argyll & Clyde, Greater Glasgow, Lanarkshire, Lothian, and Forth Valley). Cases from Forth Valley were excluded, however, because of incomplete data. To improve the potential power of the analysis and to improve our ability to detect a true difference, if one existed, we pooled all cases.

Original NHS Board case investigation records were reviewed, and the following were entered into an Excel database: age, sex, home address postal code, residence, date and age at illness onset, age at time of diagnosis, and history of risk factors (travel [foreign and domestic], recreational water contact, pet ownership, farm animal and farmland contact, and tap water consumption). Using postal codes, we linked cases to the drinking water supply zone and the water source, details of which were supplied by Scottish Water. For analysis purposes, case-patients were divided into 2 groups according to the source of drinking water at the home address (Loch Lomond or other sources). We used NHS Board resident population numbers for 2002, obtained from the General Register Office for Scotland, to identify the population at risk and calculate incidence rates.

Descriptive statistics were used to describe the characteristics of case-patients and the distribution of cases by water source, before and after the introduction of enhanced physical treatment at Loch Lomond. Cases associated with different water sources were compared by using Mann-Whitney tests for quantitative variables and χ^2 tests for qualitative variables. A χ^2 test was then performed to investigate any difference in the distribution of cases in persons who received water from Loch Lomond or from other sources before and after the introduction of enhanced treatment of Loch Lomond water. The association with other possible risk factors was investigated by using logistic regression analysis to quantify the characteristics of Loch Lomond case-patients. Unless otherwise stated, we used Minitab statistical software, version 14 (www.minitab.com) at a significance level of 5% for all analyses.

Results

Of the 2,501 reported cases, 10 had no date of diagnosis and were not further analyzed, which left 2,491 cases in the study. The period incidence of cases in patients who received water from Loch Lomond was 12.8 cases per 100,000 population before filtration (November 1999) and 6.5 per 100,000 after filtration. The period incidence of cases in patients who received water from other sources was 27.7 per 100,000 before November 1999 and 46.9 per 100,000 after November 1999. The period incidence for cases in the rest of Scotland was 39.1 per 100,000 before November 1999 and 66.1 per 100,000 after November 1999. Hence, when the incidence in Scotland as a whole increased considerably, the incidence of cases in the Loch Lomond supply zone decreased by half.

Before filtration, most case-patients were 5–14 years of age, whether they received water from Loch Lomond (62.5%) or from other sources (48.3%) (Table 1). After filtration was introduced to the Loch Lomond supply, the percentage of case-patients 5–14 years of age who received water from this supply decreased (35.1%); however, the percentage in this age group who received water from other sources (29%) also decreased. The population of case-patients 0–4 years of age increased from 5% for the Loch Lomond supply area to 31.6% and from 4.4% to 23.4% for the other-sources area. Age data for 27 case-patients were incomplete.

Age distributions of case-patients who received water from each of the 2 sources differed significantly ($p<0.001$). The mean age of case-patients in the Loch Lomond sup-

Table 1. Age of cryptosporidiosis case-patients, Loch Lomond, Scotland

Age, y	Loch Lomond, no. (%)		Other, no. (%)		All (n = 2,464)
	Before (n = 341)	After (n = 171)	Before (n = 726)	After (n = 1,226)	
≤4	17 (5.0)	54 (31.6)	32 (4.4)	287 (23.4)	390
5–14	213 (62.5)	60 (35.1)	351 (48.3)	356 (29.0)	980
15–44	93 (27.3)	43 (25.2)	283 (39.0)	464 (37.8)	883
45–64	9 (2.6)	10 (5.8)	36 (5.0)	84 (6.9)	139
≥65	9 (2.6)	4 (2.3)	24 (3.3)	35 (2.9)	72

ply area was 16.1 years before filtration was introduced and 16.4 thereafter; for the other supply area, the mean age of case-patients was 20.5 years before filtration and 20.2 thereafter (Table 2).

Sex distribution also differed significantly (χ^2 $p<0.001$). Of those case-patients living in the Loch Lomond supply zone, most (57.8%) were male; of those outside of the Loch Lomond supply zone, only 48.8% were male. With regard to sex of the study population before and after enhanced water treatment, the sex difference persisted but was reduced after water treatment (Table 3).

Before enhanced treatment of the Loch Lomond water supply, 1,068 cases were microbiologically confirmed; after enhanced treatment, 1,398 cases were confirmed. Within this cohort, 512 (21%) case-patients lived in the area served by Loch Lomond and 1,954 (79%) lived in areas served by other water sources. For areas not supplied by Loch Lomond water, 37% of cases occurred before enhanced water treatment compared with 63% after (Table 4). Data on water source were incomplete for 25 case-patients.

Comparison of the observed and expected values illustrates that fewer cases occurred in the area supplied by Loch Lomond after enhanced treatment than would have been expected if the change in treatment had no association with the incidence of cases. The analysis shows a significant association between being a case-patient and living in Loch Lomond before filtration was introduced ($p<0.001$). Therefore, statistical evidence suggests that the incidence of confirmed cases of cryptosporidiosis in the Loch Lomond area was significantly reduced after enhanced treatment was introduced. This reduction was not seen in the areas not served by Loch Lomond or anywhere else in Scotland.

Binary logistic regression was used to assess which reported risk factors were associated with being a case-patient living in the Loch Lomond supply zone (Table 5). Risk factors investigated were exposure to unfiltered Loch Lomond tap water, contact with a case-patient, contact with farm animals, travel outside the water-supply area, ownership of pets, recreational water contact, consumption of tap water, and others. These risk factors were derived from data collected from the standard cryptosporidiosis investigation forms, which are based on current understanding of risk factors for clinical illness. The frequency of exposure to the reported risk factors was consistent for some factors; e.g., contact with another case-patient was consistently cited least often, and tap water consumption was consistently cited most often. However, the variation in reported exposures over the study period generally showed consistent trends; e.g., recreational water contact increased markedly in both groups, as to a lesser extent did camping and contact with pets. Travel outside the area was cited by a very small proportion of Loch Lomond case-patients before November 1999 but increased almost 10-fold thereafter, compared with a 2-fold increase for case-patients who received water from other sources. The results of the univariate analyses are shown in Table 6.

The factors with χ^2 p values <0.10 were included in a multiple logistic regression model, corrected for age and sex. When we used backward selection by removing the least significant factor at each stage, the factors associated with being a Loch Lomond case-patient were exposure to unfiltered Loch Lomond water ($p<0.001$), tap water consumption ($p = 0.011$), and no contact with farm animals ($p<0.001$). Case-patients who received water from Loch Lomond were more likely to have become ill before filtration of tap water was introduced (odds ratio [OR] 3.5, 95% confidence interval [CI] 2.8–4.4), more likely to have consumed tap water (OR 1.93, 95% CI 1.16–3.18), and less likely to have had contact with farm animals (OR 0.48, 95% CI 0.34–0.68).

Interaction terms were fitted to the model to determine whether the effect of filtration was confounded with either consumption of tap water or farm animal contact. No significant interaction between filtration and either consumption of tap water ($p = 0.400$) or contact with farm animals ($p = 0.554$) was noted.

Table 2. Age distribution of cryptosporidiosis case-patients, Loch Lomond, Scotland

Water source	Relation to enhanced water filtration*	No.	Mean, y	Median, y	Range, y
Loch Lomond	Before	341	16.14	8.0	3–92
	After	171	16.43	8.0	1–72
Other	Before	726	20.50	13.0	3–99
	After	1,226	20.24	13.0	0–88

*Coagulation and rapid gravity filtration, introduced in November 1999.

Table 3. Sex distribution of cryptosporidiosis case-patients, Loch Lomond, Scotland

Water source	Relation to enhanced water filtration*	Male, no. (%)	Female, no. (%)
Loch Lomond	Before	197 (57.8)	144 (42.2)
	After	92 (53.8)	79 (46.2)
Other	Before	354 (48.8)	372 (51.2)
	After	581 (47.4)	646 (52.6)
Total		1,224	1,241

*Coagulation and rapid gravity filtration, introduced in November 1999.

Discussion

Effective filtration of drinking water substantially reduces the risk that infectious oocysts will pass into treated water, although residual low-level, intermittent contamination is possible. From 1991 through 1993, oocysts of the dimensions of *C. parvum* were detected in the Loch Lomond supply on 20 separate instances, 55% of which occurred in the months of November and December (*13*). The numbers of oocysts detected were low. Another study found that 11% of final treated water contained *Cryptosporidium* spp. oocysts (2–67 oocysts per 1,000 L) (*15*).

To try to eliminate this water-borne pathogen from drinking water supplies, the water industry and public health responses have focused on establishing effective multiple-barrier water treatment systems (*12*). Paradoxically, however, if drinking water transmission is an important route for maintaining immunity, efforts to eliminate *Cryptosporidium* spp. from drinking water can result in increased human susceptibility to infection with *Cryptosporidium* spp. (*16*,*17*).

Our results imply an ecologic association between illness and living in the area that received water from Loch Lomond. This association supports the view that risk for illness is increased among those living in an area supplied by minimally treated water and concurs with findings from other studies (*5*,*6*,*18*). We also found that for residents of the Loch Lomond supply area, consumption of unfiltered tap water was a significant risk factor. Of additional interest was the reduced likelihood of zoonotic transmission of *Cryptosporidium* spp. to residents of the Loch Lomond supply area because they were less likely to come into contact with farm animals. This finding provides evidence that residents were being exposed to infectious oocysts by drinking minimally treated tap water. Thus, upgrading water treatment, particularly introducing coagulation and rapid gravity filtration systems, can substantially reduce numbers of cryptosporidiosis cases and lends credence to the implementation of similar treatments for other minimally treated drinking water supplies in Scotland (*12*).

Our conclusions are valid only at the population level because the study was based on the home location of case-patients and the water supply to that location. Data on the water consumption patterns of individuals were insufficient for us to be able to comment on exposure. The ecologic study method is widely accepted in environmental epidemiology because opportunities to collect precise environmental exposure data on individuals are limited. Our use of information routinely collected by Scottish Health Boards was a practical means of improving the evidence base for an association between cryptosporidiosis and drinking tap water. Our study adds further weight to this evidence base.

Before November 1999, the incidence of illness in persons who received Loch Lomond water at home was already substantially lower (less than half) than for their counterparts who received water from other sources. Reasons for this difference are unknown. One possible, but unproven, explanation might be that continuous low-level exposure to oocysts in unfiltered water might result in a higher background level of immunity to *Cryptosporidium* spp. among consumers of Loch Lomond water (*16*) and that an unintended consequence of introducing filtration to Loch Lomond might therefore be reduced levels of immunity to *Cryptosporidium* spp. Continued surveillance of *Cryptosporidium* spp. will therefore be necessary to fully understand the long-term consequences of abrogating environmental risk factors.

Another potential confounding factor for this study might have been the 2001 outbreak of foot-and-mouth disease (FMD) (*19*). During this outbreak, reports of human cases of cryptosporidiosis were significantly reduced in southern Scotland where FMD was present. In the rest of Scotland over the same period, the reduction in cases was not significant (*20*). The reduced movement of farm animals and lower numbers of young animals in the countryside during the spring are likely to have reduced the environmental load of oocysts. Restricted public access to the countryside during the outbreak is likely to have reduced the risk for human environmental exposure to oocysts. In the study cohort, some of the reduction in the incidence of illness noted after November 1999 among Loch Lomond

Table 4. Distribution of cases by drinking water source before and after the introduction of filtration at Loch Lomond, Scotland

Relation to enhanced water filtration*	Water source, no. (%)†		
	Loch Lomond	Other	All
Before	341 (222)	727 (846)	1,068
After	171 (290)	1,227 (1,108)	1,398
Both	512	1,954	2,466

*Coagulation and rapid gravity filtration, introduced in November 1999.
†Numbers in parentheses are cases expected under the null hypothesis (i.e., no association between water supply and filtration).

Table 5. Exposure to cryptosporidiosis risk factors before and after the introduction of enhanced water filtration at Loch Lomond, Scotland*

Risk factor reported	Loch Lomond, no. (%)		Other, no. (%)	
	Before filtration	After filtration	Before filtration	After filtration
Tap water consumption	296 (95.2)	146 (96.7)	546 (89.5)	962 (95.9)
Recreational water contact	73 (23.5)	89 (59.2)	183 (29.7)	480 (47.2)
Camping	37 (11.9)	33 (22.1)	81 (13.1)	183 (18.0)
Pets	21 (6.8)	12 (8.0)	41 (6.6)	120 (11.9)
Travel outside area	12 (3.9)	55 (36.2)	91 (14.6)	295 (28.8)
Contact with case	7 (2.3)	4 (2.7)	15 (2.4)	19 (1.9)
Contact with farm animals	33 (10.6)	11 (7.3)	105 (17.0)	142 (14.0)

*Enhanced water filtration is coagulation and rapid gravity filtration, introduced in November 1999.

zone residents might have been attributable to reduced environmental exposure, associated with the FMD outbreak. However, the risk factor analysis for the Loch Lomond–zone residents indicated that exposure to farm animals was less; hence, any reduced incidence due to this confounder should be less significant than for the residents outside the Loch Lomond zone. The incidence across Scotland over the whole study period after November 1999 was actually greater than before; any effect of the FMD outbreak was temporary. In contrast, the reduction in case incidence in the Loch Lomond zone was sustained after November 1999.

A possible confounding factor may be that some case-patients from Glasgow who did not receive Loch Lomond water received water from another unfiltered supply (Loch Katrine). The variation in incidence among those who received another water supply may have been caused partially by variation in water from Loch Katrine. An outbreak of cryptosporidiosis occurred in 2000 among persons who received water from Loch Katrine (*11*). However, all known outbreak cases were excluded from this study.

In summary, our data strongly suggest that drinking unfiltered tap water from Loch Lomond transmitted *Cryptosporidium* spp. at the population level. Upgrading water treatment, and particularly introducing well-operated coagulation and rapid gravity filtration systems, can substantially reduce the numbers of cryptosporidiosis cases. These findings support implementation of similar treatments for other minimally treated drinking water supplies.

Table 6. Reported exposure variables for cryptosporidiosis, central Scotland

Variable	p value
Exposure to unfiltered Loch Lomond water	<0.001
Consumption of tap water	0.082
Contact with a case-patient	0.694
Camping	0.587
Water contact	0.028
Pets	0.074
Contact with farm animals	0.002
Travel outside water-supply area	<0.001

Dr Pollock is an epidemiologist in the Gastro-Intestinal/Zoonoses section of Health Protection Scotland. His research interests include epidemiologic, clinical, and microbiologic aspects of zoonoses, including *Cryptosporidium* spp. and *Escherichia coli* O157.

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Cross-subtype Immunity against Avian Influenza in Persons Recently Vaccinated for Influenza

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Avian influenza virus (H5N1) can be transmitted to humans, resulting in a severe or fatal disease. The aim of this study was to evaluate the immune cross-reactivity between human and avian influenza (H5N1) strains in healthy donors vaccinated for seasonal influenza A (H1N1)/(H3N2). A small frequency of CD4 T cells specific for subtype H5N1 was detected in several persons at baseline, and seasonal vaccine administration enhanced the frequency of such reactive CD4 T cells. We also observed that seasonal vaccination is able to raise neutralizing immunity against influenza (H5N1) in a large number of donors. No correlation between influenza-specific CD4 T cells and humoral responses was observed. N1 may possibly be a target for both cellular and humoral cross-type immunity, but additional experiments are needed to clarify this point. These findings highlight the possibility of boosting cross-type cellular and humoral immunity against highly pathogenic avian influenza A virus subtype H5N1 by seasonal influenza vaccination.

Influenza viruses are segmented, negative-sense RNA viruses belonging to the family *Orthomyxoviridae*. According to the antigenic differences in nucleoprotein and matrix proteins, 3 types of influenza viruses (A, B, and C) have been described. Influenza viruses A and B are associated with seasonal illness and death, whereas influenza virus C causes mild infections (*1*,*2*). Influenza A viruses are subtyped on the basis of the antigenic differences on external hemagglutinin (HA) and neuraminidase (NA) glycoproteins. Human type A influenza virus subtypes have been limited to H1, H2, and H3 and to N1 and N2 (*3*). Several HAs and NAs have been isolated from avian hosts; occasionally, they have been associated with human outbreaks (*4*,*5*).

Cytotoxic T lymphocytes play a central role in the clearance of primary influenza virus infection, peaking after 7–10 days; the peak in antibody titers occurs 4–7 weeks after primary infection (*6*–*8*). Neutralizing antibodies are completely protective against secondary challenges only with closely related strains, but they are ineffective against viruses with major antigenic divergence. For this reason, current influenza vaccines are prepared annually on the basis of World Health Organization forecasts on the most probable influenza A and B virus strains thought to be circulating in the next seasonal outbreak (*5*,*7*). By contrast, cellular responses to cross-reactive epitopes may provide a substantial degree of protection against serologically distinct viruses (*9*). The ability of influenza viruses to mutate and reassort their HA-NA genome segments between different animal species is a main concern because immunity generated by previous infections or vaccinations is unable to prevent infection by itself, although it may reduce virus replication and spread (*8*–*10*).

To date, 3 influenza subtypes have produced pandemic disease in humans: H1N1 in 1918, H2N2 in 1957, and H3N2 in 1968 (*4*,*11*,*12*). In 1997, during the avian influenza (H5N1) outbreak in Hong Kong Special Administrative Region, People's Republic of China, a cross-reactive cellular immune response induced by influenza (H9N2) was able to protect chickens from influenza (H5N1) (*13*). Moreover, adults living in the United States who were never exposed to H5N1 subtype have shown cross-type cellular immunity to influenza A virus strains derived from swine and avian species (including the H5N1 subtype isolated in Hong

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Kong) (*14*). Thus, speculation that cross-reactive T cells may decrease illness and death by reducing the replication of the new influenza virus, even if elicited by a different strain, is reasonable.

Avian influenza A viruses of the H5N1 subtype are currently causing widespread infections in bird populations. Numerous instances of transmission to humans have been recently reported in Asia and Africa, with the infection resulting in severe disease or death (>50% fatality rate). Hence, the aim of the present study was to evaluate the immune cross-reactivity between human and avian influenza (H5N1) strains in healthy donors recently vaccinated for seasonal influenza A (H1N1/H3N2). Our data indicate that influenza vaccination may boost cross-subtype immunity against influenza (H5N1), involving cellular or humoral responses or both.

Study Design and Methods

Study Population

Healthcare workers wishing to receive seasonal influenza vaccination at the Spallanzani Institute (n = 42) were enrolled. The study was approved by the local Ethical Committee; all participants gave written informed consent. Baseline characteristics of the study population are reported in the Table. Blood samples were obtained before (t0) and 30 days after vaccination (t1). The vaccine formulation was Fluarix, an inactivated and purified split influenza vaccine (GlaxoSmithKline, Verona, Italy). The antigen composition and strains were A/California/7/2004-H3N2; A/New Caledonia/20/99-H1N1; and B/Shanghai/361/2002. Each 0.5-mL vaccine dose contains 15 μg HA of each strain in phosphate-buffered saline and excipients. Vaccine was administered intramuscularly.

Cells, Viruses, and Antigens

Madin-Darby-canine kidney (MDCK) cells were maintained in Dulbecco modified Eagle medium (DMEM) containing 10% fetal calf serum (FCS), and 2 mmol/L L-glutamine, at 37°C in a 5% CO_2 humidified atmosphere. The influenza (H5N1) virus used was strain A/Hong Kong/156/97 (kindly provided by Paul Chan) (*15*). The virus stock used as challenge antigen in the hemagglutination inhibition (HI) assay was propagated in the allantoic cavities of 10-day-old embryonated hen eggs. The allantoic fluid was harvested 48 h postinoculation and clarified by centrifugation. Virus concentration was determined by HA titration as previously described (*16*), and the virus was stored at –80°C until used. The virus stock used in the microneutralization (NT) and in the cell-mediated immunity assays was propagated in MDCK cells, and the culture supernatants were collected 48 h postinoculation. The 50% tissue culture infectious dose ($TCID_{50}$), determined by titration in MDCK cells, was calculated by the Reed and Muench method (*17*).

Influenza vaccine, UV-inactivated MDCK-derived influenza virus (H5N1), or synthetic influenza (H5N1) oligo-

Table. Baseline characteristics of the study population of healthcare workers, Rome, Italy, 2005

Participant no.	Previous flu vaccine receipt	Sex	Age, y	Work position
1	None	M	37	Biologist
2	None	M	44	Administrative personnel
3	None	M	42	Administrative personnel
4	None	F	27	Laboratory technician
5	2000–2004	M	45	Physician
6	None	F	28	Laboratory technician
7	2002–2004	F	45	Nurse
8	2002–2004	M	59	Laboratory technician
9	2003–2004	M	52	Laboratory technician
10	None	M	48	Nurse
11	None	M	32	Biologist
12	2000–2004	F	42	Physician
13	2000–2004	F	54	Biologist
14	None	M	49	Physician
15	2000–2004	M	55	Physician
16	2001–2004	F	42	Nurse
17	None	F	40	Biologist
18	None	M	38	Biologist
19	None	F	34	Physician
20	2004	M	34	Biologist
21	None	F	28	Biologist
22	2003–2004	M	59	Physician
23	2003–2004	F	42	Physician
24	None	M	51	Laboratory technician
25	None	M	52	Biologist
26	None	M	28	Laboratory technician
27	2003–2004	F	40	Nurse
28	2003–2004	F	32	Nurse
29	2003–2004	M	35	Biologist
30	None	F	51	Laboratory technician
31	2003–2004	M	56	Physician
32	2003–2004	M	36	Physician
33	None	F	32	Nurse
34	2004	M	55	Physician
35	None	F	52	Administrative personnel
36	2004	M	52	Physician
37	2004	M	49	Nurse
38	2004	F	33	Biologist
39	2002–2004	F	44	Nurse
40	2004	F	43	Nurse
41	2004	M	41	Nurse
42	None	M	35	Physician

peptides were used as antigens for cell-mediated immunity. Influenza virus (H5N1) was inactivated by exposure to UV light for 10 min, and complete inactivation of UV-exposed virus was checked by infecting MDCK monolayers with undiluted preparation and by back-titrating the infectivity after 5 days postinfection. Four synthetic peptides of the influenza (H5N1) were purchased from Biodesign International (Kennebunk, ME, USA). The sequence of these peptides is specific for H5-C-terminal (15 aa), H5-middle region (14 aa), and N1-C-terminal (15 aa) and for N1-middle region (16 aa), with no cross-matching with other HA and NA sequences. These peptides can bind different HLA-DRB1 alleles, as established according to the SYFPEITHI site (www.syfpeithi.de). Specifically, N1-specific peptides can bind the following HLA-DR alleles: HLA-DRB1*0101, B1*0301, B1*0401, B1*0701, B1*1101, B1*1501. The H5-specific peptide from the N-terminal region binds several HLA-DR alleles (HLA-DRB1*0101, B1*0301, B1*0401, B1*0701, B1*1101, B1*1501). In contrast, the H5-specific peptide from the middle region did not appear to bind any HLA-DR alleles. According to the HLA-DRB1 allele frequency in the local population, these peptides can be efficiently presented by most (up to 84%) of study participants.

Cell-mediated Immunity

Cell-mediated response was assessed by detecting intracellular interferon-gamma (IFN-γ) production by effector T cells, after antigen-specific stimulation in vitro to generate effector cells from memory cells (*18*). Peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation (Ficoll-Hypaque, Pharmacia Biotech, Uppsala, Sweden) and frozen at –150°C. Thawed PBMC in culture medium (RPMI 1640, 10% FCS, 2 mmol/L L-glutamine) were stimulated with the influenza vaccine preparation (1.5 μg/mL), UV-inactivated influenza (H5N1) (MOI 0.1), or synthetic influenza (H5N1) peptides (NA and HA) (1 μg peptide/mL) for 3 days and expanded for 6 additional days in the presence of recombinant interleukin-2 (IL-2) (5 IU/mL, Boehringer-Mannheim, Mannheim, Germany). On day 9, cells were restimulated with the same antigens in the presence of 1 μg/mL αCD28 and αCD49d (immunoglobulin G1 [IgG_1], K clones CD28.2 and 9f10, respectively; Becton Dickinson, Mountain View, CA, USA) and of Brefeldin-A (10 μg/mL, Sigma, St. Louis, MO, USA). As a negative control, a mock virus preparation, obtained with uninfected MDCK cells, or irrelevant peptides were used. To control the spontaneous cytokine production, cells incubated with only αCD28 and αCD49d were included.

In addition, the frequency of IFN-γ–producing CD4 T lymphocytes from each donor in the absence of any stimulation was used to calculate the background for each stimulation. The resulting background levels were very low in every experiment, and no differences were observed between samples obtained before (t0 0.03% ± 0.04%) and after vaccination (t1 0.01% ± 0.03%). The frequency of antigen-specific CD4 T cells for each study participant was calculated by subtracting the relative background levels at t0 and t1.

Cell-mediated immunity was considered positive when the net increase was >0.2%. Although retesting samples on separate occasions gave reproducible results, t0 and t1 samples for each participant were tested simultaneously to further reduce test variability.

Immunofluorescent Staining and Flow Cytometry Analysis

Monoclonal antibodies coupled with phycoerythrin (PE), peridinin-chlorophyll protein (PerCP), allophycocyanin (APC), and phycoerythrin-Cy-7 (PE-Cy7) were combined for simultaneous staining. In this study the following were used: anti-CD4 PerCP (IgG1, clone SK3), anti-CD3 PE-cy7 (IgG2a, clone SK7), anti-IFN-γ APC (IgG1, clone B27), and anti-IL-2 PE (IgG1, clone 5344.111) (Becton Dickinson). Cells were stained as previously described (*19*).

Multiparametric flow cytometry was performed by using a FACSCanto flow cytometer (Becton Dickinson). A total of 300,000 live events were acquired, gated on small viable lymphocytes, and analyzed with FACSDiva software (Becton Dickinson). The instrument was routinely calibrated according to the manufacturer's instructions.

Microneutralization and HI Assay

The NT was performed according to a previously described procedure (*20*), in agreement with indications from the World Health Organization (*21*) and the US Department of Health and Human Services (*22*). Specifically, 2-fold serial dilutions of heat-inactivated (30 min at 56°C) human sera were performed in 50 μL DMEM without FCS in 96-well microplates. An equal volume of influenza virus (H5N1) (10^3 $TCID_{50}$/mL) was then added to each well. Uninfected-cell wells, incubated with each test serum, were included in each plate as negative controls. After 1 h incubation at 37°C, the mixtures were transferred on MDCK cell monolayers and adsorbed at 37°C for 1 h. After washing, DMEM was added, and the plates were incubated for 2 days at 37°C in 5% CO_2. NT titer was assessed as the highest serum dilution in which no cytopathic effect was observed by light microscope inspection. All serum specimens were tested in duplicate, and t0, and t1 samples from each patient were assayed in the same plate at the same time. The results were scored by persons blinded to the study participant's identification. The test results were reproducible because random replication of the assays on independent occasions gave consistent results.

The antibody titer was also established by HI test, using for challenge either the seasonal vaccine or the egg-derived influenza (H5N1) preparation. HI assays were performed in V-bottom 96-well plates with 0.5% chicken erythrocytes, as described (*16*).

Biosafety Laboratory Facilities

All experiments with live highly pathogenic avian influenza A virus (H5N1) were conducted by using Biosafety Level 3-plus (BSL3+) containment procedures (*23*). All investigators were required to wear appropriate masks with HEPA filters.

Results

Cell-mediated Immunity to Influenza Viruses

The frequency of circulating antigen-specific CD4 T cells in healthy donors enrolled in the study was analyzed by flow cytometry, by using intracellular cytokine staining assay after the in vitro expansion of effector cells. To generate effector cells from their memory precursors, PBMC were challenged with antigen in vitro for 3 days and expanded for 6 additional days in the presence of IL-2 (*18*).

Effector cells were characterized for their ability to release IFN-γ when cultured overnight in the presence of antigen. CD4 T cells were gated and analyzed for IFN-γ and IL-2 cytokine expression. A representative experiment with PBMC from a recently vaccinated healthy donor is shown in Figure 1. Without stimuli, no cytokine production in CD4 T cells was detected (Figure 1, panel A). However, the stimulation with the seasonal influenza vaccine preparation induced the production of IFN-γ by CD4 effector T cells (Figure 1, panel B: 3.2% of IFN-γ+ CD4+ T cells). Stimulation with inactivated influenza (H5N1) virus induced a CD4 T-cell response (Figure 1, panel C: 1.0% of IFN-γ+ CD4+ T cells). Finally, some CD4 T cells specific for a pool of H5 and N1 (H5/N1) peptides were also generated in this donor (Figure 1, panel D: 0.6% of IFN-γ+ CD4+ T cells). No IL-2 production was observed in these experimental conditions.

Increased Cell-mediated Immunity after Seasonal Influenza Vaccination

When the extent of CD4 T-cell–mediated immunity before and after seasonal influenza vaccination was compared in the healthy donors enrolled in the study, a nonhomogeneous pattern of responses was detected (online Appendix Figure; available from www.cdc.gov/EID/content/14/1/121-appG.htm. After vaccination (t1), a 2-fold variation of the frequency of antigen-specific T cells higher than baseline was arbitrarily considered significant. According to this threshold, an increased frequency of IFN-γ–producing CD4 T cells specific for vaccine preparation was observed after vaccination in 5 (donors 8, 11, 17, 26, 42) of 21 donors (23.8%). A slight increase of frequency of the vaccine preparation-specific CD4 T cells was observed in 5 donors (donors 9, 12, 33, 36, 40; 23.8%); a mild-to-significant decrease was observed in the remaining donors (n = 11; 52.3%).

As shown in the online Appendix Figure, panel A, most donors had a detectable level of vaccine preparation–specific CD4 T cells before vaccination. Six donors (11, 17, 22, 23, 31, 42) had a noteworthy increase over baseline of IFN-γ–producing CD4 T cells specific for the H5N1

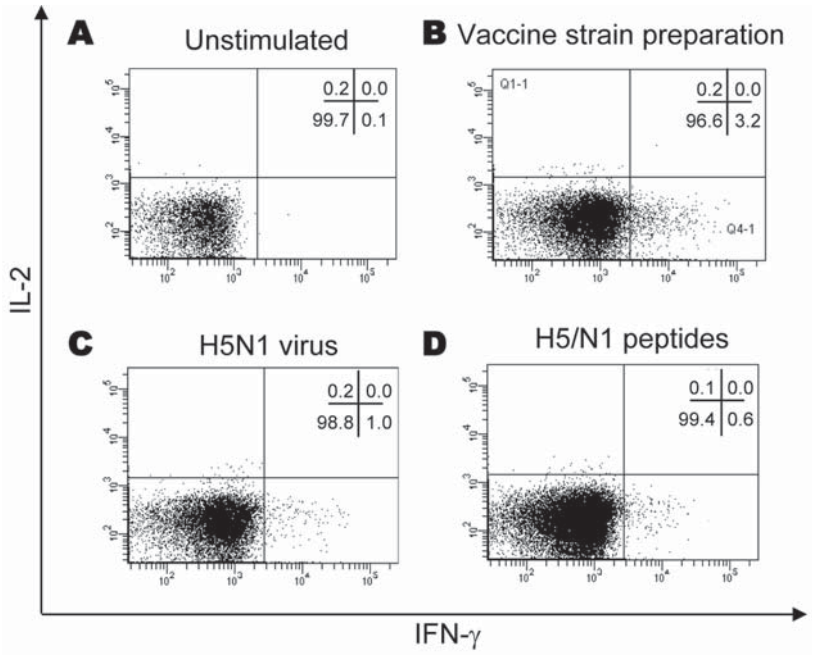

Figure 1. Detection of antigen-specific CD4 T cells against influenza viruses by flow cytometry after in vitro expansion of effector cells. Peripheral blood mononuclear cells were expanded in vitro with interleukin-2 (IL-2) for 9 days in the presence or absence of specific influenza antigens, as indicated, then analyzed by flow cytometry by using the intracellular staining assay. The effector T-cell response was analyzed for interferon-gamma (IFN-γ) or IL-2 cytokine expression. Unstimulated cultures (A), CD4 T-cell response against human influenza vaccine strain preparation (B), inactivated avian influenza (H5N1) (C), and H5/N1 peptides (D) are shown in a representative donor.

subtype (online Appendix Figure, panel B); among them, 3 were also showing an increase of the frequency of IFN-γ–producing CD4 T cells specific for vaccine preparation (donors 11, 17, 42). Two of them, donor 11 and donor 42, had a significant increase of IFN-γ–producing CD4 T cells specific for H5/N1 peptides (online Appendix Figure, panel C), which suggests that cross-type immunity may directly involve the HA/NA proteins. Furthermore, 3 other donors (donors 12, 16, 36) had an increased frequency of H5/N1 peptides–specific CD4 T cells, even if they were unable to respond to whole virus.

Indeed, in some persons we also observed a significant decrease at t1 in CD4 T cells specific for vaccine preparation (donors 2, 16, 23, 27, 30, 31, 35, 41), specific for influenza (H5N1) (donors 4, 16, 27), and specific for H5/N1 peptides (donors 2, 27, 34, 39, 41). Donors with a reduced specific response to vaccine preparation at t1 showed a higher frequency of specific CD4 T cells at t0 when compared to other donors (3.4% ± 0.88 vs. 1.29% ± 0.35, respectively, p = 0.013). Similar results were obtained when we observed the influenza virus (H5N1) (1.07% ± 0.47 vs. 0.14% ± 0.03, respectively, p = 0.0093) and H5/N1 peptides (1.19% ± 0.54 vs. 0.13% ± 0.07, respectively; p = 0.0018).

H5 versus N1 Specificity of the Cell-mediated Response

Because some study participants were reactive to inactivated influenza virus (H5N1) as well as to a peptide pool composed of 2 peptides from H5 and 2 from N1 consensus sequences, we analyzed whether this reactivity was preferentially directed against HA or NA. As shown by PBMC from a representative donor in Figure 2, the frequency of IFN-γ–producing CD4 effector T cells was appreciable after challenge with the inactivated influenza virus (H5N1) (Figure 2, panel B: 1.82% of IFN-γ+ CD4+ T cells) or with the H5/N1 peptides (Figure 2, panel C: 1.52% of IFN-γ+ CD4+ T cells). The response of PBMC from the same donor to N1 peptides was positive, whereas the response to H5 peptides was at background level (Figure 2, panel E and D: 1.49% vs. 0.14% of IFN-γ+ CD4+ T cells), a finding that suggests that N1 seems to be the main target for cell-mediated cross-type immunity against influenza (H5N1) and influenza (H3N2)/(H1N1) vaccine strains. A similar

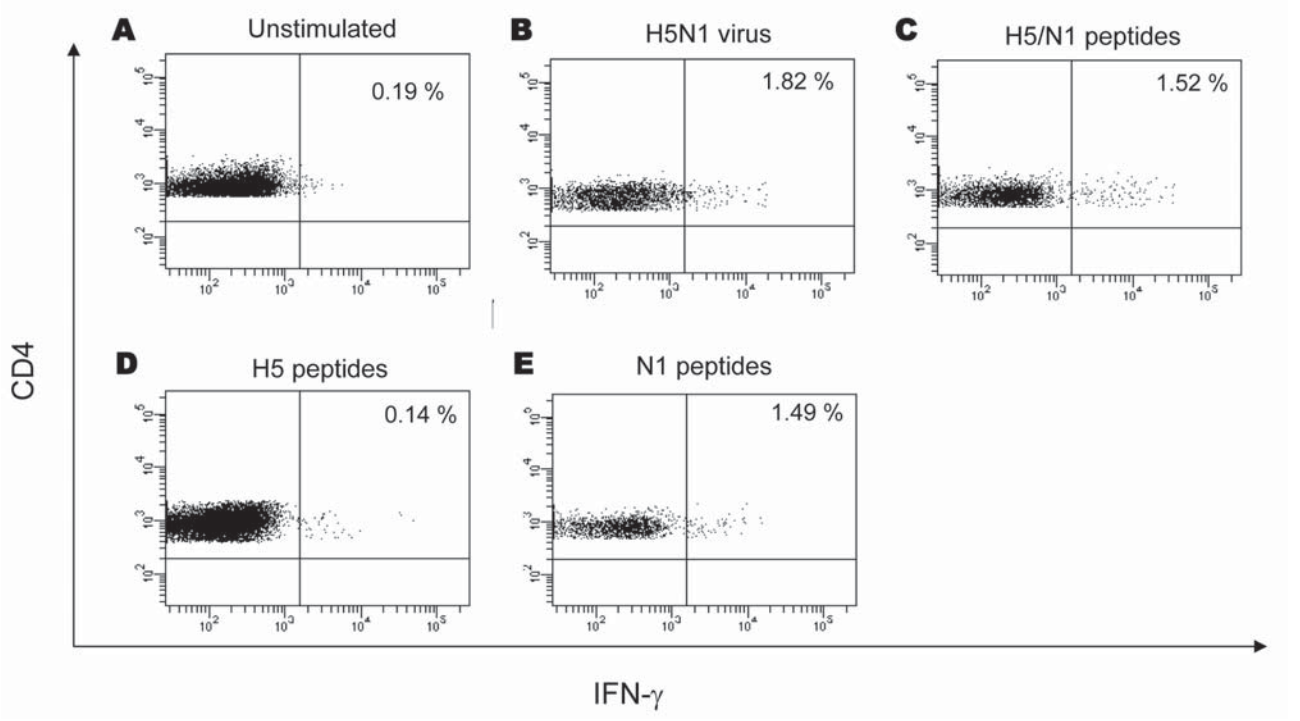

Figure 2. H5 versus N1 specificity of the cell-mediated response. Profiling of influenza (H5N1)–specific CD4 T-cell response in a representative study participant is shown. Peripheral blood mononuclear cells (PBMC) were expanded in vitro in the presence of interleukin-2 (IL-2) and stimulated with inactivated influenza (H5N1) virus (B), peptide pool composed by 4 peptides from H5 and N1 (C), H5 peptides (D) and N1 peptides (E). Panel A shows unstimulated cultures. Dot plots showed the presence, at similar frequency, of specific CD4 T cells when PBMC were stimulated with inactivated influenza (H5N1) virus (panel B, 1.82%), influenza (H5N1) peptides (panel C, 1.52%), and N1 peptides (panel E, 1.49%). No specific CD4 T cells producing interferon-gamma (IFN-γ) were observed after challenge with H5 peptides (D). As negative control, either mock-infected culture supernatants or irrelevant peptides were used, giving results very similar to unstimulated cultures (not shown). A similar pattern was observed in 4 other study participants, supporting the hypothesis that the actual target of cross-subtype immunity could be N1.

pattern was observed in 4 other study participants, which supports the hypothesis that the target of cross-subtype immunity could actually be N1.

Increased Humoral Immunity after Seasonal Influenza Vaccination

Human sera from the same donors were tested for HI activity against both vaccine and influenza (H5N1) preparations and for neutralization activity against influenza (H5N1) virus. Individual titers are reported in Figure 3. A 4-fold rise in HA antibody titer is considered noteworthy, and after vaccination most donors (28/38; 73.7%) showed a noteworthy rise of HI titers against vaccine preparation, as indicated by an asterisk (Figure 3, top panel, black bars). HI titers against influenza virus (H5N1) remained at undetectable levels after seasonal vaccination (data not shown), but a rise of neutralization titer >20-fold over baseline was observed in 13 (34.2%) of 38 donors (Figure 3, bottom panel, asterisk). All but 1 study participant also responded to seasonal vaccination by a rise in HI titers against vaccine preparation. One donor (21) showed high titers against the H5N1 subtype in NT but a low HI titer against vaccine, a unique situation in the study population. However, antibodies to both anti–influenza (H5N1) and influenza vaccine are raised by vaccination. Our findings indicate that seasonal vaccination can raise neutralizing immunity against influenza (H5N1), which shows the existence of an antibody-dependent cross-type immunity. No correlation between influenza-specific CD4 T cells and humoral responses was observed, which suggests that this type of antibody response was mainly CD4 T-cell independent.

Discussion

We observed that influenza-specific CD4-effector T cells could be generated by long-term cultures in vitro and easily monitored by flow cytometry as IFN-γ–producing cells. When this approach was used, a small frequency of CD4 T cells specific for H5N1 subtype could be detected in several persons at baseline. Seasonal vaccine administration may enhance the frequency of reactive CD4 T cells, boosting the cross-subtype cellular immunity against avian influenza (H5N1). We also observed that seasonal vaccination raised neutralizing immunity against H5N1 subtype in a large number of donors, showing the existence of an antibody-dependent cross-type immunity. Thus, cross-reactive immunity may involve cellular and/or humoral responses, but the humoral response seems to be CD4 independent.

From the present data, N1 appears to be 1 target for cross-type cellular immunity, although we could not rule out the involvement of different (i.e., internal) antigens as possible targets of immune recognition by effector CD4 T cells. Nevertheless, in animal models, cellular immunity (mainly CLT) targeting internal proteins (i.e., NP), partly responsible for heterosubtypic protection, was not induced efficiently by inactivated vaccines (*24*). We did not use live virus, only inactivated split vaccine, whole inactivated virus, or HA and NA peptides for the influenza (H5N1) A/Hong-Kong/156/97 strain. From our data, discriminating between the CD4 T-cell response against external or internal antigens in the case of vaccine preparation was not possible. For H5N1 subtype response, we can presume that the response is against the external antigens and that the results against peptides point to a specific response against NA.

Results obtained with the whole virus and those obtained with the H5 and N1 peptides are not in complete agreement (online Appendix Figure). This finding can be explained on the basis of the substantial differences in the antigen presentation underlying the whole virus and peptides. Moreover, we observed that a high activation of specific cells at baseline (t0) was associated with a reduced

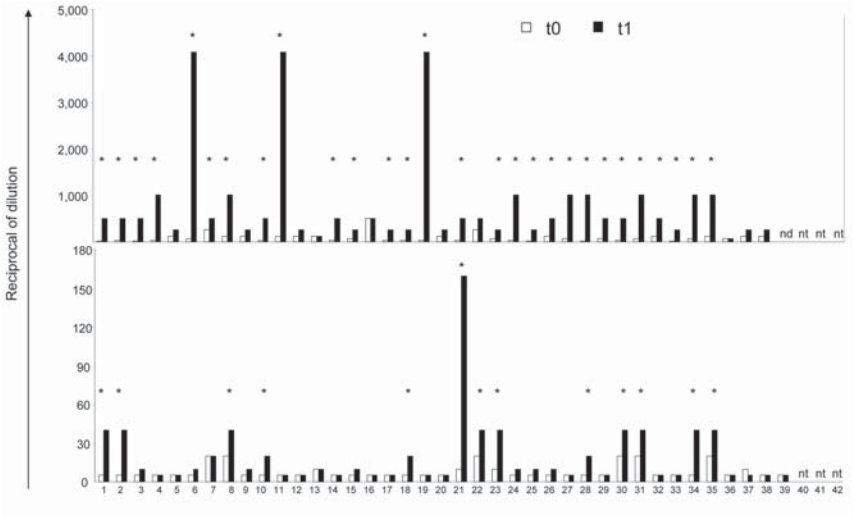

Figure 3. Humoral response against vaccine preparation and influenza virus (H5N1) before (t0) and after (t1) seasonal influenza vaccination. Hemagglutination inhibition (HI) test was used to calculate the antibody (Ab) titer against vaccine preparation (top panel), whereas a neutralization test was used to calculate the antibody titer against influenza (H5N1) (bottom panel) in healthy donors enrolled in the study at baseline (t0) and 1 month after seasonal influenza vaccination (t1). At baseline (white bars), all donors had a detectable level of human influenza antibodies. At t1 (black bars), 28 donors (73.6%) (indicated by *) showed a >4 fold increase of Ab titer against vaccine preparation (HI) over t0. After seasonal influenza vaccination, 13 serum samples (33.3%) (indicated by *) from the study population showed a 20-fold increase of neutralizing Abs against influenza (H5N1) over t0.

specific response after vaccination (t1), which suggests that stimulation of pre-activated T cells with high dose of antigen could induce T-cell anergy (*25*) with consequent loss of immune response.

Preliminary evidence also suggests that humoral cross-type immunity is targeting antigens differently from HA: sera from persons showing significant neutralizing titers against influenza (H5N1) did not recognize insect cells expressing HA from the H5N1 subtype (not shown) and did not show HI activity against H5N1 subtype. N1 may possibly also be a target of humoral immunity, but additional experiments such as Western blot analysis or inhibition of NA activity (*26*) are needed to clarify this point.

In animals, exposure to 1 specific subtype of influenza A virus can also induce protective immunity against challenges with other subtypes. This heterosubtypic or cross-protective immunity could represent a key mechanism for facing, and limiting, new influenza outbreaks. In 1997, during the Hong Kong influenza (H5N1) outbreak, an immune response induced by an influenza virus (H9N2), being T cells but not antibodies, protected chickens from lethal influenza (H5N1) (*13*). Moreover, adults living in an urban area of the United States have been described as having influenza-specific memory T cells that recognize epitopes of influenza A virus strains derived from swine and avian species, including the influenza (H5N1) strain involved in the Hong Kong outbreak in humans (*14*).

Our data confirm that persons who have never been exposed to H5N1 subtype may be able to generate a cell-mediated response against the Hong Kong influenza (H5N1) isolate. This cross-type response may be naturally occurring (probably as a consequence of exposure to seasonal influenza strains).

In mice, both CD4 T-cell–independent and –dependent antibody responses contribute to the control of influenza virus infection (*27*,*28*). Although antibodies appear to facilitate the recovery from influenza infection, it is generally believed that B cells cannot produce neutralizing, isotype-switched, influenza-specific antibodies in the absence of CD4 T-cell help (*29*,*30*). However, other data clearly demonstrate that B cells can also produce anti-influenza IgA, IgM, and IgG responses independent of CD4 helper T cells (*27*,*31*). A non–antigen-specific bystander response driven by activated CD4 T cells specific for heterologous antigen may contribute to so-called heterosubtypic immunity (*8–10*,*12*). However, the ability of influenza virus infection to promote B-cell activation and differentiation into short-lived, isotype-switched, antibody-secreting cells may result from a combination of B-cell receptor hypercross-linking, engagement of toll-like receptors, production of cytokines, as well as triggering of innate immunity.

In our study, cellular and humoral cross-reactive immunity seemed to target antigens other than HA. Influenza (H5N1) cases occur mainly in young people (*32*). This finding may be explained by hypothesizing that older people, although not previously exposed to H5N1 subtype, may have gained protective immunity by previous infections sustained by circulating influenza virus strains. It has also been shown that immunity to the N1 NA from the human influenza virus cross-reacts with the avian N1 NA virus and that this cross-reactivity protects mice against infection with the avian influenza virus (H5N1) (*26*). All these findings may be explained by hypothesizing that cross-reactive immunity is targeting the N1 NA antigen. However, whether cross-reactive antibodies to NA and CD4 T cells would be protective against illness and death, especially from influenza (H5N1) infection is not known. Further studies will be necessary to elucidate this point.

In conclusion, we demonstrated that vaccination against seasonal influenza may boost a cross-reactive immunity against an unrelated strain responsible for deadly infections in humans, i.e., the avian influenza (H5N1) strain A/Hong Kong/156/97. These data, together with previous experimental results from mice studies and epidemiologic reports, indicate that cross-type immunity should be considered an important component of the immune response against novel influenza A infections.

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Telephone Survey to Assess Influenza-like Illness, United States, 2006

Joseph L. Malone,* Mohammad Madjid, † and S. Ward Casscells*‡

Although current national response plans assume that most influenza-infected patients would stay home during a pandemic, surveillance systems might be overwhelmed and unable to monitor their health status. We explored the feasibility of using a nationwide telephone survey to monitor at-home patients. Of randomly selected adults surveyed during low influenza activity months (April–October 2006, surveillance weeks 17–41), 86% (7,268/8,449) agreed to answer questions about health status and influenza-like illness symptoms. Three percent (230/7,628) self-reported "flu." A subset (0.9%, 68/230) self-reported fever. In comparison, the Centers for Disease Control and Prevention's Sentinel Provider Network reported clinical influenza-like illness rates of 1.2%, 0.9%, and 1.2% for weeks 17, 20, and 41, respectively. The consistency between information obtained by telephone and surveillance data warrants further studies to determine whether telephone surveys can accurately monitor health status during seasonal influenza peaks and to augment current surveillance systems during a pandemic.

The US Department of Health and Human Services (DHHS), Centers for Disease Control and Prevention (CDC), and other government agencies have been working to strengthen public health systems and improve strategies for monitoring and controlling pandemic influenza. According to the US Homeland Security Council's National Strategy for Pandemic Influenza Implementation Plan, "... the public health community must have situational awareness of the evolution of disease that can only come from connectivity to the emergency departments and other acute care settings where patients with influenza are presenting. The interpandemic period presents an opportunity to establish and test these relationships" (*1*).

CDC currently supports the influenza surveillance reporting systems listed in Table 1 (*2*,*3*). Surveillance goals of the DHHS pandemic influenza plan are to 1) provide an early-warning system; 2) detect increases in influenza-like illness (ILI) at the local level; 3) monitor the impact of influenza on health (e.g., track outpatient visits, hospitalizations, and deaths); 4) track trends in influenza disease activity; and 5) identify severely affected populations (*3*). Outpatient surveillance of ILI is emphasized at the national, regional, and state levels through CDC's Sentinel Provider Network (SPN), which gathers and summarizes influenza illness surveillance reports from ≈2,300 healthcare providers nationwide (*2*). CDC plans to analyze daily reports of ambulatory patients with influenza that are accessed through the BioSense surveillance system (*4*,*5*) and use existing emergency department symptom-monitoring systems (*2*). This approach will strengthen systems that support situational awareness of influenza outbreaks, support geographic completeness and frequency of reporting, and ensure sustainable collection of ILI data during a pandemic.

The DHHS Pandemic Influenza Plan notes that "most patients with pandemic influenza will be able to remain at home during the course of their illness and can be cared for by other family members or others who live in the household" (*6*). Local health districts, although responsible for monitoring the care of these at-home patients and maintaining continuity of services, may be too overwhelmed to do so. Because of the expected high demand for medical services during a pandemic influenza emergency, SPN providers may also be overwhelmed, may have difficulty finding time to report outpatient ILI status in a timely manner, and

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Table 1. Current influenza surveillance systems reporting to the US Centers for Disease Control and Prevention*

System	Description	Reporting frequency
Sentinel Provider Network (SPN)	Reports percentage of outpatient visits for influenza-like illness and total patients seen for any reason from a network of 2,300 nationwide healthcare providers	Weekly
Emerging Infections Program (EIP)	Reports laboratory-confirmed influenza-associated hospitalizations of children <18 years of age in 11 US communities	Biweekly
New Vaccine Surveillance Network (NVSN)	Reports laboratory-confirmed influenza-associated hospitalizations of children <5 years of age in 3 US communities	Biweekly
122 Cities Mortality Reporting System	Reports pneumonia- and influenza-related deaths and total no. deaths in 122 US cities	Weekly
National Notifiable Disease Surveillance System (NNDSS)	Reports influenza-associated pediatric deaths recorded by participating state health departments	Weekly
State and Territorial Epidemiologists Report	Reports overall level of influenza activity in respective states, territories, or both	Weekly
US World Health Organization and Global Influenza Surveillance Network; National Respiratory and Enteric Virus Surveillance System (NREVSS)	Report no. influenza laboratory tests performed and no. positive results by type and, in some cases, subtype	Weekly

*See (*2*,*3*).

may be unable to monitor ILI patients at home (*4*). Inadequate surveillance will result in reduced situational awareness at local, state, and federal levels. Currently, none of the functioning federal public health systems has plans in place to identify or monitor the health status of at-home patients with ILI symptoms.

We investigated whether a national telephone survey system would be feasible to fill the gap in surveillance. To determine whether national and local situational awareness of influenza could be improved by monitoring at-home patients in a systematic way, we partnered with a public opinion research company to administer and analyze the results of a telephone survey. We report the results of that survey.

Methods

Using national pandemic influenza planning documents, CDC guidance, and the recent medical literature, we developed a telephone survey questionnaire (Table 2) that asked "Do you have the flu?" and questions about symptoms such as fever, cough, and sore throat. This design allows comparison of the survey data with the SPN's definition of ILI (i.e., fever [≥100°F (37.8°C)] and cough and/or sore throat in the absence of a known cause other than influenza). Possible risk factors for exposure to novel avian influenza strains through live poultry (*7*), foreign travel (*8*,*9*), and cats (*10*) were also addressed in the survey.

To explore the feasibility of using this telephone survey, we partnered with Zogby International (Utica, NY, USA) (*11*), a private public opinion research company experienced at collecting extensive demographic and socioeconomic information through nationwide telephone surveys. A protocol to perform telephone surveys was submitted January 11, 2006 for institutional review board (IRB) review, subsequent questionnaire modifications were approved March 20, 2006, and the first survey was completed the week of April 24, 2006, ≈110 days later. Monthly, ≈1,205 randomly selected adults, who were already providing demographic and socioeconomic information for other Zogby International telephone surveys, were asked to participate in a research study about influenza. Participants answered follow-up questions over 5-day intervals ending on each of the following dates in 2006 (corresponding to CDC influenza surveillance calendar weeks) (*12*): April 24 (week 17); May 16 (week 20); June 6 (week 23); July 25 (week 30); August 15 (week 33); September 14 (week 37);

Table 2. Telephone survey questions*

A. Do you have a cat(s)?
- A.1. How many?
- A.2. How many are recently sick?
- A.3. How many have died?

B. Do you have chickens?
- B.1. What proportion, if any, are sick?
- B.2. Have there been more deaths of chickens than usual this week?

C. Now we would like to ask you a few questions related to a research study conducted by the University of Texas Science Center at Houston. Are you willing to participate?
- C.1. Do you have the flu?
- C.1.1. Have you received your flu shot this year?
- C.1.2. What is your body temperature (fever)?
- C.1.2. Do any of your contacts have flu?
- C.1.3. Do you have shaking chills?
- C.1.4. Do you have body aches and muscle pain, in the back, arms or legs?
- C.1.5. Do you have a cough?
- C.1.6. Do you have a runny nose?
- C.1.7. Are you short-winded?
- C.1.8. Do you have a sore throat?
- C.1.9. Have you traveled to Southeast Asia recently?
- C.1.10. How long have you had these symptoms?

*Questions A–C were asked of all adults taking the telephone survey. Only respondents who answered "yes" to Question C were asked Question C.1; only respondents who answered "yes" to C.1 were asked the follow-up questions C.1.1 through C.1.10.

and October 12 (week 41). These dates were selected as a convenience sample based upon having received necessary IRB approvals and having the availability of telephone survey capacity by our telephone surveying partners. Telephone numbers for the surveys were randomly selected from commercially available national data sets of residential directory-listed telephone numbers. The probability of selection for the telephone survey was adjusted to be proportional to population sizes within area codes and telephone exchanges. As many as 6 calls were made to reach a sampled phone number, and the procedures did not result in the same telephone numbers selected to be surveyed for each subsequent month.

One adult from each randomly sampled household was asked to answer the survey questionnaire by telephone. All interviews were conducted by Zogby International's general interviewers, who are monitored by supervisors to assess adherence to surveying standards. For this study, the interviewer-to-supervisor ratio was 12:1. Quality control checks of interviewer performance were conducted on 10% of all calls. The survey was performed according to a protocol created by medical researchers at the University of Texas Health Science Center at Houston and approved by the university's Institutional Review Board. Survey results were tabulated, analyzed, and correlated by the study authors with nationwide influenza surveillance reports from CDC (Table 1) (*2*) for the 2005–06 and 2006–07 influenza seasons (*1*,*2*,*12*).

Results

Of 8,449 adults contacted, 7,268 (86%) agreed to participate in the survey. Participants were from representative socioeconomic and racial/ethnic groups (Table 3) across the United States. The overall monthly participation rate was 83%–87%. Participation rates for men and women were similar.

Of the 7,268 adults surveyed by telephone during 7 surveys (CDC weeks 17–41), 2,337 (32%) said that they had cats in the home, 184 (2.5%) lived in close contact with chickens, and 230(3.2%) answered "yes" when asked "Do you have the flu?" Of the 230 adults who answered yes to having the flu, only 49 (21%) reported having received an annual influenza vaccine, 68 (30%) reported having fever or abnormal body temperatures, 93 (40%) reported having a cough, and 89 (39%) reported having a sore throat (Table 4). According to CDC influenza surveillance reports for weeks 17, 20, and 41 (Table 5), influenza activity nationwide peaked in early March 2006 (at approximately week 10); influenza B virus was the most commonly isolated influenza virus during weeks 17 and 20, and influenza A virus was the most frequently isolated virus in week 41 (*12*). Data for weeks 21–39 in 2006 were not reported by CDC in the 2005–06 and 2006–07 influenza seasons (*12*).

Discussion

As shown in Table 4, our surveys of 7,268 adult respondents provided nationwide, prepandemic baseline information about household contact with cats (*10*) (28%–37%) and chickens (1%–4%), factors that may be relevant to the spread of currently circulating strains of avian influenza A (H5N1) virus. The data collected in 7 surveys over a 7-month period of low influenza activity were consistent with surveillance data gathered and reported by SPN.

More than 80% of persons in the populations and demographic subgroups surveyed nationwide agreed to participate in our study; this rate was similar for all regions of the country. The rate of self-reported illness from "flu" ranged consistently from 2% to 5% over the 7-month survey period, a time of low influenza activity and without reported human illnesses from highly pathogenic avian influenza A (H5N1) in the United States. Because only 30% (68/230) of respondents who self-reported the flu also self-reported fever, it follows that only a very small fraction of all respondents (0.9%, 68/7268) may have met CDC's definition of ILI (i.e., fever [≥100°F (37.8°C)] and cough or sore throat) (*12*). Similarly, during the same time frame, the SPN reported outpatient ILI rates of 1.2% and 0.9%, and the other CDC surveillance systems (Table 5) reported only regional and sporadic ILI in most states; moreover, none of the CDC-measured indicators suggested that influenza-related illness or death were excessive on the dates our surveys were conducted.

A comparison with CDC surveillance data suggests that the household telephone survey produced plausible, reproducible, and accurate results during a period when the circulation of influenza (predominantly influenza B virus) in the community was minimal. These results, when interpreted in the context of all other applicable surveillance reports, suggests that a direct telephone survey of adults at home could improve situational awareness of an influenza pandemic.Had unexpected trends or inconsistent rates of illness or apparent geographic disparities been identified, an analytic study could have been conducted to suggest possible risk factors or further investigations.

According to the DHHS Pandemic Influenza Plan, "Some states are considering the use of systematic phone surveys to supplement SPN data during a pandemic by providing estimates of local cases and affected households. CDC will explore the utility and feasibility of conducting this type of survey on a national level" (*2*). In a recent national telephone survey of 2,075 persons in Sweden, responses to the question "Did you have the 'flu' last week?" provided useful public health information regarding ongoing influenza disease activity (*13*). The survey took only 125 working hours to complete and cost approximately €3,250 (US $4,150). On the basis of our results, and assuming appropriate IRB approvals are already in place, we

estimate that a new questionnaire could be developed in conjunction with public health officials and the telephone survey partners within 1 to 2 days, and the surveys could be conducted over the next 2 days with results delivered essentially immediately to public health officials. The direct cost of conducting each of the 2- to 3-day telephone surveys involving 1,200 adults and asking 10 questions was estimated to be $14,000; the 7-survey project in Tables 2–4 had a direct cost of ≈$98,000. In the United States, CDC recently adapted an ongoing national household telephone survey project, the Behavioral Risk Factor Surveillance System (BRFSS), to measure and monitor influenza vaccination coverage during the 2004–05 influenza season (*14*). CDC surveyed 26,526 adults during February 1–27, 2005 (*14*), and reported that ≈6,363 (24%) had been vaccinated. This CDC study also showed that influenza vaccination coverage among adults through January of the 2004–05 influenza season was greatest among persons >65 years of age (62.7%). In the telephone survey reported here, 21% (49/230) of those adults who self-reported the flu had re-

Table 3. Results of a national telephone survey of US adults at home (2006) regarding influenza-like illness, cats, and live chickens in the household*

Information and questions	Survey date						
	24 Apr 2006 (wk 17)	16 May 2006 (wk 20)	06 Jun 2006 (wk 23)	25 Jul 2006 (wk 30)	15 Aug 2006 (wk 33)	14 Sep 2006 (wk 37)	12 Oct 2006 (wk 41)
No. participants/no. surveyed (%)	1,039/1,209 (86)	1,036/1,200 (86)	1,068/1,205 (89)	1,031/1,200 (86)	1,048/1,214 (86)	1,001/1,210 (83)	1,045/1,211 (86)
No. participants who answered "yes" to the following (%)							
"Do you have the flu?"	41 (4)	32 (3)	30 (3)	46 (5)	23 (2)	23 (2)	35 (3)
"Do you have any cats?"	379 (37)	316 (30)	298 (28)	331 (32)	354 (34)	330 (33)	330 (32)
"Do you have any chickens?"	21 (2)	15 (1)	39 (4)	44 (4)	18 (2)	14 (1)	33 (3)
Sex, no. participants/no. surveyed (%)							
Male	514/583 (88)	491/578 (85)	509/581 (88)	492/578 (85)	509/585 (87)	492/583 (84)	520/584 (89)
Female	525/626 (84)	545/662 (82)	558/624 (90)	539/622 (87)	539/629 (86)	509/627 (81)	526/627 (84)
Age, y, no. participants/no. surveyed (%)							
18–29	195/239 (82)	186/237 (78)	198/237 (83)	188/236 (80)	192/236 (81)	207/236 (88)	211/237 (89)
30–49	418/477 (88)	425/475 (90)	438/474 (92)	416/472 (88)	422/472 (89)	395/472 (84)	416/474 (88)
50–64	248/274 (90)	247/273 (90)	240/273 (88)	250/271 (92)	244/272 (90)	227/272 (84)	242/273 (89)
≥65	165/203 (82)	169/202 (84)	176/200 (88)	165/201 (82)	164/201 (82)	153/201 (76)	153/201 (76)
Ethnicity, no. participants/no. surveyed (%)							
White	787/896 (88)	763/878 (87)	795/887 (89)	765/879 (87)	775/884 (88)	733/884 (83)	769/885 (87)
Hispanic	96/119 (81)	90/119 (76)	107/120 (89)	93/119 (78)	104/120 (87)	105/119 (88)	101/120 (84)
African American	106/131 (81)	117/131 (89)	115/132 (87)	121/131 (92)	103/131 (79)	100/131 (76)	116/132 (88)
Asian	19/24 (79)	21/24 (88)	23/24 (96)	20/24 (83)	18/24 (75)	19/24 (79)	20/24 (83)
Other	19/24 (79)	33/36 (92)	24/36 (67)	24/36 (67)	33/36 (92)	30/36 (83)	27/36 (75)
Marital status and children, no. participants/no. surveyed (%)							
Married	594/676 (88)	556/654 (85)	582/651 (89)	576/644 (89)	567/656 (86)	597/688 (87)	606/702 (86)
Children in home	336/385 (87)	334/385 (87)	406/442 (92)	351/393 (89)	343/389 (88)	NA	NA
Home locale, no. participants/no. surveyed (%)							
Large city	259/292 (89)	285/322 (89)	286/326 (88)	262/313 (84)	267/292 (91)	273/332 (82)	296/341 (87)
Small city	270/342 (79)	300/355 (85)	265/317 (84)	281/343 (82)	280/332 (84)	310/382 (81)	282/327 (86)
Suburb	230/259 (89)	161/186 (87)	223/231 (97)	211/235 (90)	171/203 (84)	182/213 (85)	205/228 (90)
Rural	270/312 (87)	279/325 (86)	283/319 (89)	262/290 (90)	327/380 (86)	232/275 (84)	257/302 (85)
Region, no. participants/no. surveyed (%)							
East	244/278 (88)	242/276 (88)	250/277 (90)	239/276 (87)	243/279 (87)	234/278 (84)	239/279 (86)
South	263/314 (84)	278/312 (89)	262/313 (84)	274/312 (88)	266/316 (84)	284/315 (90)	276/315 (88)
Central	328/375 (88)	324/372 (87)	325/374 (87)	324/372 (87)	318/376 (85)	277/375 (74)	323/375 (86)
West	204/242 (84)	192/240 (80)	231/241 (96)	194/240 (81)	220/243 (91)	206/242 (85)	207/242 (86)
Education, no. participants/no. surveyed (%)							
Less than high school	345/52 (87)	213/240 (89)	178/240 (74)	191/240 (80)	210/242 (87)	180/241 (75)	205/242 (85)
High school graduate	221/265 (84)	266/335 (79)	304/336 (90)	279/336 (83)	285/339 (84)	273/338 (81)	297/339 (88)
Some college	306/356 (86)	250/276 (91)	259/276 (94)	242/276 (88)	236/278 (85)	238/278 (86)	241/278 (87)
More than college	466/534 (87)	305/347 (88)	323/348 (93)	318/348 (91)	314/351 (90)	308/350 (88)	300/351 (86)

*Data regarding annual income available from authors upon request. NA, not available.

ceived an annual influenza vaccine. The rates of influenza immunization reported in our August, September, and October 2006 surveys were lower than those in the early months of 2006, possibly because the 2006–07 trivalent immunizations were not yet available through healthcare providers at that time; however, this trend in coverage was similar to the monthly coverage trends reported by CDC's BRFSS in 2004–05 (*14*).

Commercial polling agencies already elicit personal information from the public on an ongoing basis. Recently, as demonstrated by its adaptation of the BRFSS system, CDC was able to obtain information in response to a new public health problem by adding a few questions to an existing telephone survey. Although this adaptation of the survey, data analysis, and reporting infrastructure of the BRFSS appears to have been successful for determining influenza health status, it has apparently not yet been translated into a permanent surveillance system or ongoing capability. Moreover, the BRFSS might be overwhelmed in a pandemic emergency and, on very short notice, be forced to use additional telephone surveys that exist in the private and academic sectors. The adaptation of a private opinion survey company's capabilities in partnership with an academic medical center through approved protocols could create a feasible, safe, inexpensive, flexible, and acceptable way of deriving public health information in emergencies and improving situational awareness.

Our study has several limitations. First, the telephone surveys were conducted only during months with low influenza activity in 2006. Second, although 7,268 adults were asked if they had "the flu," only those who responded affirmatively (230 adults) were asked further questions about their influenza vaccination status and the presence of fever, cough, and sore throat. Biases may have been introduced based on the sequential approach to these questions, because some persons with fever, cough, or sore throat may have been misclassified by answering "No" to have the flu; additional follow-up questions about these ILI-related symptoms were not asked of all those surveyed. Third, this study had limitations similar to those of CDC's BRFSS: 1) being a land-line telephone–based survey, our study excluded adults in households without telephones and adults who use only cellular telephones; and 2) because the data were self-reported and subject to recall bias, especially for questions that required recall over a longer period, frequency estimates might be less precise for some conditions or behaviors. Fourth, our estimates of the proportion of adults with nondirectory-listed telephone numbers including those who had ILI (*12*) were based on unmeasured self-reported temperatures (*13*) rather than on direct observations by healthcare providers, as in the SPN intended for ambulatory populations in healthcare facilities and clinics. Fifth, other illnesses and other viral infections besides influenza can cause ILI and can be accompanied by fever, cough, or sore throat. Finally, there was no laboratory confirmation of influenza or ILI in our survey participants.

Nonetheless, we were able to analyze our data in terms of self-reported fever, cough, or sore throat, thereby enabling us to estimate the proportion of respondents who met CDC's definition of ILI (*12*,*13*). This capability might be useful during an actual pandemic, when it might be more desirable to assess the health status of patients by telephone (*15*) rather than exposing these and other patients

Table 4. Health status information for patients answering "yes" to "Do you have the flu?" and possible risk factors for pandemic influenza illness, United States, 2006

Information and questions	Survey date						
	24 Apr 2006 (wk 17)	16 May 2006 (wk 20)	06 Jun 2006 (wk 23)	25 Jul 2006 (wk 30)	15 Aug 2006 (wk 33)	14 Sep 2006 (wk 37)	12 Oct 2006 (wk 41)
No. participants in survey	1,039	1,036	1,068	1,031	1,048	1,001	1,045
No. who answered "yes" to "Do you have the flu?"	41	32	30	46	23	23	35
If answered "yes" to "Do you have the flu?" self-responded "yes" to the following, no. (%)							
Annual influenza vaccination	12 (29)	14 (44)	14 (47)	30 (65)	5 (22)	2 (9)	2 (6)
Elevated body temperature	11 (27)	22 (69)	9 (30)	7 (15)	1 (4)	13 (57)	5 (14)
Contact with others having flu	16 (39)	6 (19)	14 (47)	24 (52)	13 (57)	8 (35)	3 (9)
Cough	15 (37)	12 (38)	16 (53)	24 (52)	5 (22)	10 (44)	11 (31)
Aches	23 (56)	25 (78)	9 (30)	32 (70)	9 (39)	16 (70)	7 (20)
Chills	9 (22)	3 (9)	4 (13)	21 (46)	9 (39)	10 (43)	9 (26)
Runny nose	22 (54)	23 (72)	8 (27)	23 (50)	5 (22)	14 (61)	9 (26)
Short-windedness	9 (22)	7 (22)	8 (27)	29 (63)	12 (52)	9 (39)	2 (6)
Sore throat	15 (37)	22 (69)	5 (17)	23 (49)	8 (35)	6 (26)	10 (29)
Southeast Asia travel	0 (0)	1 (3)	0 (0)	19 (41)	2 (9)	1 (4)	1 (3)
Illness duration <10 days	24 (59)	21 (66)	16 (53)	26 (57)	7 (30)	18 (78)	20 (57)

Table 5. Summary of CDC national influenza surveillance reporting, United States, 2006*

Influenza surveillance system	Data reported	Survey date†		
		29 Apr 2006 (wk 17)	20 May 2006 (wk 20)	14 Oct 2006 (week 41)
Sentinel Provider Network (SPN)	Outpatient visits	ILI rate = 1.2% (<2.2% national baseline)	ILI rate = 0.9% (<2.2% national baseline)	ILI rate =1.2% (<2.1% national baseline)
Emerging Infections Program (EIP)	Laboratory-confirmed influenza hospitalizations of children <18 years of age	1.08/10,000 (cumulative)	1.21/10,000	Not reported
New Vaccine Surveillance Network (NVSN)	Laboratory-confirmed influenza-associated hospitalizations of children <5 years of age	4.3/10,000 (cumulative)	5.4/10,000	Not reported
122 Cities Mortality Reporting System	Pneumonia and influenza deaths compared with all causes of death	7.1% < threshold 7.8%	6.3% < threshold 7.4%	5.6% < threshold 6.38%
National Notifiable Disease Surveillance System	Seasonal influenza-associated pediatric deaths reported by all state health departments	30	35	0
State and Territorial Epidemiologists Report	State-level assessments of influenza activity	Regional activity in 3 states (KY, NY, CT); focal activity in 4 states (HI, ME, MA, PA) and District of Columbia; sporadic or no activity in 48 states	Sporadic activity in 25 states; no Activity in 25 states	Local activity in 2 states (AL, HI); sporadic activity in 6 states (CA, ID, TX, FL, LA, NE); no activity in 43 states
National Respiratory and Enteric Virus Surveillance System	Predominant circulating virus	Influenza B	Influenza B	Influenza A
US WHO and Global Influenza Surveillance Network; National Respiratory and Enteric Virus Surveillance System (NREVSS)	Percentage of laboratory specimens positive and ILI positive for influenza virus	9.4	6.3	1.0

*CDC, Centers for Disease Control and Prevention; ILI, influenza-like illness as defined by CDC (*6, 12*); WHO, World Health Organization.
†Weeks 23, 30, 33, and 37 data were not available because CDC reported results only through week 20 in the 2005–06 influenza season and began reporting results on week 40 in the 2006–07 influenza season. The last day of the CDC week reports vary by several days from the last day of the survey results reported in Tables 3 and 4.

and healthcare workers to the risk for healthcare-associated transmission of respiratory pathogens in clinical settings. Although the SPN's nationwide estimate of ILI, determined using CDC criteria, was similar to our telephone survey-based estimate for the periods of low influenza activity in 2006, more data are needed to assess the performance of these methods during seasonal influenza peaks and during a pandemic. Others have suggested that sentinel surveillance studies typically underestimate influenza in a population; thus, telephone surveys may prove to be an increasingly important component of influenza surveillance (*13*).

Conclusion

Telephone surveys might offer a practical solution to addressing the gaps in knowledge of influenza health status that might arise during a pandemic. Further telephone surveys should be performed during the peak influenza season to determine whether such an approach to surveillance would be a useful addition to ongoing influenza surveillance systems. The usefulness of the telephone survey to gain influenza immunization history and current ILI information on all people at risk should also be explored.

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Experimental Infection of Swans and Geese with Highly Pathogenic Avian Influenza Virus (H5N1) of Asian Lineage

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The role of wild birds in the epidemiology of the Asian lineage highly pathogenic avian influenza (HPAI) virus subtype H5N1 epizootic and their contribution to the spread of the responsible viruses in Eurasia and Africa are unclear. To better understand the potential role of swans and geese in the epidemiology of this virus, we infected 4 species of swans and 2 species of geese with an HPAI virus of Asian lineage recovered from a whooper swan in Mongolia in 2005, A/whooper swan/Mongolia/244/2005 (H5N1). The highest mortality rates were observed in swans, and species-related differences in clinical illness and viral shedding were evident. These results suggest that the potential for HPAI (H5N1) viral shedding and the movement of infected birds may be species-dependent and can help explain observed deaths associated with HPAI (H5N1) infection in anseriforms in Eurasia.

The first indication of wild bird involvement in the Asian lineage highly pathogenic avian influenza (HPAI) virus (H5N1) epizootic occurred late in 2002 and 2003, when HPAI (H5N1) was isolated from captive and wild birds in Hong Kong Special Administrative Region, People's Republic of China (*1*). Since these initial outbreaks, HPAI (H5N1) has continued to cause illness and death in a variety of wild birds in Asia (*2*), and in 2005 the virus was recovered from migratory waterfowl during a wild bird die-off involving primarily bar-headed geese (*Anser indicus*) at Qinghai Lake, People's Republic of China (*3*). Although several thousand birds died in this outbreak (*4*), it is unknown how many birds, including other species, were infected and dispersed from Qinghai Lake. In the late summer through fall of 2005, Asian lineage HPAI virus (H5N1) was first detected in Europe, where it was isolated from dead wild waterfowl in several European Union member states and neighboring countries (*5*). Most of these HPAI (H5N1) isolates were recovered from a limited number of species in the order Anseriformes, including mute swans (*Cygnus olor*), whooper swans (*C. cygnus*), tufted ducks (*Aythya fuligula*), and Canada geese (*Branta canadensis*) (*5*,*6*).

Although the ability of Asian lineage HPAI (H5N1) to infect and cause death in wild birds has been documented, the epidemiology of this virus in free-ranging avian populations is unclear. Wild avian species infected by this virus in Asia have been taxonomically diverse, whereas in Europe, most deaths and HPAI virus (H5N1) isolations have been limited to only a few species of geese and swans. A growing body of genetic and epidemiologic evidence suggests that, in 2005, migratory waterfowl played a role in the geographic spread of Asian lineage HPAI virus (H5N1) to Europe (*5*,*7*). However, the virus has not been detected in clinically healthy wild birds in Europe that were not associated with ongoing bird die-offs (*8*), and no evidence has clearly shown that the virus is maintained or geographically spread by infected asymptomatic wild birds. A reliance on dead bird surveillance makes HPAI virus (H5N1) in wild waterfowl difficult to evaluate and has left several gaps in our understanding (*9*). Whether Asian lineage HPAI (H5N1) that spilled over from domestic poultry to migratory waterfowl has or can be maintained in wild avian populations or whether similar outbreaks will recur is not known.

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The objective of this study was to evaluate the susceptibility and viral shedding patterns in 4 species of swans and 2 species of geese that were experimentally infected with HPAI virus (H5N1) and to then predict the ability of each species to spread the virus into new areas. Susceptibility was determined on the basis of prevalence and onset of illness and death and distribution of microscopic lesions and viral antigen. Viral shedding patterns were based on duration, route, and concentration of viral excretion. We evaluated the potential ability of a given species to geographically move the virus on the basis of the duration and viral titers associated with asymptomatic shedding.

Methods

Animals

We used 4 species of swans and 2 species of geese in this study: whooper swan, black swan (*C. atratus*), trumpeter swan (*C. buccinator*), mute swan, bar-headed goose, and cackling goose (*B. hutchinsii*). All birds used in this study were bred in captivity and purchased from commercial breeders in the United States. The swans were 5–6 weeks of age at the time of the experiment. This age was chosen on the basis of availability of birds and size restrictions imposed by the isolation units. Geese were ≈12 weeks of age at the time of the experiment, which corresponds to the age of juvenile waterfowl during the peak prevalence of avian influenza virus (AIV) in wild waterfowl in North America (*10*). Male and female birds were included in each species in approximately equal numbers. Infected birds for each species were housed separately in groups of 4 or 5 (inoculated and contact birds) in self-contained isolation units, which were ventilated under negative pressure with HEPA-filtered air. Sham-inoculated birds were maintained in separate units from the infected birds and grouped by individual species. The birds were maintained under continuous lighting, and food and water were provided ad libitum.

All birds used in this study were cared for in accordance with the guidelines of the Institutional Animal Care and Use Committee, as outlined in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (*11*) and under an animal use protocol approved by the Institutional Animal Care and Use Committee at the Southeast Poultry Research Laboratory (SEPRL), Agricultural Research Service (ARS), United States Department of Agriculture (USDA), Athens, Georgia, USA, and at the University of Georgia (UGA), Athens, Georgia, USA. All experiments were performed in the USDA-certified Biosafety Level 3-Ag facility at SEPRL (*12*).

Viruses

A stock of the A/whooper swan/Mongolia/244/2005 (H5N1) (Mongolia/2005) HPAI virus was produced by second passage in 9-day-old embryonated chicken eggs. Allantoic fluid from the inoculated eggs was diluted in brain–heart infusion (BHI) medium to yield a final titer of 10^6 median embryo infectious doses (EID_{50}) per 0.1 mL (single-bird inoculum) as determined by standard procedures (*13*). A sham inoculum was prepared by diluting sterile allantoic fluid 1:100 in BHI. The Mongolia/2005 virus was originally isolated from a dead whooper swan during a large die-off of waterfowl (*14*), and the isolate was chosen for use in this study because of its known lethality in wild waterfowl under natural and experimental (*15*) conditions. In addition, this strain appears to be a genetically representative isolate from the wild bird HPAI virus (H5N1) (clade 2.2) that has been reported in Asia, Europe, and Africa (*15*). Extrapolations from our data herein were made with the assumption that the Mongolia/2005 virus is representative of the 2005–2006 Asian lineage HPAI (H5N1) isolated from dead wild birds in Eurasia.

Experimental Design

Preinoculation serum was collected from each bird to confirm that they were serologically negative to influenza A type–specific antigens by the agar gel precipitin (AGP) test and the H5 hemagglutinin subtype by the hemagglutinin–inhibition (HI) test. In addition, oropharyngeal and cloacal swabs were collected from each bird to ensure that they were not actively infected and shedding AIV at the start of the study. Two (whooper swan and cackling goose) or 3 (trumpeter swan, black swan, mute swan, and bar-headed goose) birds from each species were inoculated intranasally (IN) with 0.1 mL of the Mongolia/2005 virus solution. After 24 hours, 2 additional birds from each species were placed in the housing unit with the inoculated birds. All birds were monitored daily for illness or death for 14 days. Illness was defined as any clinical abnormality detectable upon physical examination after inoculation with, or contact exposure to, the HPAI virus (H5N1), including weakness, cloudy eyes, respiratory difficulty, shivering, crowding, ruffled feathers, hemorrhage on the unfeathered skin, and neurologic signs, such as tremors, seizures, severe incoordination, and torticollis. Oropharyngeal and cloacal swabs were collected and then placed in BHI with antimicrobial agents (400 μg/mL gentamicin, 4,000 U/mL penicillin, and 5 μg/mL amphotericin B) from all birds at 1, 2, 3, 4, 5, 7, 10, and 14 days postexposure (dpe). At 14 dpe, blood was collected from the surviving birds for serologic testing, and the birds were euthanized with intravenous sodium pentobarbital (100 mg/kg body weight). Blood was not collected from birds that died during the course of the study. Necropsies were performed on all birds, and routine tissues were collected for histopathologic and immunohistochemical evaluation. In addition, oropharyngeal and cloacal swabs were collected from all birds that died and

were stored in BHI with antimicrobial agents for virus isolation. In reporting the temporal data, 0 days postcontact (when the contact birds were placed into the cage with the inoculated birds) was assumed to be equivalent to 0 days postinoculation.

One bird from each species was inoculated IN with a sham solution and housed in a separate unit from the virus-exposed birds. Oropharyngeal and cloacal swabs and preinoculation serum samples were collected from these birds before inoculation to confirm that they were not actively infected with an AIV and were negative for serum antibodies to the type-specific A antigen and the H5 hemagglutinin. These birds were monitored for illness and death for the 14-day trial. At 14 DPE, serum was collected from each sham-inoculated bird for serologic testing, and the birds were euthanized as described above. A necropsy was performed on each bird, and samples were collected for histopathologic and immunohistochemical evaluation.

Histopathologic Examination and Immunohistochemical Testing

Tissues samples collected at necropsy were preserved in 10% neutral buffered formalin. After fixation, the tissues were routinely processed and embedded in paraffin. Sections were cut at 5 μm and stained with hematoxylin and eosin. Duplicate sections were cut and immunohistochemically stained by using a mouse-derived monoclonal antibody (P13C11) specific for type A influenza virus nucleoprotein antigen as the primary antibody (SEPRL). The procedures used to perform the immunohistochemical testing have been previously described (*16*). Fast red was used as the substrate chromagen, and slides were counterstained with hematoxylin. Demonstration of viral antigen was based on chromagen deposition in the nucleus, with or without chromagen deposition in the cytoplasm.

Virus Isolation

Oropharyngeal and cloacal swabs collected at necropsy were stored at –70°C until virus isolations and titrations were performed. Isolation of virus from the swabs was performed in 9- to 11-day-old embryonated chicken eggs by using standard procedures (*13*). Positive samples were also titrated in 9- to 11-day-old embryonated chicken eggs by determining the EID_{50}. The minimal detectable titer from the swabs was $10^{0.97}$ EID_{50}/mL.

Serologic Assays

Serologic testing was performed on the pre- and postinoculation serum with the AGP and HI tests by using standard procedures (*17*). The HI tests were performed by using a 0.5% suspension of chicken erythrocytes in phosphate-buffered saline. Serum was pretreated with chicken erythrocytes to neutralize any naturally occurring serum hemagglutinins, and the first dilution on the test plate was 1:8. All HI titers ≥8 were considered positive.

Results

Disease, deaths, viral distribution, and pathology differed among the swans and geese infected with the Mongolia/2005 virus strain (Table 1). Among all species of swans, 100% of infected birds died, including all birds that were directly inoculated with the virus and those that acquired the infection through contact exposure. Viral shedding was detected in each of the IN-inoculated birds (including all swan and goose species) at 1 dpe (average oropharyngeal titer at 1 day postinoculation: black swans, $10^{4.30}$ EID_{50}/mL; mute swans, $10^{3.23}$ EID_{50}/mL; trumpeter swans, $10^{4.17}$ EID_{50}/mL; whooper swans, $10^{4.10}$ EID_{50}/mL; bar-headed geese, $10^{3.83}$ EID_{50}/mL; cackling geese, $10^{3.50}$ EID_{50}/mL). Challenge virus was detected in oropharyngeal and cloacal swabs from every IN-inoculated and contact bird of each species studied except 1 bar-headed goose, which is described later. Viral shedding was detected in each of the contact swans by 1 dpe but was delayed in contact geese; virus was not detected until 3 dpe in the cackling geese and 2 dpe in the bar-headed geese. Similarly, there was no difference between the onset of detectable clinical signs and death in swans that were inoculated with virus and those that were exposed through contact. Contact geese, however, had a delayed onset of detectable clinical signs (cackling geese, 6.5 days; bar-headed geese, 6.5 days) and death (cackling geese, 7.5 days; bar-headed geese, 8.0 days) compared with

Table 1. Disease, death, and pathologic data from 4 species of swans and 2 species of geese exposed to highly pathogenic avian influenza virus (HSM) by intranasal inoculation and contact with infected birds*

Species	Disease rate (d to onset)	Duration, d (range)†	Mortality rate (d to death)	Virus distribution
Black swan	5/5 (1–2)	<1 (0–1)	5/5 (2–3)	Blood vessels
Trumpeter swan	5/5 (2)	4 (3–5)	5/5 (4–6)	Brain, skin, multiple organs‡
Whooper swan	4/4 (2–4)	3 (1–5)	4/4 (4–4)	Brain, skin, multiple organs
Mute swan	5/5 (5–7)	<1 (0–1)	5/5 (5–8)	Brain, skin, multiple organs
Cackling goose	4/4 (3–7)	3 (1–9)	3/4 (4–8)	Brain, pancreas, liver, adrenal gland
Bar-headed goose	5/5 (3–7)	4 (1–8)	2/5 (6–7)	Brain

*Exposure date for each species was adjusted so that 0 d postcontact (when the contact birds were placed into the cage with the inoculated birds) was assumed to be equivalent to 0 d postinoculation.
†Average duration of detectable clinical signs.
‡Adrenal gland, pancreas, liver, lungs, heart, spleen, kidneys, air sacs, trachea, intestinal parasympathetic ganglia, and gastrointestinal tract.

IN-inoculated geese (clinical signs: cackling geese, 3.5 days; bar-headed geese, 3.3 days; death: cackling geese, 4.0 days; bar-headed geese, 6.0 days)

Black swans were the most susceptible species examined in this study; 100% died within 2–3 dpe. Most black swans were found dead without having exhibited any clinical signs of disease. When disease was observed, it lasted for <24 hours, and clinical signs included severe listlessness and neurologic dysfunction consisting of seizures, tremors, and marked incoordination. Influenza viral antigen was detected primarily in endothelial cells lining the blood vessels throughout most visceral organs and the brain (Figure 1, panel A). Microscopic examination showed that all black swans that died had widespread multiorgan necrosis with mild acute inflammation, which was strongly correlated with the distribution of the virus. All of the black swans shed virus before death and, as with all birds in this study, titers were higher in respiratory secretions than in feces (Table 2). All waterfowl that died shed virus in respiratory secretions and feces; shedding generally increased with time and reached a maximum within 24–48 hours of death.

Susceptibility was similar in the remaining 3 species of swans. Disease and death occurred later in these species, and the duration of illness, with 1 exception, was longer. Clinical signs consisted of mild to moderate listlessness, which progressively worsened to severe listlessness with neurologic signs similar to those observed with the black swans. Viral antigen was detected in the neurons (Figure 1, panel B), astrocytes, and other parenchymal cells of the brain and most of the examined visceral organs, as opposed to the vasculotropic distribution in black swans. Microscopic lesions were strongly associated with the anatomic location of detectable viral antigen and consisted of multifocal to coalescing necrosis with mild to moderate heterophilic inflammation. Within this category, the Mongolia/2005 virus infection in mute swans was unique. Clinical signs occurred later (5–7 dpe) in mute swans than in any of the other species examined in this study. The duration of disease in mute swans was extremely short (<24 hours) and comparable to the duration of disease in black swans. The clinical signs observed in mute swans were similar to those in the whooper and trumpeter swans. Birds in all 3 of these species shed high concentrations of virus in respiratory secretions with maximum titers approximating those of the black swans.

The 2 species of geese differed in their susceptibility to the Mongolia/2005 virus and both were less susceptible than the swan species. All of the cackling geese became sick after inoculation with the Mongolia/2005 virus, but only 3 of the 4 birds died and the remaining bird slowly recovered until clinical signs of disease were no longer apparent. The cackling geese that died exhibited severe listlessness and marked neurologic signs similar to those observed in the swans. The single goose that survived became moderately listless with ruffled feathers and cloudy eyes before clinical signs resolved but did not exhibit neurologic clinical signs during the study. This goose produced postexposure antibodies to AIV that were detected by the AGP and HI tests. Cackling geese that died had a short duration of illness (average duration 1.67 days) as opposed to the goose that survived, which exhibited detectable clinical signs for 9 days before resolution. Viral antigen in the 3 geese that died was restricted to the brain, pancreas, liver, and adrenal gland. Microscopic lesions primarily involved these organs and included multiple foci of necrosis with moderate heterophilic to lymphoplasmocytic inflammation. The single goose that survived had minimal amounts of viral antigen in the neurons of the brain and mild perivascular encephalitis. The surviving goose also

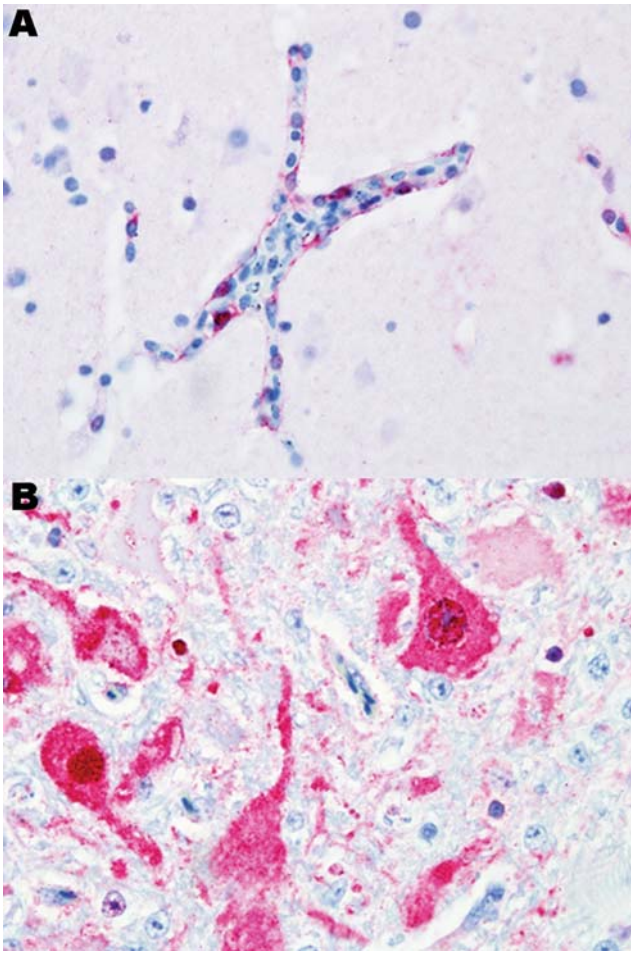

Figure 1. Photomicrograph of viral antigen (red). A) Endothelial cells lining a blood vessel in the brain of a black swan. B) Neurons in the brain of a mute swan. Both birds died after experimental infection with highly pathogenic avian influenza virus (H5N1). Immunohistochemical stain with hematoxylin counterstain. Magnification x40.

Table 2. Virus isolation data from 4 species of swans and 2 species of geese exposed to highly pathogenic avian influenza virus (H5N1) by intranasal inoculation and contact with infected birds*

	Oropharynx		Cloaca	
Species	Average duration†	AMT‡ (EID_{50}/mL)	Average duration†	AMT‡ (EID_{50}/mL)
Black swan	2 (2–3)	6.46	2 (1–2)	4.94
Trumpeter swan	5 (4–6)	6.14	4 (2–5)	3.18
Whooper swan	5 (4–6)	6.30	4 (3–5)	4.25
Mute swan	5 (3–7)	5.58	4 (3–4)	4.46
Cackling goose	5 (4–6)	5.25	3 (2–5)	3.05
Bar-headed goose	6 (5–8)	5.10	3 (0–7)	2.55§

*Exposure date for each species was adjusted so that 0 d postcontact (when the contact birds were placed into the cage with the inoculated birds) was assumed to be equivalent to 0 d postinoculation. AMT, average maximum titer; EID_{50}, median embryo infectious dose.
†Average duration of viral shedding in days (range).
‡AMT for birds that shed virus. All of the contact and inoculated birds shed detectable concentrations of virus by the oropharyngeal and cloacal route with 1 exception (noted below).
§One bar-headed goose did not excrete detectable virus by the cloacal route, and the average maximum titer for cloacal shedding in this species was calculated based on the 4 birds with detectable cloacal shedding. If all 5 geese were included in this calculation, the average cloacal shedding would be $\log_{10}$ 2.04 EID_{50}/mL.

shed lower concentrations of virus (maximum oropharyngeal titer $10^{3.9}$ EID_{50}/mL) than the 3 geese that died (average maximum oropharyngeal titer $10^{5.7}$ EID_{50}/mL), but the duration of shedding was approximately similar in both oropharyngeal and cloacal swabs.

Bar-headed geese were the least susceptible of the 6 species examined in this study. All 5 of the geese infected with the Mongolia/2005 virus exhibited clinical signs of infection; 2 of these birds died, and the remaining 3 became ill, but the clinical signs slowly resolved until they were no longer apparent. The duration of clinical signs and onset of illness and death were similar to those of the cackling geese. The bar-headed geese that died exhibited severe depression and neurologic signs. The 3 geese that survived became mildly depressed with transiently cloudy eyes but did not exhibit neurologic signs. The duration of disease was longer for the geese that survived (average duration 5.33 days) than for the geese that died (average duration 2.50 days). All 3 of these surviving geese produced antibodies to AIV that were detected by the AGP and HI tests. Viral antigen and microscopic lesions in bar-headed geese were primarily present in the brain. Viral antigen staining was more widespread in the 2 geese that died than in the 3 that survived. Microscopic lesions consisted of moderate perivascular encephalitis and neuronal necrosis in geese that died and mild perivascular encephalitis in birds that survived. The concentration and duration of viral shedding were similar between bar-headed geese that died and those that survived. Cloacal shedding was detected in all of the bar-headed geese except one, which was one of the surviving birds.

Discussion

During the outbreaks of HPAI virus (H5N1) of Asian lineage in Europe in 2005–2006, certain duck and swan species were overrepresented in the mortality reports (*5*). Although field data from the outbreaks indicated that these waterfowl species were susceptible, their contribution to the spread of HPAI virus (H5N1) is not clear. In general, asymptomatic birds can shed virus before the onset of illness or after clinical signs have resolved. In this study, all 6 waterfowl species shed virus before the onset of clinical signs, though species-related differences were apparent (Figure 2). Some geese of both species survived infection, but none of the surviving birds actively shed detectable virus after clinical signs resolved.

Black swans were the most susceptible species to HPAI virus (H5N1) infection, with illness, deaths, and viral distribution similar to results for gallinaceous poultry (*16*). Although all of the black swans shed virus before dying, the asymptomatic viral titers were low, and the rapid course of disease would most likely preclude geographic spread of virus by this species. The high susceptibility of black swans to infection, however, would make them a good sentinel species for detection of HPAI virus (H5N1) in Australia and New Zealand, where this species is found naturally. In addition, the high concentrations of virus shed after the onset of clinical signs, but before death, would allow this species to contribute to viral transmission during a local outbreak in waterfowl.

Illness and death occurred later in whooper swans, mute swans, and trumpeter swans, which would potentially allow actively infected (and shedding) birds in these species more time to spread virus during their movements. This is particularly true for mute swans, which shed moderate to high concentrations of virus for several days without showing clinical signs of disease. The longer duration of asymptomatic viral shedding would allow this species ample time to travel and have contact with other wild birds and shared aquatic habitats. Mute swans do not migrate; however, freezing temperatures may cause many populations to move during the winter season as waterways freeze. This possibility has been suggested as a factor that contributed to the spread of HPAI virus (H5N1) in Europe during 2005–2006 (*6*).

All of the swans used in this study were inoculated at 5 to 6 weeks of age, and the high virulence observed in these

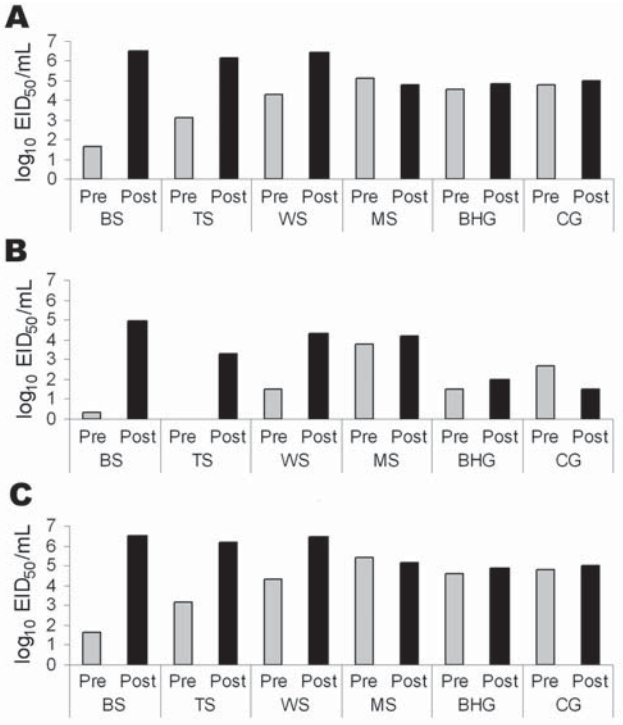

Figure 2. The average concentration of viral shedding in oropharyngeal (A), cloacal (B), and combined (C) routes before (pre) and after (post) the onset of clinical signs in 4 species of swans and 2 species of geese exposed to highly pathogenic avian influenza virus (H5N1) by intranasal inoculation or contact with infected birds. Viral concentrations were determined by adding viral titers before and after the onset of clinical signs for each individual bird and then using these values to calculate a pre- and postclinical average for each species. The single bar-headed goose that did not shed detectable concentrations of virus in the feces was included in the calculation of the averages for this species. EID_{50}, median embryo infectious dose; BS, black swans; TS, trumpeter swans; WS, whooper swans; MS, mute swans; BHG, bar-headed geese; CG, cackling geese.

species may be attributable to the young age of the birds. A negative association between age and death associated with HPAI virus (H5N1) infection occurs in ducks up to 4 weeks of age but not in chickens (*18*). Whether similar age-related differences in susceptibility to HPAI virus (H5N1) exist with swans is not known. However, the Mongolia/2005 strain was equally or more lethal for the 4 species of swans in this study as other HPAI virus (H5N1) strains have been in a variety of age-matched or younger wild avian species, including gallinaceous birds, waterfowl, and gulls (*19*,*20*). If age-related susceptibility does exist in swans, older birds may be more likely to survive the infection.

The cackling geese were more susceptible to HPAI virus (H5N1) than the bar-headed geese, as evidenced by higher proportion of deaths and more systemic viral distribution, and both of these geese species were generally less susceptible than swans. The delayed viral shedding, illness, and deaths of contact geese compared with inoculated geese provide further support that geese are less susceptible than swans. These factors may also suggest that viral transmission would occur at a slower rate within populations of these geese species than in swans. While susceptibility varied between these 2 species, both had similar onsets of disease and death, duration and concentrations of viral shedding, and duration of asymptomatic shedding. On the basis of these parameters, bar-headed geese and cackling geese would be equally efficient disseminators of HPAI virus (H5N1). Cackling geese are closely related to Canada geese, which were affected in some European outbreaks of HPAI virus (H5N1) (*6*). The lower susceptibility, relative to the other species examined in this study, of bar-headed geese was surprising considering the large number of birds that died at the Qinghai Lake outbreak in 2005. On the basis of the mortality rates in this study, many bar-headed geese may have been infected and survived; our results support the possibility that this species played a role in the transmission of HPAI virus (H5N1) to waterfowl or other wild birds outside of Qinghai Lake.

Consistent with reported wild bird mortality data from previous outbreaks, data from this study identified species-related variability in susceptibility to HPAI virus (H5N1) among wild species of waterfowl. Several important characteristics of HPAI virus (H5N1) infection differ between waterfowl species, including duration of asymptomatic shedding and duration and concentration of viral shedding. According to these characteristics, mute swans, cackling geese, and bar-headed geese may be recognized as species that pose a greater risk for transmission and spread of HPAI (H5N1). Relatively few wild avian species, rather than anseriform species as a whole, may have contributed to most of the spread of HPAI (H5N1) within Eurasia. This conclusion is consistent with observed mortality patterns during outbreaks and from the failure to detect HPAI (H5N1) in clinically normal anseriform species despite intensive sampling. This finding implies that the epidemiology of this particular lineage of AIV in waterfowl populations differs from that of low-pathogenicity AIV that naturally circulate in wild birds and that the establishment of a silent (without detectable disease and death) natural reservoir for Asian lineage HPAI virus (H5N1) strains within wild waterfowl populations may be unlikely.

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Short- and Long-term Effects of Bacterial Gastrointestinal Infections

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During 1997–2004, microbiologically confirmed gastrointestinal infections were reported for 101,855 patients in Sweden. Among patients who had *Salmonella* infection (n = 34,664), we found an increased risk for aortic aneurysm (standardized incidence ratio [SIR] 6.4, 95% confidence interval [CI] 3.1–11.8) within 3 months after infection and an elevated risk for ulcerative colitis (SIR 3.2, 95% CI 2.2–4.6) within 1 year after infection. We also found this elevated risk for ulcerative colitis among *Campylobacter* infections (n = 57,425; SIR 2.8, 95% CI 2.0–3.8). Within 1 year, we found an increased risk for reactive arthritis among patients with *Yersinia* enteritis (n = 5,133; SIR 47.0, 95% CI 21.5–89.2), *Salmonella* infection (SIR 18.2, 95% CI 12.0–26.5), and *Campylobacter* infection (SIR 6.3, 95% CI 3.5–10.4). Acute gastroenteritis is sometimes associated with disease manifestations from several organ systems that may require hospitalization of patients.

Bacterial gastrointestinal infections continue to cause illness and death and contribute to economic loss in most parts of the world, including high-income countries that have developed surveillance and control programs. The symptoms of acute bacterial intestinal infection are usually mild to moderate, and spontaneous remission occurs (*1*), but in some cases, the disease can cause rapid deterioration of a patient's condition.

An episode of acute enteric infection involving extraintestinal organs can also lead to complications and trigger chronic disease. Complications include irritable bowel syndrome (*2*), reactive arthritis (*3*), hemolytic uremic syndrome (HUS) (*4*), and Guillain-Barré syndrome (GBS) (*5*). There may be other, perhaps unusual and less documented, late effects of acute enteric infections, such as inflammatory bowel disease (*6*).

In Sweden, there is no active follow-up on reported cases of bacterial enteric infection in terms of disease outcome or long-term complications. During the 8-year period 1997–2004, >100,000 persons with acute gastrointestinal infection were reported within the national surveillance program for communicable diseases. We present a retrospective cohort study of these patients to investigate the association between exposure to a bacterial pathogen and the risk for autoimmune illness, gastrointestinal complications, and extraintestinal infectious disease.

Materials and Methods

Participants comprised persons with intestinal infection (nontyphoidal *Salmonella* spp., *Campylobacter* spp., *Yersinia enterocolitica*, *Shigella* spp., or enterohemorrhagic *Escherichia coli* [EHEC]) reported to the Swedish Institute for Infectious Disease Control during 1997–2004. We collected data on age, sex, date reported, and country of infection and used social security numbers for identification. This identification number was used to link our cohort of cases (those with short-term complications occurring within 3 months or long-term effects within 1 year after infection) to the Swedish Hospital Discharge (covers all hospital in Sweden) and Causes of Death registers. Ethics permission was obtained from the Ethical Committee, Karolinska Institute. Discharge diagnoses must be reported to the register; therefore, any study using this register is, in practice, population based. The Hospital Discharge Register was validated by using a diagnosis of acute myocardial infarction; underreporting was <1%, main diagnosis was missing for <1% of cases, and correct diagnosis was made for 86% (*7*).

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Table 1. Distribution of infectious agents, age and sex for 101,855 study participants, Sweden, 1997−2004

Characteristic	Nontyphoid *Salmonella* spp.	*Campylobacter* spp.	*Shigella* spp.	EHEC*	*Yersinia* spp.
No. participants					
Female	17,524	27,067	2,145	451	2,390
Male	17,140	30,358	1,668	369	2,743
Mean age, y (range)					
Female	37 (0−100)	37 (0−99)	33 (1−89)	25 (0−98)	28 (0−95)
Male	36 (0−97)	37 (0−98)	33 (0−83)	19 (0−85)	27 (0−94)

*EHEC, enterohemorrhagic *Escherichia coli*.

We calculated the follow-up time for each case as person-time from reported date of infection to an event, death, or study termination. Person-years were then compared with a Swedish standard population of 5-year age groups to calculate the expected number of cases for each disease. Standardized incidence ratios (SIRs) were constructed by dividing the observed number of cases with the expected number of cases. Ninety-five percent exact confidence intervals (CIs) were calculated under the assumption that the number of observed cases were Poisson distributed. CIs that do not overlap 1 indicate that the number of observed cases is significantly different from the number of cases expected in a population cohort of similar age and sex distribution. The described method is called indirect standardization, and interpretation of results is similar to relative risk interpretation, i.e., comparing the risk for disease in an exposed cohort to the risk for disease in an unexposed cohort.

We previously estimated standardized mortality ratios (SMRs) for *Salmonella* (*8*) and *Campylobacter* infections (*9*) and showed that country of infection (domestic or abroad) was an effect modifier; i.e., the SMR differed substantially between these 2 strata and no pooled SMR could be calculated. The underlying factor for this interaction was probably that the term *abroad* served as a proxy for healthiness or a healthy traveler effect. For our present analysis, we divided the cohort into 2 strata on the basis of country of infection (Sweden or abroad), but no statistical significant interaction was evident. We concluded that crude SIRs irrespective of country of infection could be estimated. All analyses were conducted by using SAS statistical software, version 8.2 (SAS Institute, Inc., Cary, NC, USA).

Results

Demographic data on the 101,855 study participants and frequency counts for infectious agents are summarized in Table 1. *Campylobacter* spp. caused the most cases, 57,425 (56%). The second most frequent pathogen was *Salmonella* spp., the causative agent in 34,664 cases (34%); distribution of serovars is shown in Table 2. Of all cases of gastroenteritis, *Yersinia* spp. accounted for 5,133 (5%) cases; *Shigella* spp. 3,813 (4%); and EHEC 820 (<1%).

Table 3 shows the number of reported case-patients with specific diseases within 3 months of an episode of bacterial gastrointestinal infection, along with expected number of cases and SIRs. Not surprisingly, the highest risks were found for HUS after EHEC infection and GBS following campylobacter infection. Although SIRs were quite elevated, absolute risks were more moderate; among 820 cases of EHEC infection, we found 13 episodes of HUS (1.6%), 57,425 cases of campylobacteriosis, 13 cases of GBS (0.02%), 5,133 cases of *Yersinia* infection, and 9 cases of reactive arthritis (0.2%). The risk for aortic aneurysm among patients with salmonellosis was significantly higher than expected (SIR 6.4, 95% CI 3.1–11.8). The absolute risk for bacteremia/sepsis was 0.02% for case-patients with *Campylobacter* infection and 0.03% for those with salmonellosis. For many complications, we did not find any statistically significant elevated risks. Other complications that we had hypothesized to be associated with gastrointestinal infections could not be shown. Only a few cases were found within 3 months, contributing to imprecise estimates of SIRs.

Within 1 year of acute bacterial gastrointestinal infection, case-patients with *Yersinia* enteritis were at increased risk for reactive arthritis (SIR 47.0, 95% CI 21.5–89.2), *Salmonella* infection (SIR 18.2, 95% CI 12.0–26.5), and *Campylobacter* infection (SIR 6.3, 95% CI 3.5–10.4) (Table 4). The risk for ulcerative colitis was elevated among patients with salmonellosis (SIR 3.2, 95% CI 2.2–4.6) and, to a lesser extent, among patients with campylobacteriosis (SIR 2.8, 95% CI 2.0–3.8). Of the 29 patients in our salmonellosis cohort who had ulcerative colitis, 13 (44%) had first experienced ulcerative colitis during the 10-year period before the acute infection. Among patients with campylobacteriosis, we found 42 with ulcerative colitis, of whom 18 (43%) had received a diagnosis of ulcerative colitis in the 10-year period before the infection. We did not find any

Table 2. Most frequent serotypes isolated among study participants with nontyphoid *Salmonella* infection, Sweden, 1997−2004

Salmonella serotype	Frequency	Relative frequency, %
S. species, not subtyped	14,643	42
S. Enteritidis	10,580	31
S. Typhimurium	2,607	8
S. Virchow	741	2
S. Hadar	734	2
Other specified serotypes	5,359	15
Total	34,664	100

Table 3. Complications associated with gastroenteritis, 3 months postinfection, among 101,855 patients with bacterial gastrointestinal infection, Sweden, 1997−2004*

Disease	Infecting organism	Obs	Exp	SIR	95% CI
Respiratory system					
Bacterial pneumonia, pneumonitis due to food and vomit	Nontyphoid *Salmonella* spp.	24	13.5	1.8	1.1–2.6
	Campylobacter spp.	17	21.4	0.8	0.5–1.3
	EHEC	1	0.3	3.1	0.1–17.2
	Shigella spp.	1	1.1	0.9	0.02–5.2
	Yersinia spp.	4	2.3	1.8	0.5–4.5
Blood					
Hemolytic-uremic syndrome	Nontyphoid *Salmonella* spp.	1	<0.05	55.5	1.4–309.1
	Campylobacter spp.	2	<0.05	81.0	9.8–292.7
	EHEC	13	<0.05	18,333.4	9,761.8–31,350.6
Circulatory system					
Aortic aneurysm	Nontyphoid *Salmonella* spp.	10	1.6	6.4	3.1–11.8
	Campylobacter spp.	5	2.4	2.06	0.7–4.8
	Yersinia spp.	1	0.2	5.2	0.1–28.9
Endocarditis	Nontyphoid *Salmonella* spp.	2	0.4	5.7	0.7–20.5
Digestive system					
Peritonitis	Nontyphoid *Salmonella* spp.	1	0.6	1.9	0.05–10.1
	Campylobacter spp.	2	0.9	2.3	0.4–8.4
Perforation of intestine (nontraumatic)	Nontyphoid *Salmonella* spp.	1	0.1	9.7	0.3–54.0
	Campylobacter spp.	2	0.2	12.39	1.5–44.7
	EHEC	1	<0.05	655.3	16.6–3,651.0
Idiopathic acute pancreatitis	Nontyphoid *Salmonella* spp.	6	2.6	2.3	0.9–5.1
	Campylobacter spp.	7	4.1	1.7	0.68–3.5
Hepatic failure	Nontyphoid *Salmonella* spp.	1	0.3	4.0	0.1–22.2
Infectious diseases					
Septicemia	Nontyphoid *Salmonella* spp.	10	2.6	3.9	1.8–7.1
	Campylobacter spp.	14	4.1	3.4	1.9–5.7
	Shigella spp.	1	0.2	5.1	0.1–28.2
Nervous system					
Guillain-Barré syndrome	*Campylobacter* spp.	13	0.2	66.6	35.5–114.0
Musculoskeletal system					
Pyogenic arthritis	Nontyphoid *Salmonella* spp.	4	0.8	5.2	1.4–13.4
	Yersinia spp.	1	0.1	10.1	0.3–56.2
Osteomyelitis	Nontyphoid *Salmonella* spp.	3	0.6	5.4	1.1–15.7

*Obs, observed number of cases; Exp, expected number of cases; SIR, standardized incidence ratio; CI, confidence interval; EHEC, enterohemorrhagic *Escherichia coli*.

increased risk for Crohn's disease in the same group of patients. We did not find any elevated risk for many of the rheumatologic diseases included in the present study in any of the participants. The distribution of *Salmonella* serotypes among patients with aortic aneurysm, reactive arthritis, and ulcerative colitis in our cohort did not differ in any substantial way from the whole salmonellosis cohort (Table 5), although the number of patients was rather small.

Discussion

Our data confirm the elevated risk for complications and long-term sequelae after an episode of acute bacterial gastroenteritis. We have presented new estimates of the absolute and relative risk for well-described complications such as HUS after EHEC infection, GBS after an episode of *Campylobacter* enteritis, and reactive arthritis after *Yersinia* enteritis. Another complication that we have been able to verify is aortic aneurysm after an episode of salmonellosis. Perhaps more unexpected, the risk for ulcerative colitis was elevated in the cohort of patients with salmonellosis and campylobacteriosis. The distribution of *Salmonella* serovars was the same among patients with and without complications. The finding of no major difference in the distribution of *Salmonella* serovars between the group of patients with and without complications indicates that factors other than *Salmonella* serovar alone determine the risk for complications.

Compared with other studies, our new estimate of the risk for HUS after EHEC infection is lower than previously reported (*10*,*11*). An explanation of our lower estimates could be that we used only International Classification of Diseases (ICD) codes specific for HUS. Several of these cases may in fact be classified under nonspecific ICD codes that also include a large proportion of cases unrelated to HUS. However, had we included them in the analysis, any association with the infections would have

Table 4. Complications associated with gastroenteritis, 1 year postinfection, among 101,855 patients with bacterial gastrointestinal infection, Sweden, 1997−2004*

Disease	Infecting organism	Obs	Exp	SIR	95% CI
Digestive system					
Crohn's disease	*Campylobacter* spp.	27	17.1	1.6	1.0–2.3
	Salmonella spp.	14	10.3	1.4	0.8–2.3
	Shigella spp.	1	1.1	0.9	0.02–5.2
	Yersinia spp.	2	1.1	1.8	0.2–6.4
Ulcerative colitis	*Campylobacter* spp.	42	14.8	2.8	2.0–3.8
	EHEC	1	0.1	6.8	0.2–37.7
	Salmonella spp.	29	9	3.2	2.2–4.6
	Yersinia spp.	3	1	2.9	0.6–8.5
Other specified/unspecified noninfective gastroenteritis and colitis	*Campylobacter* spp.	37	14.9	2.5	1.8–3.4
	Salmonella spp.	30	9.2	3.3	2.2–4.6
	Yersinia spp.	10	1.3	7.6	3.7–14.0
Irritable bowel syndrome	*Campylobacter* spp.	15	5	3.0	1.7–5.0
	Salmonella spp.	5	3	1.7	0.5–3.9
	Yersinia spp.	3	0.4	7.8	1.6–22.9
Intestinal malabsorption	*Salmonella* spp.	1	0.6	1.7	0.04–9.3
	Yersinia spp.	1	0.1	7.9	0.2–43.7
Musculoskeletal system					
Postdysenteric arthropathy, Reiter disease, other reactive arthropathies	*Campylobacter* spp.	15	2.4	6.3	3.5–10.4
	Salmonella spp.	27	1.5	18.2	12.0–26.5
	Shigella spp.	2	0.1	13.4	1.6–48.4
	Yersinia spp.	9	0.2	47.0	21.5–89.2
Rheumatoid arthritis	*Campylobacter* spp.	22	22.5	1.0	0.6–1.5
	EHEC	1	0.2	5.8	0.2–32.1
	Salmonella spp.	9	14.7	0.6	0.3–1.2
	Shigella spp.	1	1.2	0.8	0.02–4.7
	Yersinia spp.	3	1.5	2.0	0.4–5.7
Other arthritis	*Campylobacter* spp.	8	3.8	2.1	0.9–4.2
	Salmonella spp.	4	2.5	1.6	0.4–4.1
	Shigella spp.	1	0.2	4.3	0.1–24.1
	Yersinia spp.	1	0.4	2.4	0.06–13.4
Other necrotizing vasculopathies (Goodpasture syndrome, TTP, Wegener granulomatosis, giant cell arteritis)	*Campylobacter* spp.	10	3.3	3.1	1.5–5.6
	EHEC	0	<0.05	32.8	0.8–183.0
	Salmonella spp.	1	2.1	0.5	0.01–2.7
Systemic lupus erythematosus	*Campylobacter* spp.	5	3.4	1.5	0.5–3.4
	Salmonella spp.	2	2.1	1.0	0.1–3.5
Systemic sclerosis	*Campylobacter* spp.	2	1.7	1.2	0.2–4.4
	Salmonella spp.	3	1.1	2.8	0.6–8.1
Other systemic involvement of connective tissue (Sjögren syndrome, mixed connective tissue disease, polymyalgia rheumatica)	*Campylobacter* spp.	12	5	2.4	1.2–4.2
	Salmonella spp.	4	3.1	1.3	0.4–3.3
	Shigella spp.	1	0.2	4.2	0.1–23.3
Ankylosing spondylitis	*Campylobacter* spp.	2	1.1	1.8	0.2–6.4
	Salmonella spp.	1	0.7	1.5	0.04–8.1

*Obs, observed number of cases; Exp, expected number of cases; SIR, standardized incidence ratio; CI, confidence interval; EHEC, enterohemorrhagic *Escherichia coli*.; TTP, thrombotic thrombocytopenic purpura.

been diluted. Our estimate of risk for GBS and campylobacter are in line with a study in England that showed a risk of <2/10,000 that GBS will develop in a patient with campylobacteriosis (*12*). These results are also in line with a previous study in Sweden (*13*). All estimates of complications in this study are based on discharge data from the Hospital Discharge Register; this means that minor complications that either were not presented to any doctor or were handled only by general practitioners were not available for this analysis. At the population level, reactive rheumatologic symptoms associated with infection are typically mild and transient (*14*). This is probably the reason why our estimate of reactive arthritis after *Yersinia* infection is quite low, although similar low risks have been reported elsewhere (*15*).

In patients with atherosclerotic disease, or in those with preexisting aneurysms, transient bacteremia with nontyphoidal *Salmonella* infection can result in vascular infections (*16–18*). Most of these aneurysms described previously have been localized in the subrenal segment of the

Table 5. *Salmonella* serotypes among patients with aortic aneurysm, reactive arthropathies, and ulcerative colitis, Sweden, 1997−2004

Disease or condition	*Salmonella* serotype	Frequency	Relative frequency* (%)
Aortic aneurysm (n = 10)	*S.* Enteritidis	3	30 (31)
	S. Dublin	2	20 (<1)
	S. Virchow	1	10 (2)
	Other *S.* spp.	4	40 (42)
Postdysenteric arthropathy, Reiter disease, other reactive arthropathies (n = 27)	*S.* Enteritidis	10	37 (31)
	S. Typhimurium	3	11 (8)
	S. London	1	4 (<1)
	Other *S.* spp.	13	48 (42)
Ulcerative colitis (n = 29)	*S.* Enteritidis	7	24 (31)
	S. Typhimurium	4	14 (8)
	S. Kottbus	1	3 (<1)
	S. Agona	1	3 (1)
	S. Ituri	1	3 (<1)
	Other *S.* spp.	15	52 (42)

*Relative frequency in total cohort, n = 34,664.

abdominal aorta (*17*). *Salmonella* spp. in these patients can invade the arterial intima and cause a localized endothelial infection that results in an aneurysm or the enlargement of a previously existing aneurysm. This may explain the association between *Salmonella* infection and aortic aneurysm in this study.

Our findings of an elevated risk for ulcerative colitis in the cohort of patients with salmonellosis and campylobacteriosis need further study. In another large cohort study, an association between acute gastroenteritis and inflammatory bowel disease was identified (n = 43,013), where the incidence rate for ulcerative colitis was 40 per 100,000 person-years, a doubling of the risk for those unexposed to infection (*19*). We do not know why an episode of infectious gastroenteritis could contribute to the initiation or exacerbation of ulcerative colitis. Seasonal variation in the onset of ulcerative colitis, and reports that excessive childhood infections are associated with higher risk for ulcerative colitis, may support the hypothesis that infections could be triggers of disease (*20*). From this study, we cannot say whether there is a causal relationship between *Salmonella* and *Campylobacter* infections and relapse of disease in patients with known ulcerative colitis, or whether the infection could trigger ulcerative colitis in susceptible persons. We cannot entirely rule out that the findings are an artifact, resulting from an increased number of medical examinations and stool cultures in a group of patients with diarrhea because of a known or unknown inflammatory bowel disease. More study is needed to confirm or refute our findings.

Because irritable bowel syndrome is diagnosed and treated at hospital in only a minority of patients, our estimates are probably too low. Many studies have not used a control group but reported only the numbers and percentages of patients who had irritable bowel syndrome after gastroenteritis (*21*); 1 study with controls estimated a relative risk of 11.9 (CI 6.7–21) after 1 year of follow-up (*22*).

Our study has some limitations. Perhaps the most serious one is the selection bias of patients entering the gastroenteritis cohort. Only a small fraction of all patients with *Salmonella* infection, for example, seek medical care, have a stool sample taken, and are eventually reported to national surveillance (*23*). This could have an effect on the results, especially if we are collecting data on those with the most severe disease; disease severity itself affects complications and sequelae. Another limitation is the lack of information on confounding factors among study participants, especially coexisting illnesses such as malignant disease or immunodeficiencies of any cause. Such coexisting illnesses could perhaps increase to some extent the risk for complications (*6*), but our results on the effect of disease from gastrointestinal infections would not have changed. Although the quality of the Swedish Hospital Discharge Register is quite good, there is always a general problem of reliability in registry-based epidemiologic research.

In conclusion, we studied the risk for complications 3 months and 1 year after acute bacterial gastroenteritis and found disease manifestations from several organ systems that required hospitalization of patients. These findings are a reminder of, and could be an argument for, the usefulness of existing control programs targeted to control bacterial enteric disease.

This study was approved by the Regional Ethical Committee, Karolinska Institute, Stockholm, Sweden.

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Avian Influenza Virus (H5N1) Replication in Feathers of Domestic Waterfowl

Yu Yamamoto,* Kikuyasu Nakamura,* Masatoshi Okamatsu,* Manabu Yamada,* and Masaji Mase*

We examined feathers of domestic ducks and geese inoculated with 2 different avian influenza virus (H5N1) genotypes. Together with virus isolation from the skin, the detection of viral antigens and ultrastructural observation of the virions in the feather epidermis raise the possibility of feathers as sources of infection.

Since 1997, an epidemic of avian influenza (AI) virus subtype H5N1 has spread in Asia, causing fatal infections in poultry, wild birds, mammals, and humans (*1*). Wild waterfowl, including ducks and geese, are natural hosts of AI virus of all 16 hemagglutinin subtypes in nature (*2*,*3*). Generally, AI virus is transmitted by the fecal-oral route without causing clinical signs (*2*–*4*). Although current AI virus (H5N1) strains have mild to severe pathogenicity in waterfowl (*5*–*7*), these birds can still be carriers of the virus (*7*). Even asymptomatic domestic ducks can shed the virus from the cloaca and oral cavity (*7*,*8*) and contribute to viral maintenance and spread (*9*,*10*). Therefore, focusing on the epidemiologic role of domestic waterfowl in AI (H5N1) outbreaks is important.

We previously reported that the Japanese AI virus (H5N1) isolated in 2004 causes necrosis of the feather epidermis with viral antigens in domestic ducks, a finding that demonstrates the possibility of viral release from feathers (*11*). In addition, these affected feathers can cause infection in orally inoculated domestic ducks (*12*). Except for our previous studies, to our knowledge this feather lesion has not been reported in AI (H5N1)–infected waterfowl. However, if the feather lesion is common to other waterfowl species and AI (H5N1), affected feathers might involve the spread of the virus. We describe the pathologic, virologic and ultrastructual findings of the feather in domestic waterfowl infected with AI (H5N1).

The Study

Two species of domestic waterfowl, ducks (n = 4) and geese (n = 4), were used. Domestic ducks (*Anas platyrhynchos* var. *domestica*) called *Aigamo* in Japanese are a crossbreed of wild mallard and domestic ducks; they are free-ranging ducks in water-soaked rice paddy fields and are used for weed control and meat production. Domestic geese (*Anser cygnoides* var. *domestica*) are reared for food production on farms. We selected geese because wild geese (*A. indicus*) accounted for a large proportion of the deaths in AI (H5N1) outbreaks at Qinghai Lake in People's Republic of China in 2005 (*5*). These 2 species of birds were obtained from the farm at 1 day of age and raised with commercial food in an isolated facility. Birds were moved into negative-pressure isolators of Biosafety Level 3–approved laboratories (National Institute of Animal Health, Tsukuba, Japan) for acclimation 1 week before inoculation.

Two different AI virus (H5N1) genotypes were used. A/chicken/Yamaguchi/7/2004 (Ck/Yama/7/04) is classified as genotype V (*13*). A/chicken/Miyazaki/K11/2007 (Ck/Miya/K11/07) belongs to genotype Z and H5 clade 2 subclade 2 (M. Mase, unpub. data), which is now circulating from China to Japan, Europe, and Africa (*5*,*14*). The stored virus was propagated for 36–48 hours in the allantoic cavity of 10-day-old embryonated chicken eggs at 37°C. The infectious allantoic fluid was harvested and stored at –80°C until use. All experimental procedures were approved by the Ethics Committee of the National Institute of Animal Health in Japan.

For each species, two 4-week-old birds were inoculated intranasally with 0.1 mL of the inoculum containing 10^8 50% egg infectious dose (EID_{50}) per mL of each AI virus (H5N1) genotype. Each inoculated group was kept in a separate isolator. Inoculated birds were euthanized with an overdose injection of sodium pentobarbital (i.v.) on days 3 and 5 postinoculation.

For histopathology, the skin, including numerous feathers, was removed from the head, neck, back, shoulder, abdomen, thigh, and tail. Samples were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin. Immunohistochemistry was performed to detect the viral antigen with a Histofine Simple Stain PO (M) kit (Nichirei Inc., Tokyo, Japan). A mouse monoclonal antibody specific for the influenza A matrix protein (diluted 1:500; clone GA2B, AbD Serotec, Kidlington, UK) was used as the primary antibody (*11*). For the virus isolation, clean dry skin was collected from the neck and stored at –80°C (*11*). The viral titer of the samples was determined with 10-day-old embryonated chicken eggs and expressed as EID_{50}/g as previously described (*13*). The viral titer $<10^2$ EID_{50}/g was considered negative for virus isolation. For the electron microscopic examination, flesh contour feathers were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer, postfixed in 1% osmium tetroxide, and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate

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Table. Histopathology of feathers and virus isolation from the skin in domestic ducks and geese inoculated with 2 different avian influenza virus (H5N1) genotypes*

	A/chicken/Yamaguchi/7/2004		A/chicken/Miyazaki/K11/2007	
PID	Duck	Goose	Duck	Goose
3	+/+† (3.5)‡	–/– (–)	+/+ (4.5)	+/+ (4.4)
5	+/+ (–)	+/+ (4.5)	–/+ (3.8)	+/+ (2.5)

*PID, postinoculation day.
†Epidermal necrosis of feathers/detected viral antigens; +, positive; –, negative.
‡Viral titer of the skin expressed as $\log_{10}$ 50% egg infectious dose (EID_{50})/g; –, negative (<10^2 EID_{50}/g).

and examined under a Hitachi H-7500 transmission electron microscope (Hitachi Corp., Tokyo, Japan).

Inoculated birds did not exhibit apparent clinical signs, except for unilateral corneal opacity in a goose inoculated with Ck/Yama/7/04 on day 5 postinoculation. Results of histopathologic and virologic examinations are summarized in the Table. Histologically, viral antigens were occasionally detected in the feather epidermal cells with or without epidermal necrosis (Figure 1, panels A and B). Some affected feathers were accompanied by heterophilic and lymphocytic infiltration in the inner feather pulp. Other tissues in the skin were negative for influenza virus by immunohistochemical analysis with the exception of very rare positive reaction in stromal cells in the feather pulp. Virus isolation from the skin was positive in 1 duck and 1 goose inoculated with Ck/Yama/7/04; the viral titers were $10^{3.5}$ and $10^{4.5}$ EID_{50}/g, respectively. All ducks and geese inoculated with Ck/Miya/K11/07 tested positive for the isolation; the viral titers were $10^{2.5}$–$10^{4.5}$ EID_{50}/g. Ultrastructurally, round, enveloped virions 80 to 100 nm in diameter were observed between feather epidermal cells in both domestic ducks and geese (Figure 2, panels A and B). Spherical virions budding from cell surface were occasionally observed (Figure 2, panel C).

Conclusions

We found that 2 different AI virus (H5N1) genotypes that were isolated in 2004 and 2007 can replicate in the feather epidermal cells of domestic ducks and geese. To our knowledge, this is the first report of in vivo ultrastructural observation of AI (H5N1) replication in waterfowl.

The important finding is that the histologic feather finding and virus isolation from the skin were found in inoculated birds that did not exhibit apparent clinical signs. Although 1 goose inoculated with Ck/Yama/7/04 was negative for all examinations, this might have resulted from individual differences in susceptibility or the limited area of the skin used for the examination. Nevertheless, our data indicate that recent AI (H5N1) strains are likely to replicate in feather epidermal cells of domestic ducks and geese. All birds inoculated with Ck/Miya/K11/07, which belongs to the current lineage spreading to Europe and Africa, tested positive for virus isolation, compared with the results with Ck/Yama/7/04. Feathers can easily drop off, blow away, or be reduced to dust, suggesting that affected feathers of waterfowl infected with influenza (H5N1) virus can be potential sources of infection, along with their feces and respiratory secretions (*7*,*8*). At this time, it is unclear to what extent affected feathers contribute to the epidemiology of AI (H5N1) field outbreaks. However, more attention needs to be paid to persons who handle domestic waterfowl possibly infected with AI virus (H5N1).

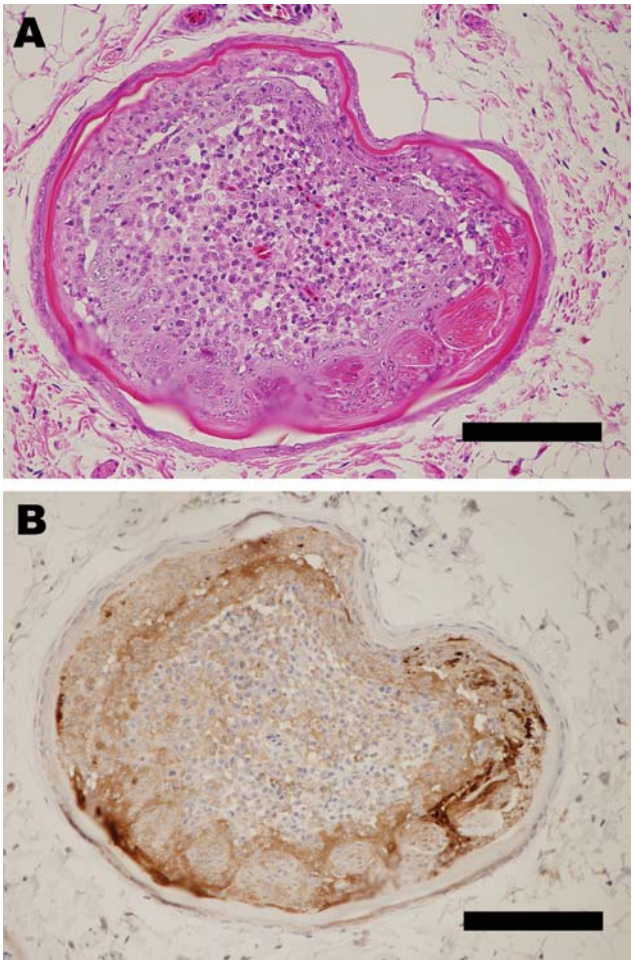

Figure 1. Pathologic changes in a goose infected with A/chicken/Miyazaki/K11/2007. A) Outer epidermal necrosis of the feather with inflammation in the inner feather pulp. Hematoxylin and eosin stain. Bar = 120 μm. B) Influenza viral antigens detected in feather epidermal cells. Immunohistochemistry. Bar = 120 μm.

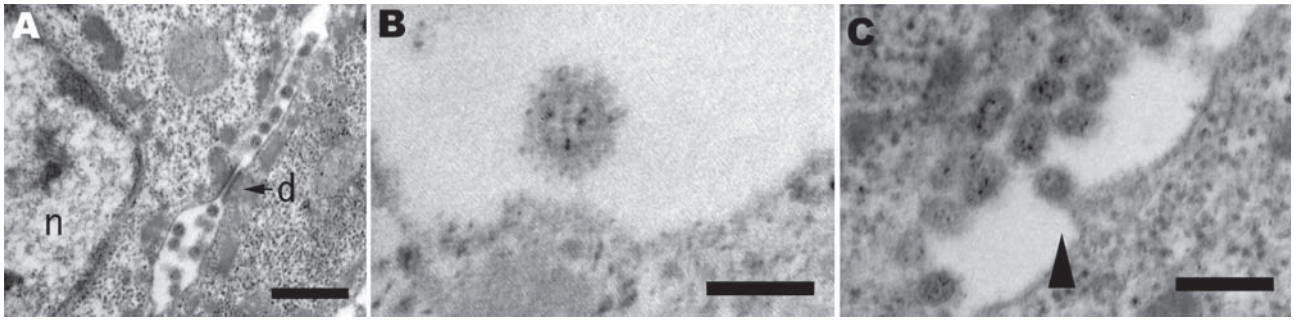

Figure 2. Pathology of a duck infected with A/chicken/Yamaguchi/7/2004. A) Electron microscopy of the feather epidermis showing virions observed between epidermal cells with the desmosome (d) and nucleus (n). Bar = 500 nm. B) Spherical virion with envelope spikes. Bar = 100 nm. C) Budding process of virion (arrowhead). Bar = 250 nm.

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Prolonged *Bartonella henselae* Bacteremia Caused by Reinfection in Cats

Mardjan Arvand,* Juliane Viezens,* and Julia Berghoff*

We analyzed the genetic relatedness of blood culture isolates of *Bartonella henselae* from 2 cats of patients with cat-scratch disease at admission and after 12 months. Isolates from each cat at different times were clonally unrelated, which suggested reinfection by a second strain.

Bartonella henselae is a zoonotic pathogen associated with a broad spectrum of disease manifestations in humans. Cat-scratch disease (CSD) is commonly encountered in immunocompetent patients; in immunocompromised patients, bacillary angiomatosis, peliosis hepatis, and recurrent bacteremia are usually seen. Domestic cats represent the main host and reservoir for *B. henselae* (*1*). Recurrent, intraerythrocytic bacteremia develops in infected cats without overt clinical symptoms (*2*). Experimental infection of specific pathogen–free cats with *B. henselae* induces recurrent episodes of bacteremia, which, in most cases, resolve spontaneously within 22–33 weeks postinfection (*3*–*5*). Prolonged bacteremia >7 months has been documented sporadically, e.g., in 1 of 12 experimentally infected cats inoculated with the highest infectious dose (this cat was bacteremic 32 weeks postinfection) (*3*), or in an unspecified number of cases in 21 experimentally infected cats that were bacteremic 48 weeks postinfection (*5*).

Few studies have investigated the course of recurrent bacteremia in naturally infected cats because follow-up investigations are difficult to conduct (*1*,*6*–*9*). Koehler et al. (*1*) detected recurrent bacteremia with a duration ≥2 months in 3 cats of patients with bacillary angiomatosis. Kordick et al. (*6*) reported positive blood cultures in cats of several CSD patients up to 14 months after collection of the initial positive culture. In the latter study, the first blood culture was collected from the index cat of 1 CSD patient 22 months after the onset of the disease in the patient and contained *B. henselae* (*7*). Sander et al. (*8*) found repeated bacteremia in the cat of a CSD patient after 5 months and in 2 other cats after 1 year. In another study, *B. henselae* was isolated from the blood culture of a cat of a patient who had an episode 2.5 years earlier of debilitating fatigue with a duration of 1 month and without fever or lymphadenopathy (*7*). *B. henselae* was isolated again from the blood culture of the index cat after 5 months (*7*).

In these studies, the question whether the cats were still infected by the initial *B. henselae* strain or had acquired a new strain was not addressed. It was concluded that the cats were persistently infected with *B. henselae* (*8*). We have recently demonstrated the appropriateness of pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) for differentiation of *B. henselae* isolates to the strain level (*10*,*11*). Therefore, we analyzed the clonal relationship between sequential *B. henselae* isolates that were obtained at different times from the blood of 2 cats to determine whether recurrences were caused by the initial strain or a new strain.

The Study

We tested 4 isolates of *B. henselae*: FR96/BK36, FR96/BK36II, FR96/BK75, and FR96/BK75II. These isolates were grown from the blood of 2 naturally infected cats (cat 36 and cat 75) of CSD patients at first consultation and after 12 months, respectively (*9*). The original colony counts were 100, 100, 120, and 100, respectively. PFGE analysis was conducted after digestion of DNA with *Sma*I, and MLST was conducted after partial sequencing of 8 genetic loci (*12*,*13*). PFGE analysis showed 9 band differences between isolates 36 and 36II and 10 band differences between isolates 75 and 75II (Figure), which suggested that isolates obtained from the same cat at different times were not clonally related (*14*). MLST analysis showed 3 and 6 different alleles between isolates 36 and 36II and isolates 75 and 75II, respectively (Table). Isolates 36 and 36II were assigned to sequence type (ST) 14 and ST5, which have the 16S rRNA alleles 1 and 2, respectively. Isolates 75 and 75II were assigned to ST5 and ST7, respectively, and both had the 16S rRNA allele 2 (Table). These data suggest that both cats were infected by a second *B. henselae* strain at the second time blood was obtained.

Conclusions

Our data indicate that recurrent *B. henselae* bacteremia in naturally infected cats does not necessarily represent a relapse but may be caused by reinfection. We were surprised to find that both cats were presumably reinfected by a different strain within 1 year. The interval between collection of the initial and follow-up blood samples was long, which might explain the high rate of reinfection. The possibility that the cats were infected by 2 *B. henselae* stains at the time blood was first obtained was examined by subjecting 5 single-colony–derived cultures of each initial isolate to PFGE analysis, which did not show evidence for

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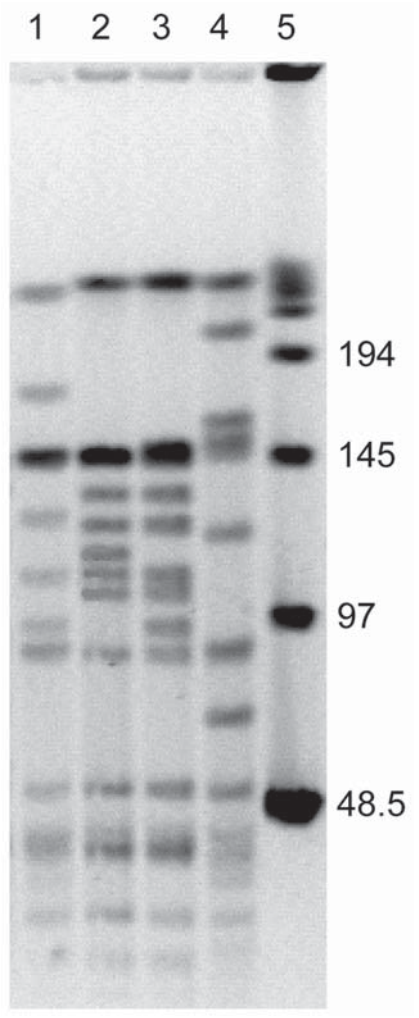

Figure. *Sma*I macrorestriction patterns of *Bartonella henselae* isolates from 2 cats. Lane 1, cat 36, first isolate; lane 2, cat 36, second isolate obtained 12 months later; lane 3, cat 75, first isolate; lane 4, cat 75, second isolate obtained 12 months later; lane 5, bacteriophage λ molecular mass pulsed-field gel electrophoresis marker. Values on the right are in kilobases.

co-infection. In addition, co-infection by 2 strains is unlikely because we would not have been able to determine a unique sequence for those genetic loci that displayed allelic polymorphism. Furthermore, co-infection by 2 strains in both cats would represent a rare coincidence. Nevertheless, we cannot completely rule out co-infection with 2 strains at the time blood was first obtained, with 1 strain being highly underrepresented among initial colonies.

To our knowledge, this is the first report to document sequential infection by different *B. henselae* strains in naturally infected cats by using 2 independent typing methods. In a previous study, Kabeya et al. (*9*) reported variations of up to 5 bands in PFGE patterns of *B. henselae* isolates obtained from naturally infected cats during different bacteremic peaks. However, we have recently demonstrated that genetic variants displaying 1- to 4-band differences frequently occur within primary *B. henselae* isolates and do not necessarily indicate infection by a different strain (*11*,*12*). Careful interpretation of PFGE typing results, use of additional restriction endonucleases, or use of other typing techniques is necessary to ensure correct classification of different patterns to the variant or strain level. *B. henselae* isolates collected from different bacteremic episodes of naturally infected cats after a long interval should by tested by molecular typing to determine their clonal relatedness.

In conclusion, our data emphasize the requirement for molecular typing to differentiate between relapse and reinfection by *B. henselae* in naturally infected cats. Studies on additional isolates are required to evaluate the frequency of reinfection by a different strain in naturally infected cats. Results of these studies would provide a better understanding of the natural course of feline infection. Our data also suggest that infection by a distinct *B. henselae* strain does not induce protective immunity against subsequent infection by a clonally unrelated strain. These results are partially consistent with those of Yamamoto et al. (*15*), who found incomplete cross-protection between isolates with different 16S rRNA alleles. Recent studies have shown that the delineation of *B. henselae* isolates into 2 genotypes on the basis of 16S rRNA sequence is not consistent with phylogenetic classifications using other genetic loci and does not reflect clonal lineage of isolates (*11*,*14*). Reevaluation of induction of cross-protection between different *B. henselae* strains in an experimental infection model is needed.

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Dr Arvand is a professor of medical microbiology at the Institute for Medical Microbiology, Virology and Hygiene, Univer-

Table. Multilocus sequence typing data of 8 genetic loci of 4 *Bartonella henselae* isolates from 2 naturally infected cats*

Isolate	16S rRNA	*batR*	*gltA*	*ftsZ*	*groEL*	*nlpD*	*ribC*	*rpoB*	ST
FR96/BK36	1	2	1	2	2	1	1	1	14
FR96/BK36II	2	1	1	1	2	1	1	1	5
FR96/BK75	2	1	1	1	2	1	1	1	5
FR96/BK75II	2	4	2	3	1	2	2	1	7

*Values are allele numbers. ST, sequence type.

sity of Rostock, Rostock, Germany. Her research interests include pathogenesis, persistence, and clinical and epidemiologic aspects of *B. henselae* infections.

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Human Case of *Streptococcus suis* Serotype 16 Infection

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Streptococcus suis infection is an emerging zoonosis in Southeast Asia. We report a fatal case of *S. suis* serotype 16 infection in a Vietnamese man in 2001.

Streptococcus suis is a gram-positive, facultatively anaerobic coccus that may cause pneumonia, meningitis, septicemia, and arthritis in pigs. The pig can also be a healthy carrier of *S. suis* in the upper respiratory tract (particularly the tonsils and nasal cavities), the genital tract, and the alimentary tract (*1*,*2*). The first case of *S. suis* infection in humans was reported in Denmark in 1968. Since then, increasing numbers of cases have been reported in many countries, including the Netherlands; the United Kingdom; France; Hong Kong Special Administrative Region, People's Republic of China; Thailand; Taiwan; and the United States (*3*–*7*). A recent outbreak of *S. suis* infections in Sichuan Province, People's Republic of China, emphasized the importance of *S. suis* as an emerging zoonosis (*8*). *S. suis* is also the most important cause of bacterial meningitis in adults at the Hospital for Tropical Diseases (HTD) in Ho Chi Minh City, Vietnam (N.T.H Mai et al., unpub. data). The number of cases is likely underreported and will likely increase further with increased awareness and enhanced capacity to culture and identify *S. suis*.

The Patient

A 57-year-old unemployed man from Long An Province, southern Vietnam, who had a history of alcohol abuse, had a 10-day history of abdominal pain, jaundice, anorexia, and weight loss. At the time of admission to HTD in 2001, the patient was lethargic, his vital signs were stable, and his neck was not stiff. Physical examination showed cutaneous spider naevi, jaundice, hepatosplenomegaly, and ascites. Leukocyte count was 15.3 $\times 10^3$ cells/μL (70% neutrophils), blood urea nitrogen 12.1 mmol/L (reference range 3.57–7.14 mmol/L), creatinine 150 μmol/L (<115 mmol/L), sodium 127 mmol/L (135–145 mmol/L), potassium 6.67 mmol/L (3.5–5.1 mmol/L), serum aspartate aminotransferase 87 IU/L (12–30 IU/L), serum alamine aminotransferase 41 IU/L (13–40 IU/L), and albumin 20 g/L (35–52 g/L). An abdominal ultrasound examination showed hepatosplenomegaly and ascites. Ascitic fluid was cloudy and contained 5 g/L protein. Results of Gram stain and culture of ascitic fluid were negative. A diagnosis of spontaneous bacterial peritonitis associated with alcoholic liver cirrhosis was made, and the patient was treated with 2 g/day of ceftriaxone.

Twenty-four hours after admission, acute respiratory distress developed. The patient's family decided to take him home because they were unable to pay for further treatment; the patient died on the same day. The blood culture (BACTEC 9050 system; Becton Dickinson Microbiology Systems, Sparks, MD, USA), which was taken at the time of admission, grew *S. suis* 24 hours after collection. Further inquiries into potential pig exposure, after the blood culture results were reported, indicated that the patient kept pigs near his house and was known to regularly consume portions of the pig that had a high risk of being contaminated, such as the intestine.

S. suis was identified on the basis of colony morphology, negative katalase reaction, optochin resistance, and APIStrep (bioMérieux, Marcy l'Etoile, France). Serotyping was performed by slide agglutination using specific antisera (Statens Serum Institute, Copenhagen, Denmark) and was positive for serotype 16. Confirmation of the serotype was performed at the International Reference Laboratory for *S. suis* Serotyping, Quebec City, Quebec, Canada (M. Gottschalk). This strain was susceptible to penicillin (MIC 0.032 mg/L), ceftriaxone (0.064 mg/L), rifampin (0.032 mg/L), chloramphenicol (2 mg/L), erythromycin (0.064 mg/L), levofloxacin (0.38 mg/L), and vancomycin (0.5 mg/L) but resistant to tetracycline (64 mg/L) by Etest (AB-Biodisk, Solna, Sweden) when Clinical Laboratory Standard Institute breakpoints were used. On pulsed-field gel electrophoresis (PFGE) that used restriction enzyme *Sma*I, this strain showed little similarity with a representative set of serotype 2 isolates from Vietnam (Figure). Multilocus sequence typing (MLST) (www.mlst.net) showed that the sequence of 5 of 7 alleles of the included housekeeping genes had not been previously described. Thus, this strain was assigned the new sequence type 106. On eBURST analysis (www.mlst.net), this sequence type does not belong to any of the clonal complexes but is a singleton. PCR for detection of the genes encoding the putative virulence factors extracellular protein factor (EF) and suilysin were negative. Results of Western blot for detection of muramidase-released protein (MRP) and EF, using rabbit polyclonal antibody against MRP and EF (provided by

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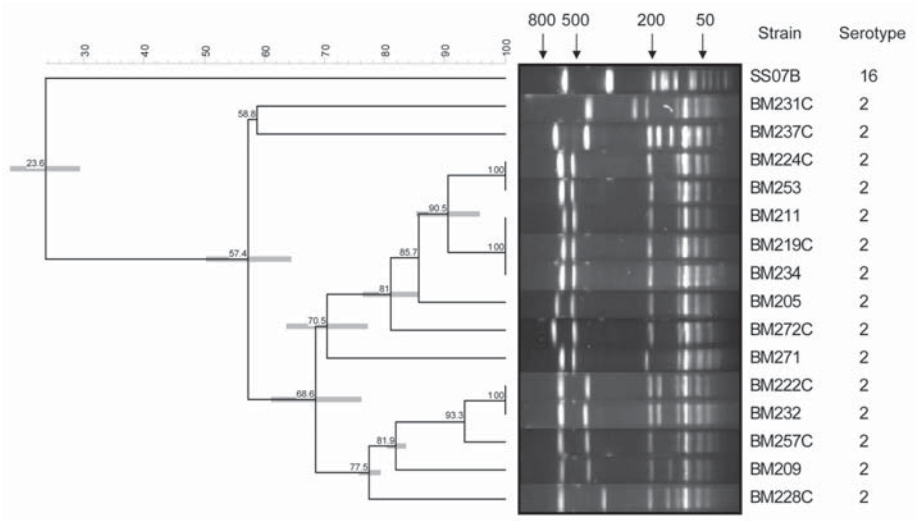

Figure. Pulsed-field gel electrophoresis after *Sma*I digestion of *Streptococcus suis* serotype 16 strain SS07 and a representative set of *S. suis* serotype 2 strains isolated from patients with meningitis in southern Vietnam. A dendrogram was generated by Dice analysis (optimization 0.5%, band tolerance 1%) and cluster analysis with unweighted pair group method with arithmetic average, using Bionumerics software (Applied Maths, Sint-Martens-Latem, Belgium). Numbers in dendrogram indicate percentage of similarity. Arrow numbers indicate molecular size (kb).

H. Smith, the Netherlands), were also negative. *S. suis* serotype 2 strains 31533 and 89-1591 (provided by M. Gottschalk, Canada) were used as positive and negative controls respectively .

The total number of human *S. suis* infections reported until August 2006 was ≈400, and nearly 90% of these cases occurred in China, Thailand, Hong Kong, Taiwan, and the Netherlands (*4*). These data did not include at least 200 cases of *S. suis* infection in Vietnam (C. Schultsz, unpub. data). At present, 33 capsular serotypes of *S. suis* have been recognized (*1*,*4*). *S. suis* serotype 2 is considered to be the most pathogenic to pigs and humans. All human cases of *S. suis* infection for which serotyping was available were caused by *S. suis* serotype 2, except for 1 case of serotype 1 (*9*), 1 case of serotype 4 (*3*), and 1 case of serotype 14 (*6*). Among 116 cases of *S. suis* meningitis seen at HTD from 1997 through 2005, 115 were caused by serotype 2 and 1 by serotype 14.

S. suis serotype 2 can cause meningitis, septicemia and septic shock, arthritis, endocarditis, pneumonia, endophthalmitis, and cellulitis in humans (*3*,*5*,*8*). The mortality rate for *S. suis* serotype 2 meningitis is <10% (*10*), but it can reach 70% among patients with septicemia and septic shock (*11*). The exact route of infection for humans is not known. Cases have been linked to accidental inoculation through skin injuries, for example, during occupational exposure to pigs and pork, but inhalation of aerosols and ingestion of contaminated food have also been suggested (*3*,*9*,*11*,*12*). Preexisting medical conditions, such as alcoholism and liver cirrhosis, as was present in our patient, or a prior splenectomy may predispose to severe infection.

Serotype 16 has never been isolated from humans and, to our knowledge, has also rarely been reported as a cause of invasive disease in pigs. One isolate of *S. suis* serotype 16 was reported from a diseased pig in Germany and 4 isolates from slaughter pigs in South Korea (*13*,*14*). The *S. suis* serotype 16 strain was sensitive to all antimicrobial agents tested except tetracycline, as has been reported for serotype 2 isolates (*5*). PFGE results showed that this human serotype 16 isolate was unrelated to human serotype 2 isolates. On MLST, this isolate had a new sequence type that did not belong to any of the clonal complexes. In contrast, most serotype 2 isolates reported so far belong to clonal complex 1 (www.mlst.net). Taken together, these results suggest that capsule switch, such as has been observed for *S. pneumoniae*, does not explain the emergence of invasive isolates of a different serotype. In addition, the PCR and Western blot analyses indicate that the serotype 2 capsule, EF, MRP, or suilysin is not required for virulence of *S. suis* in humans, as has also been shown in pigs.

Conclusions

S. suis infection is an emerging zoonosis in Asia. Strains with serotype 16 are among those capable of infecting humans.

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Dr Nghia is an infectious disease specialist. His research interests include the epidemiology, diagnosis, clinical manifestations, and treatment of *Streptococcus suis* infection in Vietnam.

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Magpies as Hosts for West Nile Virus, Southern France

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European magpies (*Pica pica*) from southern France were tested for antibodies to West Nile virus (WNV) and viral shedding in feces during spring–autumn 2005. Results suggest that this peridomestic species may be a suitable sentinel species and a relevant target for additional investigations on WNV ecology in Europe.

West Nile virus (WNV, Flaviviridae, *Flavivirus*) is an arbovirus that principally infects a wide range of bird species, but spillover infections may occur in mammals, including horses and humans. In southern France, WNV was first reported during the 1960s in the Camargue, a wetland area with many types of birds. This virus was recently detected in the same area. It was responsible for 76 equine cases in 2000 and 32 equine cases in 2004. On the basis of ornithologic and epidemiologic data, several bird species were suggested as candidates for WNV amplification and emergence in the Camargue (*1*). Among these species, corvids may be of particular interest because several species of the family Corvidae have experimentally been shown to be highly competent for WNV transmission (*2*,*3*).

We studied the European magpie or common magpie (*Pica pica*) because this species is territorial and abundant in both wet and dry areas. Pilot serologic investigations conducted in the 2000 and 2004 Camargue outbreaks in horses suggested a high WNV seroprevalence in magpies (*4*,*5*). Furthermore, WNV was isolated in 2004 from a yearling magpie near a farm with clinical equine cases (*5*). The aim of our study was to better assess WNV seroprevalence in magpies in the Camargue area and detect WNV circulation during the postepizootic year of 2005.

The Study

The study was conducted from late spring to early autumn 2005. Multicatch magpie traps, i.e., circular traps that catch ≤4 birds simultaneously, were set 1 day per week from July to September in different places within 3 areas (Figure, top panel). Area A contained dry and wet habitats in which some WNV equine cases were reported in 2004. Area B was a wetland in which most clinical equine cases occurred in 2004, and a WNV-positive magpie was detected in October 2004. Area C was a wetland in which a WNV-positive house sparrow (*Passer domesticus*) was detected in October 2004 (*5*). Additionally, some magpies were obtained in July and August 2005 from a crow ladder trap permanently set in area D, a dry area in which some horses had WNV infection in 2004. A few magpie nestlings were also sampled from their nest in May, June, and July 2005.

Flying birds were classified as juveniles or adults by using plumage criteria (*6*). All magpies were ringed, sampled (blood and cloacal swab), and released. Serum samples were first screened for immunoglobulin (Ig)G to WNV by using an indirect ELISA with horseradish peroxidase–conjugated anti-wild bird IgG (A140–110P; Bethyl Laboratories, Montgomery, TX, USA). Positive and doubtful samples were further tested by microneutralization by using the France 05.21/00 equine WNV strain (GenBank accession no. AY268132) and staining with crystal violet (*5*). Because the recapture rate of wild birds is usually low and WNV is excreted in feces of infected birds over a short period (*2*), we also tested for WNV RNA in feces of all 29 seropositive birds (i.e., 35 samples because some birds were captured several times) and 4 seronegative birds. Nucleic acid was extracted from cloacal swabs by using the QIAamp viral RNA mini kit (QIAGEN S.A., Courtaboeuf, France) and amplified with WNV-specific primers (*7*).

Of 271 magpies captured, 29 had WNV neutralizing antibodies at a titer ≥20, which confirmed a relatively high WNV seroprevalence (10.7%, 95% binomial confidence interval 7.3%–15.0%) in the Camargue magpie population. No seroconversion, i.e., a 4-fold increase in measured antibody titer, was detected in 46 (17%) magpies recaptured during the field season. Most titers were <80 (Figure, bottom panels), and WNV-positive birds recaptured in the summer (n = 5) showed titers stable over time. These findings, and the fact that adults (26/76, 34.3%) were more frequently seropositive than juveniles (3/173, 1.7%) ($p<0.001$, by χ^2 test), suggest past exposure to the virus.

Because antibodies to WNV are believed to remain detectable in birds for >1 year (*8*,*9*), adult magpies that were seropositive in this study had probably been exposed to WNV within the past few years either during the recent 2004 epizootic circulation or before this time. The first possibility is supported by the fact that WNV-positive birds were particularly abundant at site B, in which most clinical equine cases were detected in 2004. Because maternal transmission of antibodies to WNV was reported in birds (*10*,*11*), detection of 3 juvenile magpies with low antibody titers may also be explained by the 2004 WNV circulation.

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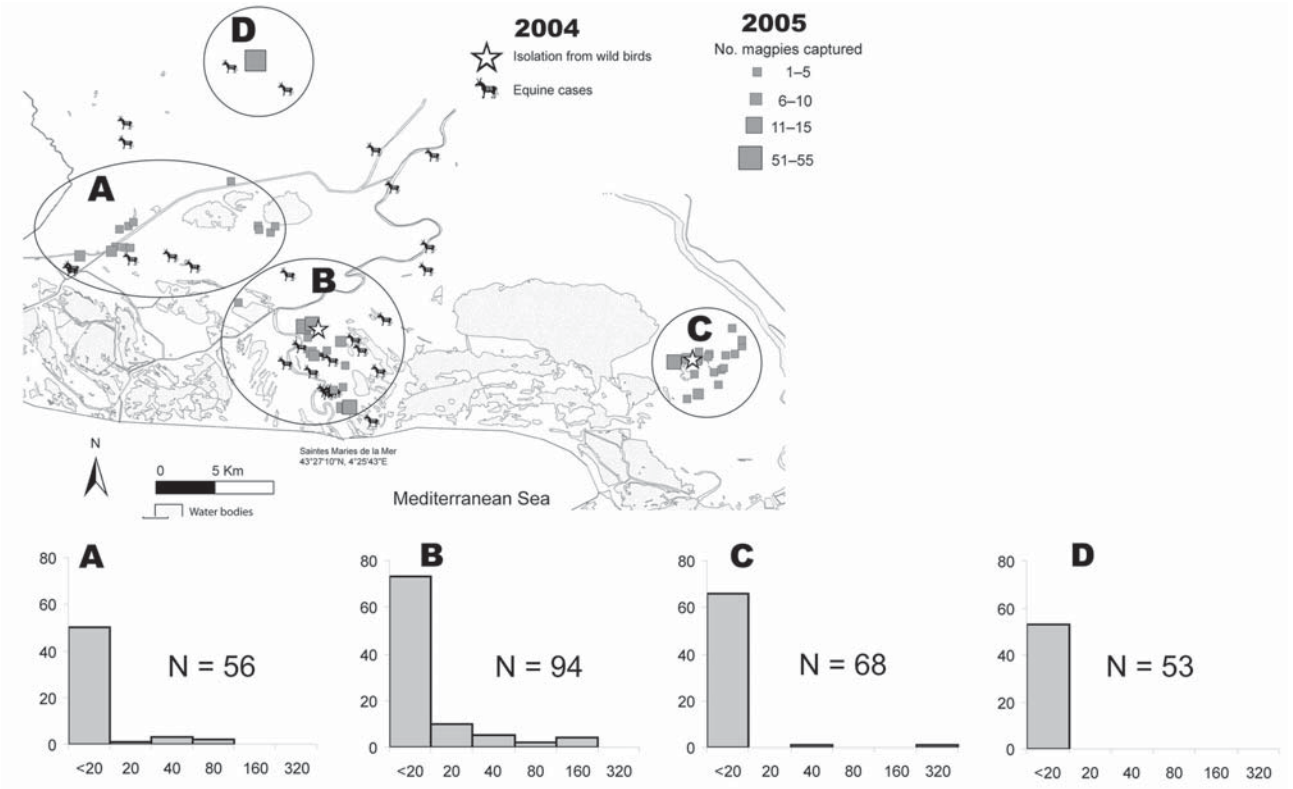

Figure. Area of Camargue, France, showing locations of magpie capture sites: site A (n = 56, with 24 adults, 32 juveniles, and 0 nestlings), site B (n = 94, with 34 adults, 57 juveniles, and 3 nestlings), site C (n = 68, with 17 adults, 34 juveniles, and 17 nestlings), and site D (n = 53, with 1 adult, 52 juveniles, and 0 nestlings). Confirmed equine and avian cases infected with West Nile virus in 2004 are also indicated. Histograms correspond to site designations and indicate serologic titers (x-axes) measured by using a microneutralization test plotted against no. birds (y-axes).

However, a cloacal sample from a seropositive juvenile magpie captured in early August 2005 was positive by nested PCR, which suggests that WNV was circulating among wild birds in 2005. Unfortunately, infectious WNV could not be isolated from this sample.

Conclusions

This serosurvey confirmed that a relatively high proportion of the Camargue magpie population has been exposed to WNV. Because magpies are sedentary, with only limited dispersal, seropositive birds in this study had likely been exposed to WNV in the Camargue area. Although serologic data suggested past exposure to the virus, detection of WNV-specific RNA by nested PCR in a cloacal swab suggests that WNV was circulating in the Camargue in 2005. No other evidence of WNV transmission was obtained in this serosurvey or by the national surveillance network of captive sentinel ducks or chickens, and no clinical equine cases were reported.

Our results suggest that magpies might be sensitive indicators of WNV enzootic activity in the Camargue. Although trapping magpies may be challenging because these birds are extremely wary and quickly learn how to avoid traps, surveillance of juvenile birds might be useful. Permanently set crow ladder traps with captive sentinel magpies to attract wild birds might be the most efficient way to capture large numbers of juvenile birds. This method would enable detection of seroconversion in captive magpies, and analysis by reverse transcription–PCR of cloacal swabs from free-ranging birds could be used as a supplemental WNV surveillance tool.

Further investigations are needed to better understand whether magpies are frequently exposed to WNV or whether observed seroprevalence is merely the result of a long history of virus circulation in the Camargue. Blood meal analyses of likely mosquito vector species, e.g., *Culex pipiens* L. and *Cx. modestus* Ficalbi (*12*,*13*), may help answer this question, although nonvectorial transmission may occur in this scavenger bird species (*14*). Other serologic surveys in the Camargue suggest that magpies have higher prevalence levels of WNV than other sedentary species (*4*,*5*; E. Jourdain, unpub. data). However, because only a few selected species were targeted and sample size was small for most of them, a survey of a wider range of bird

species is needed to enable better comparisons. Other corvid species, such as the carrion crow (*Corvus corone*) or the jackdaw (*C. monedula*), would be relevant targets.

WNV has also been reported in magpies in Russia (*15*), which suggests that these birds might be useful for WNV surveillance in other European transmission foci. However, because WNV epidemiology is complex and highly variable between sites, local epidemiologic studies should be performed before magpies are used as sentinel birds in other areas.

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Emerging Angiostrongyliasis in Mainland China

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Our review of angiostrongyliasis in China found that the disease is emerging as a result of changes in food consumption habits and long-distance transportation of food. Enhanced understanding of angiostrongyliasis epidemiology, increased public awareness about the risks associated with eating raw food, and enhanced food safety measures are needed.

Angiostrongylus cantonensis was first described as a parasite of the Norway rat (*Rattus norvegicus*) and the black rat (*R. rattus*) in Guangzhou (formerly Canton), People's Republic of China, in 1933. The first human case was reported from Taiwan in 1945. Transmission to humans is primarily by consumption of raw snails. Contaminated vegetables and paratenic hosts such as freshwater prawns, crabs, and frogs may also play a role in transmission (*1*).

The first case of human angiostrongyliasis in mainland China was diagnosed in 1984 (*2*). During the past decade, the number of cases has sharply increased (*3*). A large outbreak occurred in Beijing during 2006. The outbreak peaked during August and involved 160 persons, 100 of whom were hospitalized. This number of patients is similar to the total number of infections recorded in China over the past decade. Our aim was to briefly review angiostrongyliasis outbreaks in mainland China, update angiostrongyliasis epidemiology, and recommend measures to prevent and control angiostrongyliasis.

The Study

The first reported outbreak of angiostrongyliasis in China occurred in 1997 in the city of Wenzhou in the eastern coastal Zhejiang province; it affected 65 persons (*4*). Since 2000, 6 more outbreaks have been reported, along with numerous individual cases. A literature review found 334 recorded cases; only 4 cases were reported between 1984 and the 1997 outbreak in Wenzhou city. The 7 outbreaks summarized in Table 1 accounted for 86.5% of the total cases.

The Figure depicts the 9 provinces in China where angiostrongyliasis has been reported thus far. The youngest patient was 11 months of age, and the oldest was 70 years of age. In the recent Beijing outbreak, 99 of the 100 hospitalized patients were available for interview; 59 were male and 40 were female, age range was 13–57 years, median age was 36 years. The causative agent was confirmed for 16 (4.8%) of 334 cases. Four children died. Outbreak investigations found that 75.1% of all patients had eaten raw apple snails (*Pomacea canaliculata*) or raw giant African land snails (*Achatina fulica*).

A. cantonensis–endemic foci have been discovered in the provinces of Fujian, Guangxi, Hainan, Yunnan, and Zhejiang (Figure). Jinhua city in Zhejiang province is the parasite-endemic setting furthest north. Infected *A. fulica* were found in a farm (used for temporary cultivation and selling of snails) in Liaoning province in northeast China; however, the infected snails might have been imported from provinces located further south.

Thirty-two species of wild mollusk in China have been screened for *A. cantonensis;* 22 of these species (68.8%) harbored the parasite (Table 2). The highest rate and intensity of infections were recorded in *A. fulica*, followed by slugs (*Vaginulus* spp.) and *P. canaliculata*. Terrestrial snails and slugs showed higher rates and intensities of infections than freshwater mollusks. However, at least 1 freshwater snail, *P. canaliculata,* plays an important role in the epidemiology of angiostrongyliasis.

Of 15 wild rodent species captured in mainland China, 11 harbored *A. cantonensis*. The prevalence and intensity of infection were generally higher in *R. norvegicus* than in other rodents. Infections were also found among nonhuman primate, equine, and canine species (*5*,*6*). However, the prevalence in domestic animals and nonrodent wildlife remains to be fully investigated.

During surveys of 12 potential paratenic host species in China, *A. cantonensis* larvae were recovered from frogs (*Hylarana guentheri*, *Rana limnocharis,* and *R. plancyi*) and toads (*Bufo melanostictus*). In contrast to the situation on different Pacific islands (*7*,*8*), *A. cantonensis* has not yet been found in freshwater shrimp, fish, crabs, and planariae in mainland China.

The *A. cantonensis*–endemic area in China is rapidly expanding, and causes are multifactorial. First, *A. fulica* and *P. canaliculata* have invaded the southern part of China after being imported from East Africa in the 1930s and South America (through Taiwan) in the 1980s, respectively. While both snail species were imported as food, they also became established in the wild fauna and are now common in southern China. Second, vast habitats with suitable environmental conditions can be found in many parts of the country. Recent studies indicate suitable habitats not yet colonized; hence, further expansion of *A. cantonensis*–endemic settings is cause for concern (*9*). Finally, the low host specificity of *A. cantonensis* further supports the expansion of this parasite into new areas.

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Table 1. Reported angiostrongyliasis outbreaks in mainland China since 1997

Year	Location (city, province)	No. infections	Source of infection	Reference*
1997	Wenzhou, Zhejiang	65	*Pomacea canaliculata*†	Zheng RY et al., 2001
2002	Changle, Fujian	8	*P. canaliculata*†	Lin JX et al., 2003
2002	Fuzhou, Fujian	9	*P. canaliculata*†	Yang FZ et al., 2004
2002	Fuzhou, Fujian	13	*Achatina fulica*‡	Wu CH et al., 2004
2004	Kunming, Yunnan	25	*P. canaliculata*†	Han JH et al., 2005
2005	Kunming, Yunnan	9	*P. canaliculata*†	Wei LP et al., 2005
2006	Beijing, Beijing	160	*P. canaliculata*†	Not available

*Full reference details are available from the corresponding author upon request.
†Common name, apple snail.
‡Common name, giant African land snail.

The predominant freshwater and land snail species found in Chinese markets are *P. canaliculata, A. fulica, Cipangopaludina chinensis,* and *Bellamya aeruginosa*. The first 2 account for most *A. cantonensis* infections in mainland China. In Taiwan, *C. chinensis* plays an important role in the epidemiology of angiostrongyliasis (*10*). Recently, *B. aeruginosa* was found to harbor natural infections with *A. cantonensis;* several infections have been linked to this species (*11*,*12*). Consumption of freshwater shrimp and crabs has increased over consumption of snails. Although only few *A. cantonensis* infections could be traced to shrimp and crabs and none yet in China, their infection potential merits attention.

Conclusions

The booming economy and rapid infrastructure development in China have affected food production and trade. For example, these developments enabled the countrywide marketing of aquaculture products and snails produced in the southern provinces, which might also be responsible for the emergence of foodborne trematodiasis (*13*). After the recent angiostrongyliasis outbreak in Beijing, the source of infection was traced to *P. canaliculata* originating from Guangxi province in southwest China. Socioeconomic changes have also increased the popularity of specific consumption habits across China. Although the residents of the southeastern coastal areas have a long history of eating raw food, including snails and seafood, similar dishes have recently become popular among the urban middle and upper classes in inland China.

After the 2006 outbreak in Beijing, different measures have been proposed for preventing and controlling angiostrongyliasis. First, food safety and transportation must be improved to avoid human infections and the further spread of intermediate host snails to areas in which the disease is not endemic. In snail farms, rigorous control of rats should be implemented. Collection and marketing of wild snails should be limited. Second, hygiene and food preparation techniques in restaurants should be improved to prevent cross-contamination of other food items. Third, these efforts should be accompanied by sound information in communication and education campaigns to raise public awareness. The basic message, that consumption of raw or undercooked snails is a key risk factor for the transmission of a serious disease, can be easily conveyed and is readily understood by the public. After the first outbreak in Wenzhou city in 1997, a comprehensive health education campaign about safe food consumption habits and the most prominent symptoms associated with angiostrongyliasis was launched in Zhejiang province. Few cases were reported thereafter.

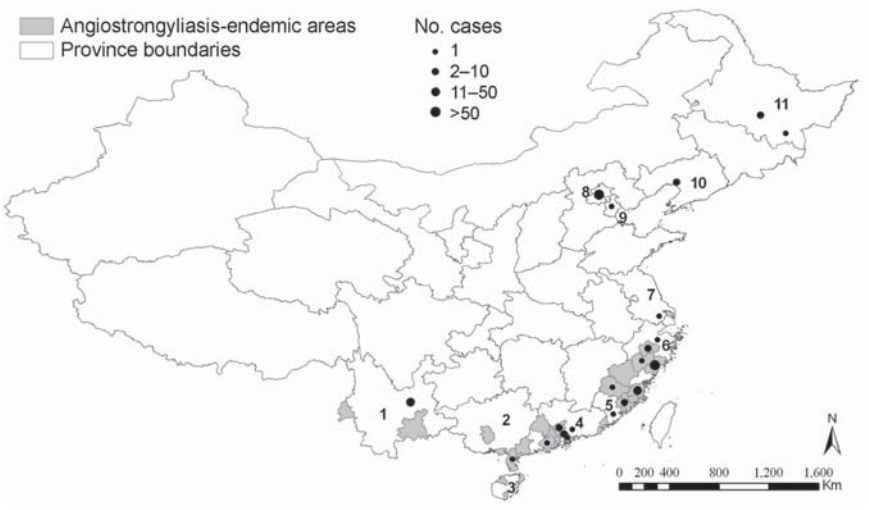

Figure. Provinces in People's Republic of China where cases of angiostrongyliasis has been reported and locations where outbreaks occurred. Province names: 1, Yunnan; 2, Guangxi; 3, Hainan; 4, Guangdong; 5, Fujian; 6, Zhejiang; 7, Jiangsu; 8, Beijing; 9, Tianjing; 10, Liaoning; 11, Heilongjiang.

Table 2. Known intermediate hosts of *Angiostrongylus cantonensis* in mainland China

Species	First investigation, y, place	First observation of positive specimens, y, place	Highest recorded prevalence, %, place	Reference*
Achatina fulica†‡	1979–1982, Guangzhou	1979–1980, Guangzhou	96.8, Zhongshan	Ding BL et al., 1982 Liang HK et al., 1984 Liang HK et al., 1992 Zhang HM et al., 1996
Pomacea canaliculata§¶	1988, Hekou	1997, Cangnan	69.4, Cangnan	Li FH et al., 1989 Pan CW et al., 2002 Li YS et al., 2001
Camaena cicatricosa‡	1979–1982, Guangzhou	1979–1982, Guangzhou	50.0, Zhongshan	Ding BL et al., 1982 Liang HK et al., 1984 Liang HK et al., 1992
Vaginulus sp.#	1980–1982, Guangzhou	1980–1982, Guangzhou	49.2, Guangzhou	Ding BL et al., 1982
Phiolomycus bilineatus#	1979–1982, Guangzhou	1979–1982, Guangzhou	100, Guangzhou	Ding BL et al., 1982 Liang HK et al., 1984
Deroceras leave#	2005, Lianjiang/Nan'an	2005, Lianjiang/Nan'an	23.8, Lianjiang/Nan'an	Li LS et al., 2006
Vaginulus yuxjsjs sp. nov#	1988, Hekou	1988, Hekou	21.0, Hekou	Li FH et al., 1989
Ariophantidae spp.‡	1988, Hekou	1988, Hekou	12.0, Hekou	Li FH et al., 1989
Macrochlamys loana#	2005, Lianjiang/Nan'an	2005, Lianjiang/Nan'an	11.2, Lianjiang/Nan'an	Li LS et al., 2006
Limax flavus#	2005, Lianjiang/Nan'an	2005, Lianjiang/Nan'an	10.1, Lianjiang/Nan'an	Li LS et al., 2006
Bradybaena brevispira‡	1979–1982, Guangzhou	1979–1980, Guangzhou	8.3, Nanhai	Liang HK et al., 1984 Liang HK et al., 1992 Pan CW et al., 2002
Meghimatium bilinestum‡	2005, Lianjiang/Nan'an	2005, Lianjiang/Nan'an	5.9, Lianjiang/Nan'an	Li LS et al., 2006
Trichochloritis rufopila‡	1986–1990, Zhuhai	1986–1990, Zhuhai	5.8, Zhuhai	Liang HK et al., 1992
Trichochloritis hungerfordianus‡	1986–1990, Zhuhai	1986–1991, Zhuhai	5.7, Zhuhai	Liang HK et al., 1992
Vaginulus alte#	1988, Hekou	1988, Hekou	4.2, Hekou	Li FH et al., 1989
Bellamya spp.¶	2005, Lianjiang/Nan'an	2005, Lianjiang/Nan'an	4.1, Lianjiang/Nan'an	Li LS et al., 2006
Bellamya aeruginosa¶	2004, Minhou/Lianjiang	2004, Minhou/Lianjiang	3.8, Minhou	Lin JX et al., 2005
Bradybaena similaris‡	1980–1982, Guangzhou	1980–1982, Guangzhou	3.2, Guangzhou	Ding BL et al., 1982
Bradybaena ravida‡	2005, Lianjiang/Nan'an	2005, Lianjiang/Nan'an	3.1, Lianjiang/Nan'an	Li LS et al., 2006
Plectotropis appanata‡	2005, Lianjiang/Nan'an	2005, Lianjiang/Nan'an	2.6, Lianjiang/Nan'an	Li LS et al., 2006
Bellamya quadrata¶	1986–1990, Panyu	1986–1990, Panyu	2.5, Panyu	Liang HK et al., 1992

*Full reference details are available from the corresponding author upon request.
†Common name, giant African land snail.
‡Land snail.
§Common name, apple snail.
¶Freshwater snail.
#Slug.

Awareness of angiostrongyliasis needs to be improved for consumers and health professionals. Education campaigns should inform consumers about the risk of contracting angiostrongyliasis, e.g., by eating raw snails, and professional knowledge among healthcare providers should be improved to ensure timely detection of infections and adequate medical response.

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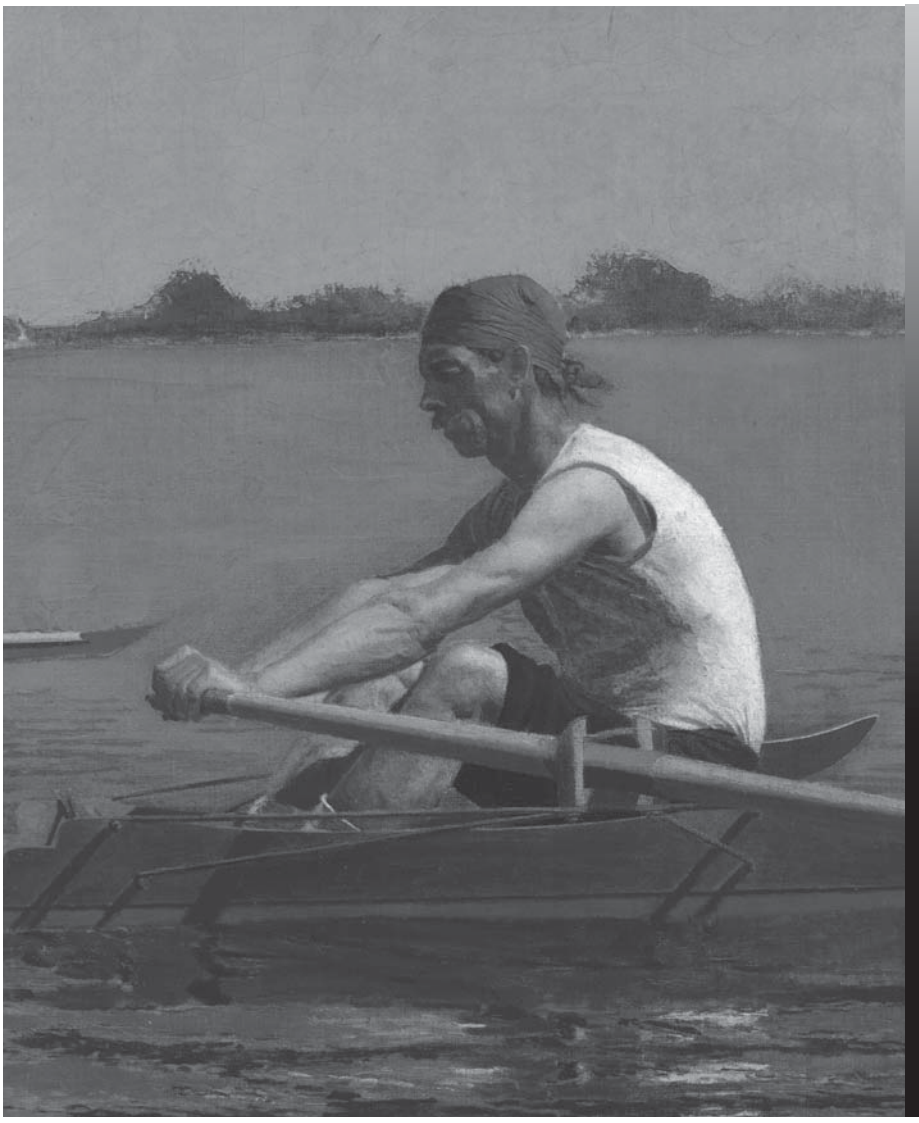

Hepatitis E in England and Wales

Hannah C. Lewis,* Sophie Boisson,* Samreen Ijaz,* Kirsten Hewitt,* Siew Lin Ngui,* Elizabeth Boxall,† Chong Gee Teo,* and Dilys Morgan*

In 2005, 329 cases of hepatitis E virus infection were confirmed in England and Wales; 33 were confirmed indigenous infections, and a further 67 were estimated to be indigenous infections. Hepatitis E should be considered in the investigation of patients with hepatitis even if they have no history of travel.

Acute hepatitis caused by hepatitis E virus (HEV) has historically been considered an imported disease associated with travel to a disease-endemic area. However, an increasing number of sporadic hepatitis E cases not associated with travel have been reported from industrialized countries, including England and Wales (*1*).

Four HEV genotypes have been identified that correlate with the geographic origin of the virus (*2*). Genotype 1 is regularly identified with disease-hyperendemic areas such as Africa and southern Asia; genotype 2, with Mexico and West Africa; genotype 3, with industrialized countries such as North America, Europe, and Japan; and genotype 4, with eastern Asia and India.

The epidemiology of non–travel-associated hepatitis E is largely unknown. Although the main route of transmission in disease-hyperendemic areas is the consumption of fecally contaminated water, risk factors for HEV infection in non–disease-hyperendemic countries have included occupational exposure to pigs (*3*) and consumption of raw or undercooked pork products (*4*), shellfish (*5*), and venison (*6*).

HEV is endemic in pig populations worldwide (*7*), including England and Wales, where HEV genotype 3 is widespread in pigs, and subgenomic sequencing studies have shown a close relationship between pig and human HEV strains (*8*). Although zoonotic transmission from swine to humans appears plausible, the possibility of a reservoir common to both swine and humans cannot be excluded.

The Study

The reference laboratory for hepatitis E at the Health Protection Agency's Centre for Infections reported 17 cases of non–travel-associated hepatitis E in England and Wales from 1996 through 2003 (*5*). During 2004, that laboratory and the other hepatitis E reference laboratory in Birmingham received an increased number of samples for HEV testing with a corresponding increase in numbers of hepatitis E cases diagnosed by testing for HEV immunoglobulin (Ig) M and IgG by using ELISAs (Genelabs Technologies Inc, Redwood City, CA, USA). These observations led to this study, the aims of which were to describe the epidemiology of hepatitis E in England and Wales, estimate the number of non–travel-associated cases, and identify risk factors for HEV infection in indigenous cases.

During 2005, the reference laboratories confirmed 329 cases of acute hepatitis E infection. This number represented a substantial increase from 125 cases in 2003 and 150 in 2004. Information on the age groups and sex of all patients who received a diagnosis during 2003, 2004, and 2005 is shown in Figure 1. This figure shows a progressive increase in the number of acute cases of hepatitis E in older male patients over the period, and panel B demonstrates the overrepresentation of older men among those with established indigenous cases during 2005 compared with those who were known travelers.

The travel history of the 329 patients is summarized in Figure 2. For 102 (31%) patients, pre-illness travel information was recorded on the laboratory request form. Travel status was obtained for 44 additional patients through follow-up, and 33 patients who had not traveled outside the United Kingdom were considered to be indig-

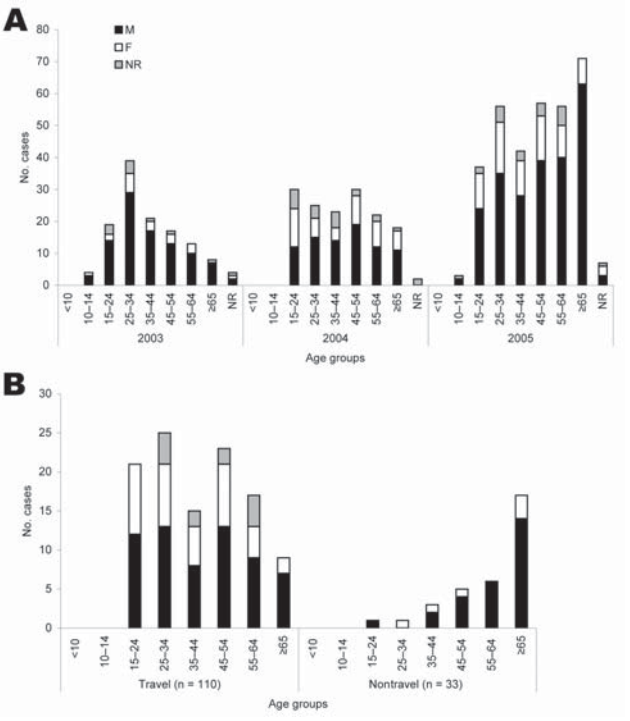

Figure 1. A) Age and sex distribution of acute hepatitis E patients, 2003–2005 (n = 604). B) Age, sex distribution, and travel associated with indigenous acute hepatitis E case-patients (n = 143). NR, not recorded.

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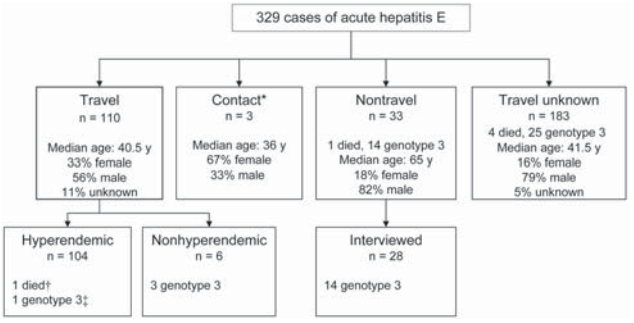

Figure 2. Details of cases of acute hepatitis E (HEV) infections, 2005. *Contact with hepatitis E patients who recently returned from hyperendemic countries; †24-year-old woman infected with HEV genotype 1; ‡45-year-old Caucasian man who traveled to Iraq.

enous case-patients. Twenty-three (70%) indigenous case-patients were >55 years of age, compared to 26 (24%) of 110 travel-associated case-patients; the median age was 65 years (interquartile range [IQR] 50–74 years) and 41 years (IQR 29–54) ($p<0.0001$) respectively. Thirty-two of the indigenous case-patients (97%) had Caucasian names (name classification was used as a proxy for ethnicity) compared with 21 (19%) of 110 travelers ($p<0.001$).

The number of indigenous HEV infections was estimated for those with an unknown travel history by fitting a logistical regression model that used Caucasian name and age as predictor variables. An estimated 67 (95% confidence interval 58–75) of the 176 patients (when the date of birth was known) in this group acquired their infection in England and Wales.

Twenty-eight indigenous case-patients were available for telephone interview in which a detailed structured questionnaire was used to identify potential risk factors over the 9-week period before illness. The Table summarizes the results obtained from responses. All patients were white, and 23 (82%) were male, with a median age of 65 years. Twenty (71%) were referred from southern England and Wales; a similar proportion of interviewed patients lived at inland or coastal addresses, and a high proportion of patients lived in densely populated urban areas. No common risk factors were identified apart from 17 (60%) owning pets.

HEV RNA was detected in 14 of the 33 patients with indigenous hepatitis E, and all virus strains belonged to genotype 3. All but one of the genotype 3 nucleotide sequences clustered with previously described strains from the United Kingdom and Europe. Three distinct sequence groups were noted, but subsequent detailed analysis showed no correlation between these groups and geographic distribution or time, which indicated that a common source was unlikely.

Conclusions

The number of hepatitis E cases diagnosed in England and Wales began increasing substantially after 2004. In 2005, acute hepatitis E was diagnosed in 329 patients; 33 of these definitely had acquired their infection in England and Wales, and a further 67 were estimated to have acquired their infection there. These figures may nevertheless be an underestimation since we also found that a high proportion of clinicians do not test for HEV unless the patient reports a recent history of travel to a disease-endemic area. This practice may be changing, and the progressive increase in the number of older patients with diagnoses of hepatitis E may reflect a greater awareness among clinicians. Local interest in hepatitis E may well explain the geographic distribution of indigenous cases.

The demographic features of patients who acquired their infection in England and Wales are striking. Most patients were Caucasian men >55 years of age. These find-

Table. Main risk factors for cases of indigenous hepatitis E, England and Wales, 2005*

Risk factor	Yes/ possibly	No
Residence		
Coastal address (<20 miles from sea)	15	13
Urban area (>10,000 inhabitants)	27	1
Animal exposure		
Occupation involving animal exposure	1	27
Own pets	17	11
Live on/visited a farm	4	24
Food preferences (i.e., likely to have consumed in the 9 weeks before onset of illness)		
Pork	22	5
Raw/undercooked pork	0	27
Pig liver	7	19
Raw/undercooked pig liver	0	26
Venison	0	25
Chicken	24	2
Raw/undercooked chicken	0	27
Other meat (beef, lamb, turkey)	18	8
Raw/undercooked other meat	4	21
Fish	18	9
Shellfish	5	22
Food preparation		
Handle raw meat for cooking	13	15
Wash fruits	18	10
Wash vegetables	20	5
Drinking water supply		
Mains	21	6
Bottled water	15	13
River/stream or well	2	26
Recreational water exposure	5	23
Dinghy sailing	2	25
Fishing	4	24
Swimming (pool)	1	26

*n = 28; some respondents did not answer all questions, so not all cells add up to 28.

ings corroborate other studies in the literature (*9*). Possible reasons for this high attack rate in older adults remain unclear. Men infected with HEV are more likely to access healthcare than women in both disease-hyperendemic and non–disease-hyperendemic countries (*5*,*10*). Some researchers have suggested that risk factors for disease may be linked to male occupational or societal roles (*2*). These unique demographic findings need to be emphasized to the medical community because older Caucasian men may not be perceived as being at risk by physicians.

The epidemiology of hepatitis E in non–disease-endemic countries remains poorly understood, and the results from seroprevalence studies vary greatly, which suggests that commercially available serologic tests may not be reliable for population surveys on genotype 3 infections. Despite in-depth telephone interviews, we did not identify a likely common source of infection, and genotyping data suggest multiple sources of exposure to HEV. We found that 60% of patients reported owning pets, a higher proportion than the proportion of animal-owning households in the United Kingdom for cats (25%) and dogs (21%) (*11*). Similar findings have been reported in the Netherlands (*12*). Although this difference might just reflect the fact that pet ownership is higher among older persons, a high prevalence of anti-HEV antibodies has been found among dogs in India (*13*) and cats in Japan (*14*), and a patient with hepatitis E from Japan owned a pet cat who was positive for antibodies to HEV (*15*), all of which indicate that domestic animals may be a potential reservoir for infection.

With the growing recognition of hepatitis E as an increasingly important zoonotic infection in England and Wales, HEV should be considered an etiologic agent of acute and fulminant hepatitis even in patients who report no travel history. We need to raise awareness among health professionals to consider hepatitis E when investigating non–travel-associated cases of hepatitis.

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Ms Lewis is a fellow in the European Program for Intervention Epidemiology Training at Statens Serum Institut in Denmark. This study was undertaken while she worked as an epidemiologist at the Centre for Infection, Health Protection Agency, London. Her main interests are zoonoses and emerging infections with an emphasis on outbreak investigation and field epidemiology.

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Protochlamydia naegleriophila as Etiologic Agent of Pneumonia

Nicola Casson,* Rolf Michel,† Karl-Dieter Müller,‡ John David Aubert,* and Gilbert Greub*

Using ameba coculture, we grew a *Naegleria* endosymbiont. Phenotypic, genetic, and phylogenetic analyses supported its affiliation as *Protochlamydia naegleriophila* sp. nov. We then developed a specific diagnostic PCR for *Protochlamydia* spp. When applied to bronchoalveolar lavages, results of this PCR were positive for 1 patient with pneumonia. Further studies are needed to assess the role of *Protochlamydia* spp. in pneumonia.

Recently, a *Naegleria* endosymbiont (KNic) was observed but remained uncultivable, precluding precise identification (*1*). We grew a large amount of strain KNic by using *Acanthamoeba castellanii,* which enabled phenotypic, genetic and phylogenetic analyses that supported its affiliation as *Protochlamydia naegleriophila*. This new ameba-resistant intracellular bacteria might represent a new etiologic agent of pneumonia because it is likely also resistant to human alveolar macrophages (*2*,*3*). Because other *Parachlamydiaceae* were associated with lung infection (*4*–*6*), we assessed the role of *Pr. naegleriophila* in pneumonia by developing a diagnostic PCR and applying it to bronchoalveolar lavages.

The Study

KNic growth in *A. castellanii* was assessed by immunofluorescence (*7*) with in-house mouse anti-KNic and Alexa488-coupled anti-immunoglobulin antibodies (Invitrogen, Eugene, OR, USA). Confocal microscopy (LSM510; Zeiss, Feldbach, Switzerland) confirmed the intracellular location of KNic and demonstrated its rapid growth within *A. castellanii*. To precisely assess the growth rate, we performed PCR on *A. castellanii*/KNic coculture by using PrF/PrR primers and PrS probe. After 60 hours, we observed an increased number of bacteria per microliter of 4 logarithms (online Appendix Figure, available from www.cdc.gov/EID/content/14/1/167-appG.htm).

A. castellanii/KNic and *N. lovaniensis*/KNic cocultures were processed for electron microscopy as described (*7*). Ameba filled with bacteria exhibiting 3 developmental stages already described in other *Parachlamydiaceae* (*8*) were observed (Figure 1).

To measure the serologic differentiation index (SDI) between strain KNic and other *Chlamydia*-like organisms, we immunized Balb/c mice to produce anti-KNic antibodies. Purified *Pr. amoebophila* (ATCC PRA-7), *Simkania negevensis* (ATCC VR-1471), *Parachlamydia acanthamoebae* strain Seine, *Waddlia chondrophila* (ATCC 1470), *Neochlamydia hartmannellae* (ATCC 50802), *Criblamydia sequanensis* (CRIB 18), and *Rhabdochlamydia crassificans* (CRIB 01) antigens were tested by micro-immunofluorescence against mouse anti-KNic antibodies, whereas KNic antigen was tested with serum against all these different *Chlamydia*-like organisms (*9*). SDIs were calculated as described (*9*,*10*). Serum from mice immunized with KNic showed strong reactivity against autologous antigen (titers of 4,096). Significant cross-reactivity between KNic and *Pr. amoebophila* (SDI = 7) and *P. acanthamoebae* (SDI = 10) was observed. Mouse anti-KNic serum did not react with other *Chlamydia*-like organisms (Table 1). Because cross-reactivity between members of the order *Chlamydiales* was proportional to the relatedness between each species (*9*), the strong cross-reactivity between KNic and *Pr. amoebophila* supports the affiliation of KNic in the genus *Protochlamydia*.

Taxonomic position of KNic was further defined by sequencing 16Sr RNA (*rrs*, DQ635609) and ADP/ATP translocase (*nnt*, EU056171) encoding genes. The *rrs* was amplified/sequenced using 16SIGF/RP_2Chlam primers (*11*). The *nnt* was amplified/sequenced using nntF2p (5′-TGT(AT)GAT(CG)CATGGCAA(AG)TTTC-3′) and nntR1p (5′-GATTT(AG)CTCAT(AG)AT(AG)TTTTG-3′) primers. Genetic and phylogenetic analyses were conducted by using MEGA software (*12*). The 1,467-bp *rrs* sequence showed 97.6% similarity with *Pr. amoebophila*, 91.8%–93.2% with other *Parachlamydiaceae*, and 85.7%–88.6% with other *Chlamydiales*. Based on the Everett genetic criteria (*13*), KNic corresponds to a new species within the *Protochlamydia* genus because its sequence similarity with *Pr. amoebophila* is >95% (same genus) and <98.5% (different species). Phylogenetic analyses of *rrs* gene sequences showed that KNic clustered with *Pr. amoebophila*, with bootstraps of 98% and 95% in neighbor-joining and minimum-evolution trees, respectively. The 569-bp *nnt* sequence exhibited 91.1% similarity with *Pr. amoebophila*, 65.5%–72.6% with other *Parachlamydiaceae*, and 55.4%–72.6% with other *Chlamydiales*. Phylogenetic analyses of *nnt* sequences showed that KNic clustered with *Pr. amoebophila*. On the basis of these analyses, we propose to name strain KNic "*Protochlamydia naegleriophila*."

We then developed a specific diagnostic PCR for *Protochlamydia* spp. Primers PrF (5′-CGGTAATACG

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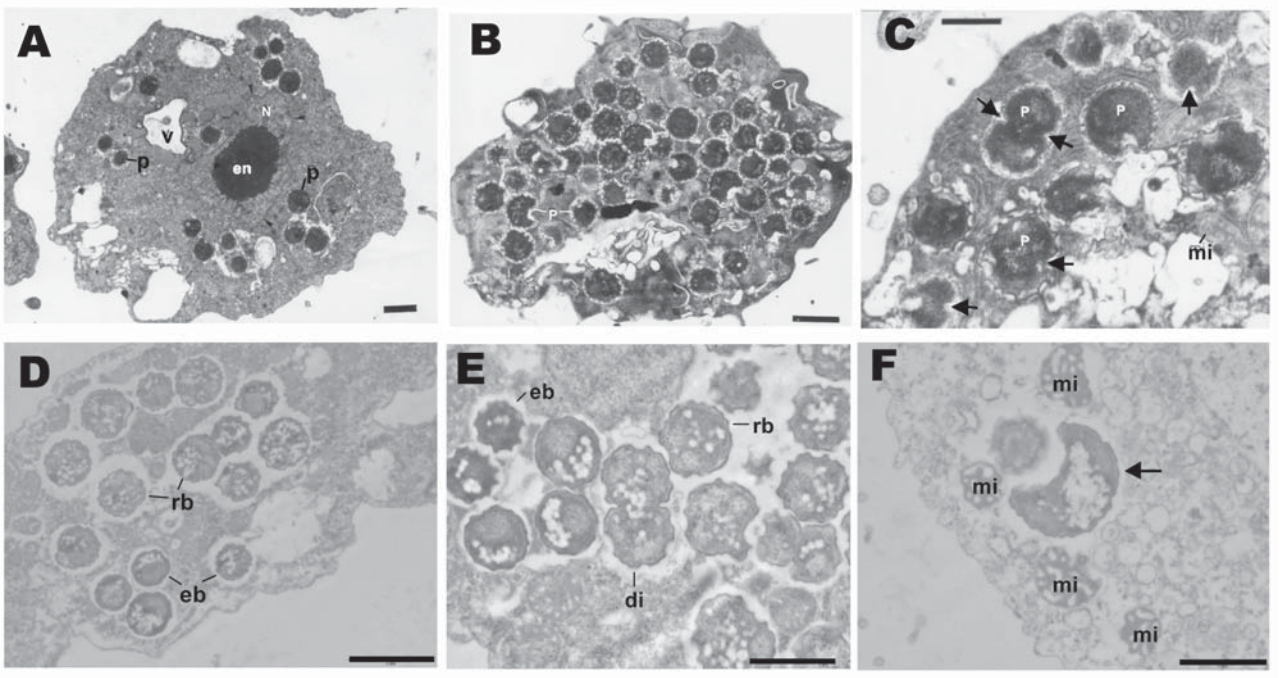

Figure 1. Transmission electron microscopy of *Protochlamydia naegleriophila*. A) *Naegleria lovaniensis* trophozoite after transfer of endocytobionts; strain KNic (p) from the original host strain showing 15 coccoid bacteria distributed randomly within the cytoplasm of the host ameba. N, nucleus; en, endosome (karyosome) within the nucleus; v, empty food vacuoles. Magnification ×10,500; bar = 1 μm. B) *N. lovaniensis* trophozoite jammed with numerous endoparasitic stages of *Pr. naegleriophila*. Magnification ×16,800; bar = 1 μm. C) Enlarged detail of *N. lovaniensis* trophozoite with intracytoplasmic stages of *Pr. naegleriophila*. Some stages show binary fission indicated by the fission furrow (arrows). The endoparasites have a wrinkled gram-negative outer membrane rendering a spiny appearance to the endoparasites. Signs of damage are obvious within the cytoplasm of the host ameba. mi, mitochondria. Magnification ×43,500; bar = 0.5 μm. D) *Pr. naegleriophila* within vacuoles of *Acanthamoebae castellanii* ameba 2 days postinfection. Elementary bodies (eb) and reticulate bodies (rb) are visible. Elementary bodies harbor a smooth membrane compared with the reticulate bodies, which have a spiny shape. Magnification ×10,000; bar = 2 μm. E) Enlarged detail of *A. castellanii* trophozoite with intracytoplasmic stages of *Pr. naegleriophila* 3 days postinfection. Binary fission is observed (di). Magnification ×20,000; bar = 1 μm. F) Crescent body (arrow) within *A. castellanii* observed 3 days postinfection. Magnification ×20,000; bar = 1 μm.

GAGGGTGCAAG-3′) and PrR (5′-TGTTCCGAGGTT GAGCCTC-3′) as well as probe PrS (5′-TCTGACTGAC ACCCCCGCCTACG-3′) were selected. The 5′-Yakima-Yellow probe (Eurogentec, Seraing, Belgium) contained locked nucleic acids (underlined in sequence above). The reactions were performed with 0.2 μM each primer, 0.1 μM probe, and iTaqSupermix (Bio-Rad, Rheinach, Switzerland). Cycling conditions were as described (*14*), and PCR products were detected with ABIPrism7000 (Applied Biosystems, Rotkreuz, Switzerland). Each sample was amplified in duplicate. Inhibition, negative PCR mixture, and extraction controls were systematically tested.

To allow quantification, a plasmid containing the target gene was constructed by cloning PCR products into pCR2.1-TOPO vector (Invitrogen, Basel, Switzerland). Recombinant plasmid DNA quantified using Nanodrop ND-1000 (Witech, Littau, Switzerland) was 10-fold diluted and used as positive controls.

The analytical sensitivity was 10 copy/μL (Figure 2, panel A). Intra-run variability was good (Figure 2B) with a Bland-Altman bias of 0.99 and a limit of agreement of 2.87 (Figure 2, panel A). Inter-run variability was low at high concentration, 1.12, 1.71, 0.82, 1.77 cycles for 10^5, 10^4, 10^3, 10^2 copies/μL, respectively. Inter-run variability was higher at low concentration, 4.22 cycles for 10^1 copies/μL (Figure 2, panel A). Analytical specificity was tested with bacterial and eukaryotic DNA (Table 2). The PCR slightly amplified DNA from *R. crassificans*, another *Chlamydia*-like organism. No cross-amplification was observed with any other bacteria or with human cells. The absence of cross-amplification of *P. acanthamoebae* is important because this *Chlamydia*-related bacteria is considered an emerging agent of pneumonia (*4*–*6*).

We tested 134 bronchoalveolar lavage samples from patients with (n = 65) and without (n = 69) pneumonia and extracted DNA by using a Bio-Rad Tissue Kit. One sample was positive, with 543 and 480 copies/μL. This positive result was confirmed using the 16sigF/16sigR PCR (*13*), which targets another DNA segment. This sequence exhibited 99.6% (284/285) similarity with *Pr. naegleriophila*

Table 1. Antibody titers and serologic differentiation index (SDI) obtained from reciprocal cross-reactions of mouse antiserum with different *Chlamydia*-like organisms, as determined by immunofluorescence*

Strains	Antigen titers (SDI)							
	Pr. naegleriophila	*Pr. amoebophila*	*Simkania negevensis*	*P.a.* strain Seine	*Waddlia chondrophila*	*Neoclamydia hartmannellae*	*Criblamydia sequanensis*	*R. crassificans*
Pr. naegleriophila	4,096 (0)	256 (7)	<4 (14)	512 (10)	4 (20)	16 (14)	<4 (20)	<4 (13)
Pr. amoebophila	32 (7)	256 (0)	<32 (5)	1,024 (3)	64 (10)	32 (0)	128 (9)	64 (3)
S. negevensis	32 (14)	128 (5)	512 (0)	64 (12)	128 (12)	<32 (12)	512 (8)	256 (3)
P. strain Seine	128 (10)	512 (3)	32 (12)	16,384 (0)	64 (19)	64 (12)	<32 (19)	<32 (12)
W. chondrophila	32 (20)	128 (10)	32 (12)	<32 (19)	32,768 (0)	<32 (18)	512 (13)	128 (10)
N. hartmannellae	32 (14)	128 (0)	<32 (12)	128 (12)	<32 (18)	2,048 (0)	<32 (18)	<32 (11)
C. sequanensis	32 (20)	128 (9)	128 (8)	64 (19)	256 (13)	<32 (18)	32,768 (0)	128 (8)
R. crassificans	32 (13)	128 (3)	64 (3)	64 (12)	64 (10)	<32 (11)	256 (8)	256 (0)

**Pr., Protochlamydia*; *P.a., Parachlamydia acanthamoebae*; *R., Rhabdochlamydia.* Titers highlighted in gray, previously published in (*9*), are provided here because these data were used to calculate SDI between *Pr. naegleriophila* strain KNIC and the other *Chlamydia*-related bacteria.

strain KNic and 95.1% (269/283) with *Pr. amoebophila*. The presence of *Protochlamydia* antigen in the sample was confirmed by immunofluorescence performed using rabbit anti-KNic antibody directly on the bronchoalveolar lavage sample and by ameba coculture (online Appendix Figure).

The positive sample was taken from an immunocompromised patient who had cough, dyspnea, and a lung infiltrate. Bronchoscopy examination of the lower respiratory tract showed mucosal inflammation localized at the middle lung lobe. Cytology and Gram stain of the bronchoalveolar lavage showed many leucocytes with macrophages (65%) and neutrophils (23%). Although no antimicrobial treatment was administered prior to bronchoscopy, no other etiologic agent was identified despite extensive microbiologic investigations of bronchial aspirate and bronchoalveolar lavage. Results of Gram stain, auramine stain (for *Mycobacterium* spp.), and silver stain (for *Pneumocystis carinii*) tests were negative. Only physiologic oropharyngeal flora could be grown on sheep-blood and chocolate-bacitracin agars. Cell culture, as well as culture for fungi and mycobacteria, remained sterile. Moreover, results of PCRs specific for the detection of *Legionella pneumophila*, *Chlamydophila pneumoniae*, and *Mycoplasma pneumoniae* (*15*) were all negative. The patient recovered and remained free of symptoms of acute lung infection during the next 20 months.

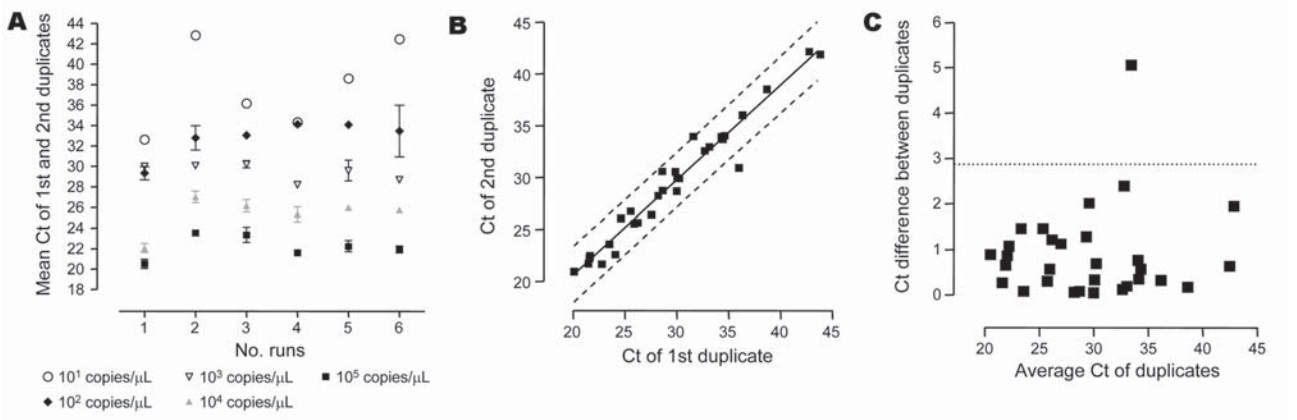

Figure 2. A) Intra and inter-run reproducibility of the real-time PCR assessed on duplicate of plasmidic positive controls performed at 10-fold dilutions from 10^5 to 10^1 plasmid/µL during 6 successive runs. Standard deviations show the intra-run reproducibility of the real-time PCR. B) Plots of the cycle threshold (Ct) of first and second duplicates, showing intra-run and inter-run variability of the real-time PCR between duplicates of positive control. 95% confidence interval is shown by the dashed lines. C) Bland-Altman graph showing the difference of Ct of both duplicates according to the mean of the Ct of duplicates. The dashed line shows the 95% confidence interval (i.e., limit of agreement).

Table 2. Bacterial and eukaryotic DNA used to determine the specificity of the real-time PCR

Bacterial DNA	Source/strain
Bordetella pertussis	Clinical specimen
Chlamydia trachomatis	Clinical specimen
Chlamydophila pneumoniae	ATCC VR-1310
Criblamydia sequanensis	CRIB-18
Enterococcus faecalis	ATCC 29212
Escherichia coli	ATCC 35218
Gardnerella vaginalis	Clinical specimen
Haemophilus influenzae	ATCC 49247
Klebsiella pneumoniae	ATCC 27736
Lactobacillus spp.	Clinical specimen
Legionella pneumophila	Clinical specimen
Listeria monocytogenes	Clinical specimen
Moraxella catharralis	Clinical specimen
Mycobacterium tuberculosis	Clinical specimen
Neisseria lactamica	Clinical specimen
Neisseria weaveri	Clinical specimen
Neochlamydia hartmanella	ATCC 50802
Parachlamydia acanthamoebae strain BN9	ATCC VR-1476
Parachlamydia acanthamoebae strain Hall's coccus	ATCC VR-1476
Protochlamydia amoebophila strain UWE25	ATCC PRA-7
Pseudomonas aeruginosa	ATCC 27853
Rhabdochlamydia crassificans	CRIB-01
Simkania negevensis	ATCC VR-1471
Staphylococcus epidermidis	Clinical specimen
Streptococcus agalactiae	ATCC 13813
Streptococcus mutans	Clinical specimen
Streptococcus pneumoniae	Clinical specimen
Streptococcus pyogenes	ATCC 19615
Waddlia chondrophila	ATCC VR-1470
Eukaryotic DNA	Source/strain
Acanthamoeba castellanii	ATCC 30010
Candida albicans	ATCC 10231
Human cells	ATCC CCL-185

Conclusions

Isolating new species from environmental and clinical samples is important to better define their epidemiology and potential pathogenicity. We defined the taxonomic position of a novel *Naegleria* endosymbiont and proposed its affiliation within the *Protochlamydia* genus as *Pr. naegleriophila* sp. nov. Moreover, we developed a new PCR targeting *Protochlamydia* spp., applied it to clinical samples, and identified a possible role of *Pr. naegleriophila* as an agent of pneumonia.

Protochlamydia naegleriophila (nae.gle.rio′.phi.la Gr. fem.n. *Naegleria*, name of host cell, Gr. adj. *philos, -a* friendly to, referring to intracellular growth of *Protochlamydia naegleriophila* strain KNic within *Naegleria* amebae). The 16Sr RNA sequence (DQ635609) of KNic is 97.6% similar to that of *P. amoebophila,* making this organism a member of the genus *Protochlamydia*. KNic does not grow on axenic media (*1*) but grows by 4 logarithms in 60 h within *A. castellanii*. KNic exhibits a *Chlamydia*-like developmental cycle, with reticulate, elementary, and crescent bodies. The reticulate body is about 900 nm and has a spiny appearance similar to that of *P. amoebophila* (Figure 2, panel B). To be classified within the *Pr. naegleriophila* species, a new strain should show a 16Sr RNA similarity ≥98.5% (*13*) and similar phenotypic traits.

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Ms Casson is completing a PhD thesis at the University of Lausanne. Her research is dedicated to defining the role of the obligate intracellular *Chlamydia*-like organisms to humans, discovering new species, and defining their role in pneumonia.

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Adamantane-Resistant Influenza Infection During the 2004–05 Season

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Adamantane-resistant influenza A is an emerging problem, but infections caused by resistant and susceptible viruses have not been compared. We identified adamantane resistance in 47% of 152 influenza A virus (H3N2) isolates collected during 2005. Resistant and susceptible viruses caused similar symptoms and illness duration. The prevalence of resistance was highest in children.

During the past 4 decades, antiviral drug therapy has been a useful strategy for both prophylaxis and treatment of influenza A (*1*,*2*). The most widely used drugs have been 2 adamantane derivatives (amantadine and rimantadine), which are effective for prophylaxis and reduce illness duration if started within 48 hours after symptom onset (*3*,*4*). These drugs block the M2 ion channel protein, preventing viral replication (*5*). From 1997 through 2004, few influenza A (H3N2) isolates were resistant to adamantanes, although resistance among isolates from Asia increased substantially in 2003 (*6*). In the United States, the proportion of influenza A (H3N2) isolates with adamantane resistance was 0.8%–2.2% during this period and 11% in the 2004–05 influenza seasons. However, in the 2005–06 season, >90% of influenza A (H3N2) isolates from patients in 26 states contained a mutation conferring resistance to adamantane drugs (*7*).

Ferret models suggest that virulence is not increased by adamantane resistance in influenza A (H3N2) infections (*8*), although experimental studies with recombinant influenza (H1N1) in mice have suggested that the mortality rate is increased by a double mutation conferring adamantane resistance (9). Little is known regarding clinical effects in humans infected with adamantane-resistant influenza viruses. We compared clinical and demographic characteristics of patients infected with either adamantane-susceptible or -resistant strains of influenza A during the 2004–05 season.

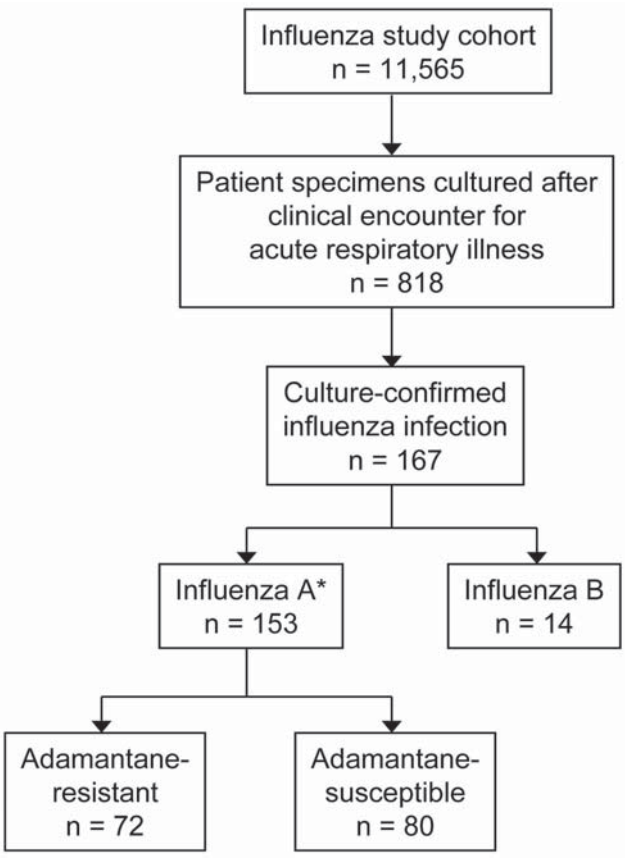

Figure. Results of patient recruitment and influenza cultures. *One isolate was not characterized.

The Study

Study participants were derived from a study of influenza vaccine effectiveness conducted within a 14–Zip code region surrounding Marshfield, Wisconsin, during January–March 2005. Eligible participants included children 6–23 months of age, adults ≥65 years of age, and persons 24 months–64 years of age with a high-risk medical condition. Research coordinators recruited eligible patients and obtained samples for influenza culture during inpatient and outpatient encounters for acute respiratory illness. Each participant (or parent) completed a short interview form to assess symptoms and onset date, and culture-confirmed patients were contacted again to determine the illness recovery date. The study was approved by the Marshfield Clinic institutional review board, and all participants provided written informed consent.

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Influenza virus was isolated by inoculation and incubation in monolayered rhesus monkey kidney cells. Cultures were examined for cytopathic effect, and cultures showing no cytopathic effect were tested twice for hemagglutination titers. Influenza A and B were confirmed by immunofluorescence (Chemicon International, Inc., Temecula, CA, USA). All influenza A isolates were tested for adamantane resistance at the Centers for Disease Control and Prevention (CDC). Procedures for RNA extraction and pyrosequencing have been previously described (*7*). A medical record review was performed to assess any use of antiviral drugs by influenza A patients from 60 days before through 14 days after the enrollment date.

Univariate comparisons were performed, and crude and adjusted prevalence ratios were computed to evaluate the association between adamantane resistance and age, gender, vaccination status, date of clinical encounter, and presence of a high-risk medical condition. Enrollment dates were grouped into 4 consecutive 3-week periods beginning January 3, 2005. The referent period was the second 3-week period when the number of influenza cases peaked. All analyses were conducted with SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

Influenza virus was isolated in 167 (20%) of 818 cultures from ill cohort members; 153 (92%) were influenza A (H3N2) and 14 (8%) were influenza B (Figure). Influenza A (H1N1) was not isolated from any patients. Adamantane resistance was present in 72 (47%) of 152 influenza A isolates; 1 additional isolate was not characterized. All resistant isolates contained a point mutation resulting in a serine to asparagine change at amino acid 31 (S31N) of the M2 protein. The median age was 43.6 years among patients with resistant isolates and 64.7 years among those with susceptible isolates ($p = 0.002$). The proportion of patients with adamantane-resistant viruses was significantly higher in 6- to 23-month-old children (70%) compared with adults ≥65 years of age (39%) (unadjusted prevalence ratio 1.8; 95% confidence interval [CI] 1.2–2.6; $p = 0.003$).

Number of symptoms and duration of illness were similar for patients with adamantane-resistant and -susceptible influenza isolates (Table 1). The proportion of patients hospitalized was nearly the same in each group,

Table 1. Clinical features of patients with adamantane-resistant or -susceptible influenza A, 2004–05 season

Clinical feature	Adamantane-resistant influenza, n = 72	Adamantane-susceptible influenza, n = 80	p value*
Median duration of illness†	12 days	13 days	0.96
No. (%) hospitalized	7 (10)	9 (12)	0.74
No. (%) fully recovered at time of follow-up interview	63 (88)	65 (81)	0.29
Median no. symptoms‡	9	8	0.59
No. (%) receiving antiviral treatment§	15 (21)	31 (39)	0.02
Median time from symptom onset to healthcare encounter	3 days	2 days	0.34
Single symptom, no. (%)‡			
Fever	34 (69)	52 (74)	0.56
Cough	48 (98)	68 (97)	0.78
Headache	41 (84)	53 (76)	0.29
Muscle pain	37 (76)	45 (64)	0.57
Nasal congestion	40 (82)	54 (77)	0.55
Hoarseness	35 (71)	55 (79)	0.37
Fatigue	45 (92)	66 (94)	0.72
Ear pain	16 (33)	24 (34)	0.85
Sore throat	29 (59)	40 (57)	0.82
Difficulty breathing	28 (57)	35 (50)	0.44
Wheezing	25 (51)	42 (60)	0.33
Nausea	16 (33)	27 (39)	0.51
Vomiting	10 (20)	9 (13)	0.27
Diarrhea	12 (24)	15 (21)	0.70
Rash	2 (4)	2 (3)	1.00
Combined symptoms, no. (%)‡			
Fever and cough	33 (67)	52 (74)	0.41
Fever and headache	31 (63)	43 (61)	0.84
Fever, cough, and headache	30 (61)	43 (61)	0.98
Fever and muscle pain	29 (59)	39 (56)	0.71
Fever, headache, and muscle pain	27 (55)	35 (50)	0.58
Fever, headache, muscle pain, and cough	26 (53)	35 (50)	0.74

*χ^2, Fisher exact, or Wilcoxon rank sum tests performed as appropriate.
†Based on 128 influenza A patients who had recovered fully at the time of follow-up interview.
‡Children 6 to 23 months of age were excluded from the numerator and denominator for symptoms.
§One patient had received antiviral treatment prior to influenza culture.

and the distribution of individual symptoms did not differ significantly. Forty-five patients received antiviral therapy (37 adamantanes, 8 oseltamivir), but only 1 was treated with amantadine before influenza culture was obtained. Clinical features and duration of illness were similar whether or not patients received adamantane therapy. In a multivariable model that included patients of all ages, adamantane resistance was not associated with gender, time of clinical encounter, or influenza vaccination status (Table 2). Children 6–23 months old without a high-risk medical condition were significantly more likely to be infected with adamantane-resistant influenza A than were adults ≥65 years of age who had a high-risk condition. In a secondary analysis**,** no association was found between adamantane resistance and number of symptoms, duration of illness, or hospitalization.

Conclusions

This study provides epidemiologic evidence that the point mutation (S31N) conferring adamantane resistance has not altered influenza A (H3N2) virulence as measured by clinical symptoms and duration of illness. Hospitalization rates were also similar, although the power to detect a difference was low. These results are consistent with prior research in ferrets, which demonstrated that virulence was similar for adamantane-resistant and -susceptible influenza A (H3N2) isolates (*8*). The source population for this study included persons who were eligible for influenza vaccination based on age group or the presence of a high-risk medical condition. Consequently, participants were more likely to experience influenza complications relative to the general population. The absence of any difference in clinical severity in this higher risk population provides some reassurance that adamantane resistance will not affect overall influenza morbidity.

Factors contributing to rapid emergence of adamantane-resistant influenza viruses are not fully known. Resistance has been observed in a variety of outbreak and institutional settings for many years (*10–12*) but did not spread into the general population until recently (6,*7*). The proportion of human influenza A (H3N2) isolates with adamantane resistance increased dramatically in People's Republic of China and Taiwan in 2003 (*6*). However, the degree to which the spread of resistant viruses from Asia contributed to the rapid escalation of adamantane resistance in the United States is uncertain.

The 2004–05 influenza season provided an opportunity to learn more about the epidemiology of adamantane resistance during a period when drug resistance was increasing in Wisconsin but was not yet universal. Children had the highest risk of acquiring an adamantane-resistant influenza infection and are an important reservoir for transmission of influenza to adult household members and the community (*13*). Still, we cannot explain why children were preferentially infected with adamantine-resistant strains in 2004–05. Only 1 patient was treated with amantadine before enrollment. The higher prevalence of resistance in children was therefore not attributable to individual adamantane use. There may have been multiple introductions of adamantane-resistant influenza viruses from other geographic areas during the 2004–05 season. This phenomenon was described in a recent phylogenetic analysis of influenza A

Table 2. Demographic features of patients with adamantine-resistant or -susceptible influenza A, 2004–05 season*

Variable	No. (%) resistant isolates, n = 72	No. (%) susceptible isolates, n = 80	Unadjusted prevalence ratio (95% CI)	Adjusted prevalence ratio (95% CI)
Age and high-risk status†				
6–23 mo without high risk	20 (74.1)	7 (25.9)	2.0 (1.3–3.0)	2.1 (1.4–3.1)
6–23 mo with high risk	3 (50.0)	3 (50.0)	1.3 (0.6–3.2)	1.4 (0.5–3.5)
2–17 y with high risk	8 (61.5)	5 (38.5)	1.7 (0.9–2.9)	1.4 (0.8–2.4)
18–49 y with high risk	7 (36.8)	12 (63.2)	1.0 (0.5–2.0)	0.9 (0.5–1.9)
50–64 y with high risk	8 (38.1)	13 (61.9)	1.0 (0.5–2.0)	1.0 (0.6–1.9)
≥65 y without high risk	7 (46.7)	8 (53.3)	1.3 (0.7–2.4)	1.2 (0.7–2.3)
≥65 y with high risk	19 (37.2)	32 (62.8)	Referent group	Referent group
Sex				
Male	37 (50.7)	36 (49.3)	1.1 (0.8–1.6)	1.1 (0.8–1.5)
Female	35 (44.3)	44 (55.7)	Referent group	Referent group
Dates of clinical encounter				
2005 Jan 3–23	10 (47.6)	11 (52.4)	0.9 (0.5–1.4)	0.9 (0.5–1.5)
2005 Jan 24–Feb 13	44 (55.0)	36 (45.0)	Referent period	Referent period
2005 Feb 14–Mar 6	15 (35.7)	27 (64.3)	0.6 (0.4–1.0)	0.6 (0.4–1.0)
2005 Mar 7–25	3 (33.3)	6 (66.7)	0.6 (0.2–1.6)	0.5 (0.2–1.3)
Received 2004–05 influenza vaccine?				
Yes	45 (42.9)	60 (57.1)	0.7 (0.5–1.0)	0.8 (0.6–1.1)
No	27 (57.4)	20 (42.6)	Referent group	Referent group

*CI, confidence interval.
†For the multivariate model, a composite variable was created based on age and the presence or absence of a high-risk medical condition as defined by Advisory Committee on Immunization Practices criteria.

(H3N2) isolates from New York state over a 9-year period (*14*). The analysis suggested that viral evolution within epidemic seasons is dominated by random importation of distinct viral strains from other geographic areas.

CDC continues to recommend that adamantane drugs not be used for influenza treatment in the United States. Given the recent emergence of oseltamivir-resistant influenza A and B infections (*15*,*16*), ongoing monitoring of influenza virus susceptibility to adamantanes and neuraminidase inhibitors is essential.

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Chikungunya and Dengue Viruses in Travelers

To the Editor: Chikungunya virus (CHIKV), an arthropod-borne virus transmitted to humans by *Aedes spp*. mosquitoes, was first isolated in Tanzania (Tanganyika) in 1953 (*1*). Various outbreaks have since occurred in Africa, Southeast Asia, and India (*2*).

CHIKV has recently been reported in a large area in the Indian Ocean islands and the Indian subcontinent. After an outbreak in Kenya in 2004, other outbreaks occurred in early 2005 on the Comoros Islands, Réunion, and other islands in the southwestern Indian Ocean; the epidemic then spread to India (*3*,*4*). Molecular analysis showed that the epidemic was caused by a variant of the Central/East African CHIKV genotype (*5*,*6*).

Internet surveillance networks provided information on epidemics in real time, alerting clinicians in the industrialized world to the spread of CHIKV and enabling them to more easily diagnose infection among travelers with fevers (*7*). We report results of diagnostic tests and analysis of predictors of infection among persons in Italy with symptoms suggestive of CHIKV infection who had traveled to potentially affected areas. Dengue virus (DENV) is endemic to many of these areas.

We studied travelers or migrants from areas to which CHIKV infection is endemic (i.e., sub-Saharan Africa) or areas currently affected by outbreaks (i.e., the Indian Ocean islands, India) who had symptoms suggestive of infection (i.e., fever and arthralgia with or without a rash) from January 2006 through March 2007. At least 1 blood sample was collected from each patient and stored at –80°C before testing for CHIKV and DENV. Median lag between onset of symptoms and date of blood collection was 22 days (range 3–179 days). Two samples (acute phase and convalescence phase) were available from 5 patients. Serologic diagnosis of CHIKV infection was determined by hemagglutination inhibition (HI) test and confirmed by plaque-reduction neutralization test (*8*). Serodiagnosis of DENV infection was conducted by using the HI test and an immunoglobulin M ELISA (Focus Diagnostics, Cypress, CA, USA). A case-report form containing information about age, sex, countries visited, travel dates, and date of onset of symptoms was completed for each patient.

Seventy-six persons participated in the study; 55.3% were male, median age was 39 years (range 1–69 years), and most (80.3%) were Italian (Table). A total of 29 (38.2%) were positive for CHIKV, and 13 (17.1%) were positive for DENV; 34 (44.7%) were negative for both viruses. Of the 29 CHIKV-positive persons, 22 (75.9%) had visited the Indian Ocean islands (Mauritius, Réunion, and Madagascar), 5 had visited Asia, and 2 had visited Africa. Travelers from Indian Ocean islands had a higher risk for CHIKV infection than those who had visited Africa (odds ratio [OR] 11.0, 95% confidence interval [CI] 1.60–119.13) or Asia (OR 17.05, 95% CI 4.31–73.05). Persons who had visited Asia had a higher risk for DENV infection (OR 8.36; 95% CI 1.58–81.73) than those who had visited other areas.

The 5 persons who were infected with CHIKV in Asia had visited India (i.e., the most visited country [21 travelers]). However, persons who visited the Indian Ocean islands had a higher risk of being CHIKV positive than those who had visited India (OR 8.8, 95% CI 2.09–39.86). A rash was associated with CHIKV infection and was >8× more likely to be reported by CHIKV-positive persons than CHIKV-negative persons (OR 7.03, 95% CI 2.23–22.93). Moreover, rash was observed in 65% of CHIKV-positive cases and 31% of DENV-positive cases, but the difference was not statistically significant because of the small sample size (OR 4.28, 95% CI 0.88–23.23). None of the other patient's characteristics was associated with infection with CHIKV or DENV.

Table. Characteristics of 76 travelers studied

Characteristic	Chikungunya virus–positive, no. (%)	Dengue virus–positive, no (%)	Seronegative, no. (%)
Total	29 (100.0)	13 (100.0)	34 (100.0)
Sex			
Male	17 (58.6)	6 (46.2)	19 (55.9)
Female	12 (41.4)	7 (53.8)	15 (44.1)
Age, y			
0–35	8 (27.6)	4 (30.8)	16 (47.0)
36–50	11 (37.9)	6 (46.1)	14 (41.2)
>50	10 (34.5)	3 (23.1)	4 (11.8)
Days spent abroad			
0–15	18 (62.1)	4 (30.8)	20 (58.8)
>15	11 (37.9)	9 (69.2)	14 (41.2)
Area visited*			
Africa	2 (6.9)	2 (15.4)	6 (17.6)
African islands	22 (75.9)	0	8 (23.5)
Asia	5 (17.2)	11 (84.6)	20 (58.8)
Nationality			
Italian	23 (79.3)	9 (69.2)	29 (85.3)
Other	6 (20.7)	4 (30.8)	5 (14.7)
Rash			
Yes	19 (65.5)	4 (30.8)	6 (17.6)
No	10 (34.5)	9 (69.2)	28 (82.3)

*Africa, continental Africa; African Islands, Western Indian Ocean islands; Asia, India and Southeast Asia.

A limitation of our study was that only 5 patients had documented seroconversion for CHIKV. However, high titers were found in all but 1 patient (>1,280 in 21 patients and 640 in 2 patients). This patient, who had a titer of 80, was an Italian who had probably not been previously exposed to CHIKV. Thus, the risk for misclassification was low. PCR for early detection of infection was not used because only 3 persons were tested within 10 days of symptom onset. Two of these persons, who were tested 7 days after symptom onset, already had antibodies to CHIKV.

In conclusion, a high proportion of travelers with symptoms of CHIKV infection who returned from areas with outbreaks of this infection or where this virus was endemic were seropositive. A lower proportion of patients had antibodies to DENV. CHIKV-positive patients were more likely to have a rash than those negative for both CHIKV and DENV. As suggested by previous studies (*9*), a rash was more common among CHIKV-positive patients than in DENV-infected patients, but the difference was not significant. Our study suggests that identification of predictors of infection with CHIKV is feasible, although it is complicated by cocirculation of DENV in the same areas.

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Acinetobacter spp. in Gunshot Injuries

To the Editor: Challenges posed by *Acinetobacter* spp. result from multidrug resistance, nosocomial spread, and hospital-wide outbreaks (*1*–*3*). We evaluated *Acinetobacter* spp. infections from gunshot injuries received during the April 2006 East Timor conflict (for a description of these events and further reading, see http://en.wikipedia.org/wiki/2006_East_Timorese_crisis).

We reviewed records of 15 injured East Timorese police officers. Median age was 29 years (range 25–45 years); 13 were male. Typical injuries were from multiple high-velocity gunshots and shrapnel. All patients had undergone surgery for stabilization and wound debridement before evacuation to the Royal Darwin Hospital (RDH) in Australia; most had likely received antimicrobial drugs including ampicillin, gentamicin, metronidazole, and ceftriaxone. They arrived at RDH a median of 3 days after injury (range 2–12 days).

The patients were separated from other hospital inpatients on arrival; they were managed as a cohort, they had dedicated nursing staff, and barrier contact precautions were practiced. However, the patients were not routinely screened for colonization with microbiologic organisms. Additional surgical management, including further wound debridement, was performed on 12 of the 15 patients (11 within 48 hours of arrival at RDH); intraoperative samples of bone, soft tissue, and wounds were submitted for culture.

From 13 patients (including all 11 with gunshot wounds), 19 *Acinetobacter* spp. isolates were recovered. *Acinetobacter* spp. was cultured from deep wound tissue obtained during surgery from 9 patients. Substantial antimicrobial drug resistance was demonstrated by automated testing (Vitek 2, bioMérieux, Marcy l'Etoile, France) (Table). All 19 *Acinetobacter* spp. isolates were classified as multidrug resistant (resistant to ≥3 drug classes) (*4*). Isolates from 10 of the 13 culture-positive patients (12 of 19 isolates) were resistant to all tested drugs except meropenem and amikacin. Susceptibility testing for tigecycline and tetracycline was not performed. No isolate was metallo-β-lactamase positive by phenotypic analysis according to tablet disk diffusion method using imipenem and imipenem plus EDTA Neo-Sensitabs (Rosco Diagnostica, Taastrup,

Table. Susceptibility of *Acinetobacter* spp. isolates to antimicrobial drugs, % (n = 19)*

Drug	Susceptible	Intermediate	Resistant
Amikacin	100	0	0
Ampicillin	0	0	100
Ceftazidime	21	26	53
Ceftriaxone	0	0	100
Ciprofloxacin	26	16	58
Gentamicin	21	0	79
Meropenem	100	0	0
Piperacillin/tazobactam	26	32	42
Ticarcillin/clavulanic acid	21	21	58
Tobramycin	21	16	63
Trimethoprim/sulfamethoxazole	11	0	89

*Isolates from gunshot wounds of 15 persons injured in East Timor, 2006.

Denmark). Isolation of *Acinetobacter* spp. (15 isolates) far exceeded that of other organisms: *Stenotrophomonas* (5 isolates); *Pseudomonas aeruginosa* (3 isolates); *Staphylococcus aureus* and *Enterococcus* spp. (2 isolates each); and *Pseudomonas putida*, *Enterobacter cloacae*, *Staphylococcus hemolyticus*, and *Mycoplasma hominis* (1 isolate each).

On the basis of clinical assessment by the treating surgeon and infectious diseases physician, 11 patients were treated for *Acinetobacter* spp. infection. Patients 1–5 had comminuted compound fractures associated with intraoperative deep wound tissue that was culture positive for *Acinetobacter* spp. and were treated for osteomyelitis; patients 6–11 were treated for wound infections; patients 6–8 had intraoperative deep wound tissue culture positive for *Acinetobacter* spp.; patients 9–10 had superficial wound swabs that were culture positive; and patient 11 had a positive culture from a nonsurgical site. Of these 11 patients, 4 had fever >38°C on the day of admission to RDH (2 of whom had a leukocyte count >20,000/μL), and another 2 had visible pus, necrotic tissue, or both. The surgical approach to these patients involved delayed wound closure; fracture fixation; vacuum dressings; and skin, bone, and nerve grafts. Choice and duration of antimicrobial drug therapy was guided by susceptibility testing and experience (*4*). Presumed osteomyelitis caused by multidrug-resistant (MDR) *Acinetobacter* spp. was treated with meropenem in combination with amikacin for at least 2 weeks, followed by another 2 weeks of meropenem monotherapy. Wound infections were similarly treated with combination therapy initially, but amikacin was stopped earlier. No aminoglycoside toxicity was observed. Treatment was stopped at 4 weeks if no signs of infection were present (healed wound plus apyrexia and a C-reactive protein level <20 mg/L). Patient 12 was colonized with MDR *Acinetobacter* spp. and was treated for aspiration pneumonia; patient 13 had MDR *Acinetobacter* spp. colonization of a central venous catheter.

Follow-up after completion of therapy ranged from 4 to 23 weeks. No patients had recurrence of infection or isolation of *Acinetobacter* spp. Defining osteomyelitis and wound infection caused by *Acinetobacter* spp. was problematic for clinicians (*4*–*6*), and some assumed infections may have represented colonization. Because treatment for MDR *Acinetobacter* spp. in this setting can be protracted and toxic (e.g., from aminoglycosides), our review highlights the potential benefits of applying prospectively documented criteria such as abnormal bone histologic findings for osteomyelitis and a workable definition of deep tissue infection to better guide treatment decisions.

RDH had not experienced outbreaks of healthcare-associated infection or colonization with MDR *Acinetobacter* spp. before or after (as of January 1, 2007) the 2006 East Timor conflict, except for positive isolates from 5 patients evacuated from the Bali bombings of 2002 and 2005. Because all but 1 isolate were recovered within 48 hours of admission, primary inoculation of *Acinetobacter* spp. into wounds is assumed to have occurred either at the time of injury (from environmental sources or preexisting skin colonization), from nosocomial transmission in East Timor (before transfer to RDH), or during evacuation to RDH. Environmental and patient-based screening at sites of primary care may help resolve the uncertainty of which source is most likely.

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Case Cluster of Necrotizing Fasciitis and Cellulitis Associated with Vein Sclerotherapy

To the Editor: Varicose vein sclerotherapy is a commonly performed cosmetic surgical procedure in which a sclerosing agent is injected into small varicose veins of the leg by using small gauge needles. It is regarded as a minor, safe procedure, usually performed in an office clinic (*1*). We describe a cluster of infections with group A *Streptococcus* spp. associated with throat carriage in a cosmetic surgeon.

In early December 2006, 3 patients were seen over a 10-day period at Geelong Hospital with infections following varicose vein sclerotherapy. All patients had undergone varicose vein sclerotherapy with polidocanol (Laurath-9; Aethoxysklerol, BSN Medical, Mount Waverley, Victoria, Australia) at a clinic of a single cosmetic surgeon. The index patient (patient A) had toxic shock syndrome and necrotizing fasciitis of the treated legs. The 2 other patients (patients C and D) had multifocal cellulitis directly correlating to the injection sites. The time between sclerotherapy and disease onset was 1–2 days.

A case-patient was defined as a patient who had undergone sclerotherapy at the clinic and subsequently had infection directly related to the site of sclerosant injection. Events were dated from the day on which the index patient had her surgical procedure. We reviewed clinic notes and infection control procedures in conjunction with the Department of Human Services of the State Government of Victoria, Australia. Specimens, where available, were collected for culture from patients by the treating clinicians. A throat swab was taken from the cosmetic surgeon. Specimens were transported and cultured by using standard methods.

During the outbreak period, 44 patients had vein sclerotherapy with 3% polidocanol at the cosmetic surgeon's clinic. In addition to the 3 patients identified on admission to hospital, a fourth patient (patient B) sought treatment from her general practitioner for medical care for a postprocedure infection. All patients had procedures on day 1 or day 7 (Figure); patients A and B were seen consecutively on day 1, and 2 patients were treated between patients C and D on day 7.

Patient A required surgical debridement, intravenous antimicrobial drugs, intensive care, and hyperbaric oxygen therapy. Intraoperative specimens taken from her during debridement cultured group A *Streptococcus* spp. Patients B, C, and D had cellulitis, but no specimens suitable for microbiologic diagnosis of cellulitis were taken for culture. Patient B was treated with oral antimicrobial agents as an outpatient. Patient C was admitted to hospital for intravenous antimicrobial therapy, and patient D showed no improvement on oral antimicrobial therapy as an outpatient and was subsequently admitted to hospital for intravenous antimicrobial agents.

Group A *Streptococcus* spp. was isolated from a throat swab taken on day 16 from the cosmetic surgeon. He reported no upper respiratory tract infection symptoms before the outbreak. He also reported that antiseptic skin preparation was not routinely used during the procedures; nor were gloves used. However, alcohol hand rubs were used between patients. The surgeon had not changed his infection control procedures recently and had not been aware of any infective complications previously. Environmental surface swabs taken on day 14 from 3 different areas (procedural trolley, surgical spotlight, and examination couch) in the clinic during the assessment yielded no pathogenic organisms. The infection control assessment team noted overall cleaning, disinfection, and hand hygiene to be inadequate.

Decolonization of the surgeon was performed by using rifampin 600 mg daily and amoxicillin 500

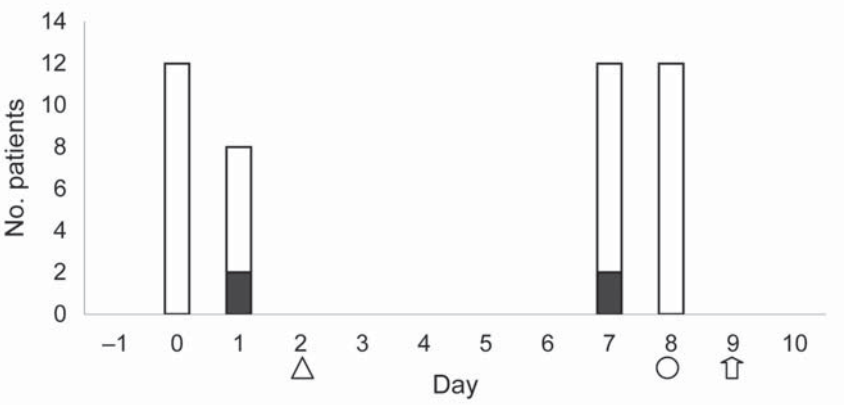

Figure. Days of procedures for infected and noninfected patients and their first manifestations of infection. □, uninfected; ■, infected; Δ, patients A and B seen with infection; O, patient C seen with infection; and ⇧, patient D seen with infection.

mg. every 6 hours for 10 days, during which time the surgeon suspended surgical procedures. Recommendations were made regarding infection prevention practices; these were undertaken by the surgeon.

Although soft tissue infection following sclerotherapy may be underreported, large case series have not noted this complication in the past (*2*,*3*); this finding suggests that any soft tissue infection following sclerotherapy should be investigated. These cases highlight the need for vigilance when considering infection control for minor procedures that take place outside of the support of hospital-based infection control services.

Soft tissue infections as complications following varicose vein sclerotherapy appear to be rare (*1*–*3*). The Australian Aethoxysklerol study reported no cellulitis in 16,804 legs injected with the sclerosing agent, and superficial thrombophlebitis occurred at a rate of 0.08% at 2-year review (*2*). Likewise, a multicenter registry with 22 European phlebology clinics reported no cellulitis or necrotizing fasciitis in 12,173 sessions (*3*).

Similarly, surgical site infections with Group A *Streptococcus* spp. are uncommon. A multicenter survey of 72 centers worldwide reported all β-hemolytic *Streptococcus* spp. (including group A and group G) accounted for <5% of infections (*4*), while surveillance in the 1990s by Centers for Disease Control and Prevention reported <1% of all surgical wound infections was caused by group A *Streptococcus* spp. (*5*). A Canadian study reported invasive group A *Streptococcus* infections following surgery in 1.1 cases per 100,000 admissions (*6*). Outbreaks have been infrequently described (*5*,*7*–*10*), and sources of colonization range from throat to anus and vagina.

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Streptococcus suis in Humans, Thailand

To the Editor: *Streptococcus suis* is an important zoonotic pathogen for swine and humans. Among 33 serotypes, serotype 2 is more frequently isolated from diseased pigs than other serotypes (*1*). However, not all serotype 2 strains are virulent, and degree of virulence varies among strains (*2*). Previous studies have reported several *S. suis* putative virulence factors, including the polysaccharide capsule, the muramidase-released protein, the extracellular factor, and suilysin (*3*–*5*). Some of these factors have been used as virulence-associated markers, and the association of the factors of *S. suis* isolates with virulence or clinical background has been suggested in Europe (*2*,*5*). However, because many virulent isolates lacking these factors have also been isolated from clinical cases in Canada (*6*), they cannot be used as virulence markers in North America.

Recent analysis of *S. suis* isolates by multilocus sequence typing (MLST) suggested the association of some clonal groups with particular clinical manifestations. That is, most invasive isolates belonged to the sequence type (ST) 1 complex, while the ST27 and ST87 complexes were found to include a higher proportion of lung isolates (*7*). Although *S. suis* has been prevalent worldwide, the geographic location of the isolates used so far was mainly Europe, North America, and East Asia (*7*–*9*). Moreover, the clonal association with virulence of *S. suis* has been discussed mainly on the basis of clinical and experimental data in swine (*7*). In this report, to broaden understanding of the population structure of *S. suis* as a zoonotic agent, we characterize 20 *S. suis* isolates (Table) recovered from humans in Thailand in 1998–2002.

Serotyping by coagglutination tests showed that 19 of the 20 isolates

belonged to serotype 2, while the remaining 1 (MNCM07) was serotype 14. MLST analysis resolved the 20 isolates into 8 STs (Table). By using eBURST (http://eburst.mlst.net), we assigned 4 isolates (MNCM01, MNCM06, MNCM07, and MNCM16) from 1 case of endocarditis and 3 cases of meningitis to the ST1 complex. The remaining isolates were assigned to the ST27 complex with a less-stringent group definition (Table), although ST101 (MNCM21) and ST104 (MNCM50) shared only 2 alleles with ST27 and were incorporated into this complex by a chaining effect. Regarding the clinical cases from which the ST27 complex isolates were recovered, the patients had meningitis, endocarditis, septicemia, septic shock, diarrhea, and respiratory involvement. The 2 ST complexes both contained isolates from deceased patients (Table).

All the isolates assigned to the ST1 complex were positive for the suilysin gene *sly*, the extracellular factor gene *epf* or its variant, and the muramidase-released protein gene *mrp* or its variant. With the exception of MNCM21 and MNCM50, which had only *sly*, all isolates classified into the ST27 complex were negative for *sly* and *epf* but positive for *mrp* or its variant. These results showed the congruence between STs and the virulence-associated gene profiles and further support the usefulness of MLST for epidemiologic studies of *S. suis*.

Of the 3 major clonal complexes identified so far in *S. suis* (ST1, ST27, and ST87), the ST1 complex particularly attracts considerable public attention as a clonal group that may have the potential for a higher degree of virulence than the others (*7*), and most (96%) of the human isolates investigated so far, including ST7 isolates, which caused the largest outbreak in China, belong to the ST1 complex (*7–9*). In this study, although no ST7 isolate was found, 4 isolates were assigned to the ST1 complex. This further confirmed the gravity of the ST1 complex not only for swine industries but also for public health.

In contrast to the ST1 complex, only 4 human clinical isolates have so far been reported to belong to the ST27 complex. Three of the 4 are isolates from Canada that belong to ST25

Table. Epidemiologic data of *Streptococcus suis* isolates from patients in Thailand, 1998–2002*

Isolate no.†	Year of isolation	Site of isolation	Virulence-associated genes‡§	Serotype	Diseases and symptoms	ST (ST complex)
MNCM01	2000	Blood	*cps2J*+/*sly*+/*epf*+/*mrp*+	2¶	Endocarditis	1 (1)
MNCM06	2000	Blood, CSF	*cps2J*+/*sly*+/*epf*+/*mrp*+	2	Neck stiffness, deafness (meningitis)	1 (1)
MNCM16	2000	CSF	*cps2J*+/*sly*+/*epf*+/*mrp*+	2	Neck stiffness (meningitis)	1 (1)
MNCM07	2000	Blood, CSF	*cps1J*+/*sly*+/*epf**/*mrp*^{S}+	14	Neck stiffness (meningitis), death	11 (1)
MNCM04	2000	Blood	*cps2J*+/*sly*–/*epf*–/*mrp***+	2	Neck stiffness, deafness (meningitis)	25 (27)#
MNCM10	2000	Blood	*cps2J*+/*sly*–/*epf*–/*mrp***+	2	Septicemia	25 (27)#
MNCM24	2001	Blood	*cps2J*+/*sly*–/*epf*–/*mrp***+	2¶	Endocarditis	25 (27)#
MNCM26	2001	Blood	*cps2J*+/*sly*–/*epf*–/*mrp***+	2	Endocarditis, deafness (meningitis)	25 (27)#
MNCM51	2002	Blood	*cps2J*+/*sly*–/*epf*–/*mrp***+	2	Septicemia, diarrhea, death	25 (27)#
MNCM55	2002	Blood	*cps2J*+/*sly*–/*epf*–/*mrp***+	2	Septic shock, death	25 (27)#
LPH4	2001	Blood	*cps2J*+/*sly*–/*epf*–/*mrp***+	2	Septicemia, diarrhea	25 (27)#
LPH12	2002	Blood	*cps2J*+/*sly*–/*epf*–/*mrp***+	2	Septic shock, death	25 (27)#
MNCM43	2002	Blood	*cps2J*+/*sly*–/*epf*–/*mrp*+	2	Endocarditis	28 (27)
MNCM21	1998	CSF	*cps2J*+/*sly*+/*epf*–/*mrp*–	2	Meningitis	101 (27)#
MNCM25	2001	Blood	*cps2J*+/*sly*–/*epf*–/*mrp***+	2	Neck stiffness (meningitis), diarrhea, death	102 (27)#
MNCM54	2002	Blood	*cps2J*+/*sly*–/*epf*–/–*mrp***+	2	Neck stiffness (meningitis), diarrhea	102 (27)#
MNCM33	2002	Blood, CSF	*cps2J*+/*sly*–/*epf*–/*mrp***+	2	Neck stiffness (meningitis)	103 (27)#
LPH3	2001	Blood	*cps2J*+/*sly*–/*epf*–/*mrp***+	2	Meningitis	103 (27)#
LPH5	2001	Blood	*cps2J*+/*sly*–/*epf*–/*mrp***+	2	Septicemia	103 (27)#
MNCM50	2002	Blood	*cps2J*+/*sly*+/*epf*–/*mrp*–	2	Pulmonary edema, death	104 (27)#

*ST, sequence type; CSF, cerebrospinal fluid.
†Isolates with MNCM number and LPH number were isolated from patients at Maharaj Nakorn Chiang Mai Hospital and Lamphun Hospital, Thailand, respectively.
‡Virulence-associated gene profiling was done as described previously (*10*). *cps1J* and *cps2J*, serotype 1 (and 14) and 2 (and 1/2) specific genes, respectively, involved in the capsular biosynthesis; *sly*, suilysin gene; *epf*, extracellular factor gene; *mrp*, muraminidase-released protein gene; +, positive; –, negative.
§*epf**, an *epf* variant that produces an ≈3,000-bp fragment by PCR with primers described previously (*10*); *mrp*** and *mrpS*, *mrp* variants that produce ≈1,800-bp and ≈750-bp fragments, respectively, by PCR with primers described previously (*10*).
¶Coagglutination reaction using anti-serotype 2 serum was weak.
#ST25, ST101, ST102, ST103, and ST104 belong to the ST27 complex, only with a less-stringent approach that defines an ST complex by sharing of alleles at ≥5 of the 7 loci.

(*7*). The remaining 1 is from Japan and assigned to ST28 (*8*). Unlike in previous reports, 80% of the human clinical isolates (16 isolates) characterized in this study were assigned to the ST27 complex. Although previous studies suggested that members of the ST27 complex may have lower potential to cause invasive diseases in swine (*7*), all the isolates were isolated from blood or cerebrospinal fluid of the patients, suggesting a high degree of invasiveness (Table). Because it is unknown whether the ST27 complex is also dominant among isolates from diseased pigs in Thailand, future surveillance will be necessary to know the situation in pigs. However, our data indicate that the ST27 complex is another clonal group that should be assessed for its importance for human infection. Because *mrp*, *epf*, and *sly* are not appropriate as virulence markers for the ST27 complex members, development of novel virulence markers will be needed for efficient discrimination of *S. suis* strains virulent for humans.

This study made use of the *Streptococcus suis* Multilocus Sequence Typing website (http://ssuis.mlst.net); this site is hosted at Imperial College and development is funded by the Wellcome Trust. The study was supported by a grant-in-aid from the Zoonoses Control Project of the Ministry of Agriculture, Forestry and Fisheries of Japan and the Endowment Fund for Medical Research, Faculty of Medicine, Chiang Mai University.

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Streptococcus suis Meningitis, United States

To the Editor: *Streptococcus suis,* commensal and opportunistic pathogens of swine, and prevalent zoonotic agents worldwide, are α-hemolytic gram-positive cocci with 35 different serotypes (*1*). In humans, *S. suis* infection has been associated with bacterial meningitis, septic shock, arthritis, pneumonia, endocarditis, endophthalmitis, and spontaneous bacterial peritonitis (*2*,*3*). Most at risk are those who handle or eat undercooked pork, e.g., farm workers, butchers, and slaughterhouse workers (*4*). Most cases have been reported in Europe or Southeast Asia (*2*,*3*). Meningitis, first recognized in 1968 in Denmark (*1*), is the most common clinical manifestation of human infection with *S. suis*. A case of *S. suis* meningitis in a pig farmer was reported in the United States (*5*). Here, we describe another case in a 60-year-old man from San Francisco who had consumed raw pork while traveling in the Philippines.

In June 2003, this man became ill with fever, diaphoresis, headache, nausea, and anorexia. He had just returned from a 7-month vacation in the Philippines. Three days after symptoms onset, his physician prescribed doxycycline. Symptoms continued and he was admitted to a local hospital 5 days later with a fever of 38.9°C, nuchal rigidity, headache, and general malaise.

The patient described no recent contact with sick persons; past medical

history was unremarkable. On physical examination, he was somnolent but fully oriented, with no focal findings on neurologic examination and only slight nuchal rigidity. He had a leukocyte count of 21,000/mm^3, including 16,400/mm^3 neutrophils. Cerebrospinal fluid (CSF) showed leukocyte count of 487/μL with 80% polymorphonuclear cells and 18% lymphocytes, and glucose and protein levels <20 mg/dL and <167 mg/dL, respectively. Gram stain of CSF showed gram-positive cocci in pairs. Empiric therapy (ceftriaxone, vancomycin, and ampicillin) for bacterial meningitis was begun. Computed tomographic scan of the head showed only sinusitis; findings of chest radiograph and transesophageal echocardiogram were negative.

On hospital day 2, blood cultures grew gram-positive cocci in pairs and chains (Figure). The organism was catalase-negative, bile esculin-negative, and pyrrolidonyl aminopeptidase-negative, consistent with *Streptococcus* spp. A latex agglutination test did not detect *Streptococcus pneumoniae* antigen. Antimicrobial susceptibility testing showed that the isolate was sensitive to penicillin (MIC = 0.03), ceftriaxone, and vancomycin but resistant to tetracycline and clindamycin. Antimicrobial therapy was changed to penicillin G, 24 million units intravenously per day.

On hospital day 5, the patient complained of hearing loss in his left ear. Results of nasopharyngeal endoscopy were negative. By hospital day 7, the organism was identified by the API 20 Strep System (bioMerieux, Marcy l'Etoile, France) as S. *suis* serotype 2. The patient subsequently stated that he was a butcher with a culinary preference for partially cooked pork, which he had eaten in the Philippines until the week prior to onset of symptoms. On hospital day 9, a formal audiology evaluation showed severe bilateral sensorineural high-frequency hearing loss (–70 dB). The patient completed a 10-day course of parenteral antimicrobial drugs and was discharged on continued oral therapy with close followup. Two months after discharge, the patient reported much improved hearing without other sequelae.

Most *S. suis* infections occur in older men and patients who report contact with pigs or eating undercooked pork products. Invasion of the bloodstream can occur directly through skin abrasions or the oral or respiratory route (*6*). Once bloodborne, *S. suis* can cause toxic shock syndrome and sepsis (*7*). The mechanism by which the organism traverses the blood-brain barrier to cause meningitis is not known, although bacterial toxins and host inflammatory mediators may play a role (*8*).

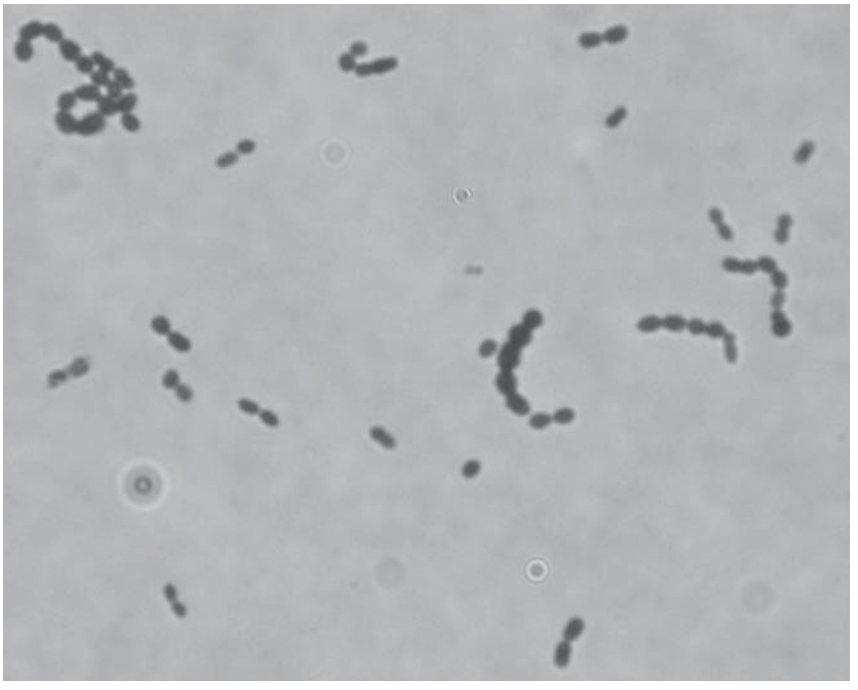

Figure. Gram-positive cocci in pairs in a 60-year-old man with meningitis. Magnification x1,000.

Hearing loss from *S. suis* meningitis, although not specific for the organism, occurs frequently in half to two thirds of patients and can be irreversible (*3*,*7*,*9*). Administering dexamethasone may ameliorate hearing loss in some cases (*10*). Penicillin G is the preferred treatment for *S. suis* infection, although penicillin resistance has emerged in *S. suis* because of the farm practice of supplementing feeds with antimicrobial drugs. As an alternative therapy, vancomycin may be used (*6*). Thus, empiric therapy for adult bacterial meningitis (ceftriaxone and vancomycin with or without ampicillin) would likely be sufficient to treat *S. suis* meningitis. Although the death rate from this disease can be high, varying from 7% in one study (*3*) to 30% in another (*6*), infection can be prevented by treating abrasions promptly, wearing gloves when handling pork, adhering to proper hand washing techniques, and sufficiently cooking pork products (*3*).

S. suis infection may go unrecognized since many laboratories do not routinely speciate α-hemolytic streptococci. However, in the United States, specialized tests such as the API 20 Strep System (API System; La Balme Les Grottes, Montalieu-Vercieu, France) or reference laboratories are readily available for diagnosis of all unidentified streptococci. In severe cases where infection is suspected, physicians may request that laboratories conduct definitive tests to identify the organism. In countries that lack these resources and where under-

cooked pork is a diet staple, underdiagnosis of *S. suis* infection is likely. Greater understanding of this organism and its disease spectrum would promote earlier diagnosis and prevention of sequelae.

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Parvoviruses in Blood Donors and Transplant Patients, Italy

To the Editor: Parvoviruses (PARV) 4 and 5 are 2 genotypes of a novel human parvovirus, with 92% nucleotide identity, identified in the plasma sample of a patient screened for acute HIV infection and in samples of manufactured plasma pools (*1*,*2*). Recently, PARV4 and PARV5 were identified in blood samples from 3 of 26 cadavers from the United Kingdom, all of whom were positive for hepatitis C virus RNA and had a history of intravenous drug use (*3*). PARV4/5 were also found in bone marrow (BM) and lymphoid tissues from 17 of 24 HIV-positive cadavers from Scotland (*4*) and in BM aspirates from 16 of 35 Italian patients with AIDS (*5*). Little or no information is available about the epidemiology and clinical correlates of infection with these novel viruses. To provide insights into their pathogenic potential in vivo, we assessed the frequency of PARV4/5 viremia in healthy patients, transplant patients, and those with suspected viral disease.

We performed a retrospective molecular study for the presence of PARV4/5 sequences in 4 groups of 417 Italian HIV-negative persons. Group 1 consisted of 100 blood donors recruited from the Transfusion Centre of Modena (northern Italy); group 2, 84 patients with hematologic diseases showing clinical signs of viral etiology but negative results for the most common viruses (herpesviruses, adenovirus, hepatitis virus, and coxsackie virus). For both of these groups, DNA was extracted for analysis from serum specimens and peripheral blood mononuclear cells (PBMCs). Groups 3 and 4 comprised recipients of kidney and allogeneic BM/peripheral blood stem cell (PBSC) transplants, for which DNA was extracted from serum specimens collected at 6 and 12 months, respectively, after transplantation. The nested PCR method was used to amplify a shared sequence of PARV4 and its variant PARV5 and was specific for the open reading frame 1. First step PCR was performed as previously described (*2*) with a sensitivity of 1–10 copies, on 1 μg PBMC DNA and on one fifth of DNA extracted from 0.25 mL of serum. Primers for second round PCR were PV4NS1Fn2 (5′-GTTGATGGYCCTGTGGTTAG-3′) and PV4NS1Rn2 (5′-CCTTTCATATTCAGTTCCTGTTCAC-3′). All positive results were confirmed by direct sequencing.

We found 3 positive case-patients, including 2 renal transplant recipients and 1 patient with a suspected viral disease; none of the blood donors tested positive on single-round PCR. On nested PCR, 1 blood donor had positive results; the positivity rate did not increase in the other groups (Table). In the first 2 groups, PARV4/5 sequences were detected only in the serum samples, not in the PBMCs collected at the same time. These sequences suggest that PBMCs are not a major site of viral replication. Similar to B19 infection, which is rarely reactivated in the setting of BM/PBSC transplantation (*6*,*7*), none of the BM/PBSC transplant patients were PARV4/5 positive. The detection of PARV4/5 sequences in the serum collected at 12 months after transplantation was not associated with the occurrence of any symptoms in the 2 renal recipients. Of note, the available serum samples collected from both recipients before transplantation, and at 6 and 24 months after

Table. Analysis of 417 patients tested for parvoviruses 4/5 by PCR*

Group	No. cases positive on serum samples/ no. tested (%)		No. cases positive on PBMCs/ no. tested	
	First-round PCR	Nested PCR	First-round PCR	Nested PCR
Blood donors	0/100	1/100 (1)	0/100	0/100
Patients with suspected viral diseases	1/84 (1.2)	1/84	0/84	0/84
BM/PBSC transplant recipients	0/107	0/107	ND	ND
Kidney transplant recipients (%)	2/126 (1.6)	2/126	ND	ND

*PBMCs, peripheral blood mononuclear cells; BM, bone marrow; PBSC, peripheral blood stem cell; ND, not done.

transplantation, were PARV4/5 negative, which suggests that asymptomatic PARV4/5 infection may transiently occur after solid organ transplantation or may be acquired throughout transfusion or transplantation. Similarly, the rate of B19 infection in solid organ transplant recipients is low (1.4%–1.8%), and most B19 DNA–positive patients remain asymptomatic (*8*,*9*).

PARV4/5 sequences were detected in the serum collected from 1 patient affected with Wegener granulomatosis. This patient was under long-term treatment with steroids, concomitant with the development of a clinical syndrome for which a viral cause was suspected, including fever, severe anemia, a histologic-examination–proven postinfectious glomerulonephritis, and eryhtroid hypoplasia, with dsyserythropoiesis and dismegakaryopoiesis on BM examination. Serologic and molecular tests for the most common viruses, including B19, were negative and the patient died of multiple organ failure 1 month later. Single-cell PCR performed on the DNA extracted from isolated BM erythroid and myeloid progenitors in the formalin-fixed, paraffin-embedded BM tissue biopsy specimens, collected 2 days before death and at autopsy, were PARV4/5 negative. While the PARV4/5 viremia, in the absence of other known viral agents, suggests a possible contribution of this novel parvovirus to the patient's clinical syndrome, the absence of the virus in the BM cells suggests that its in vivo tropism may markedly differ from that of B19.

In conclusion, although the frequency of PARV4/5 viremia is very low in the general Italian population, it is slightly higher in certain subgroups of iatrogenically immunosuppressed patients and it is not clear to which extent immunosuppression enhances viral reactivation and/or primary infection. Failure to detect PARV4/5 DNA in all but 4 study patients does not necessarily indicate a rarity of past viral exposure or infection in transplant patients or indeed in the general population. Further studies are needed to confirm a possible pathogenic role of PARV4/5 infection.

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Antimicrobial Drug Use and Antibiotic-Resistant Bacteria

To the Editor: The article by Harris et al., published in the August 2007 issue of Emerging Infectious Diseases, examined the risk factors for selecting extended-spectrum β-lactamase–producing *Enterobacteriaceae* in intensive-care patients and found that exposure to piperacillin/tazobactam and vancomycin were independent risk factors (*1*). Although antimicrobial drug use has been historically linked to antibiotic resistance in bacteria, we should not miss the perspective that such a risk factor mostly favors the cross-transmission of preexisting antibiotic-resistant bacteria, taking into account the disruption of the endogenous microflora, rather than the selection of "de novo" resistant mutants (*2*). This supposition is supported by many articles that have found genetic similarity between antibiotic-resistant microorganisms that occur in hospitalized patients, as well as by the fact that most of these pathogens exhibit cross-resistance with different classes of drugs, which should be extremely rare on a mutation basis.

This hypothesis is also supported by the evidence that healthcare workers frequently do not obey simple infection control precautions such as practicing hand hygiene between contact with different patients (*3–6*). That is likely why Larson et al., in a multicenter study in the United States, recently found no relationship between antimicrobial drug control policies and level of antibiotic resistance in bacteria, but did find an association between lower levels of antibiotic resistance in *Staphylococcus aureus* and enterococci and high compliance with hand hygiene (*7*).

Therefore, perhaps we should start looking for risk factors for being colonized or infected by any antimicrobial drug–resistant bacterium, including in our analysis some infection control measures adopted commonly during outbreak investigations, such as exposure to doctor A or nurse B, proximity to a known colonized patient, understaffing during the period of the study, and so forth. If we do so, we will likely find that antimicrobial drug use is not a completely independent risk factor for the mentioned outcome, but a risk factor closely related to the availability of the antibiotic-resistant microorganism in the local environment or on our own hands.

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In Response: We appreciate the comments by Dr. Bellissimo-Rodrigues regarding our article analyzing risk factors for surveillance-culture positivity with extended-spectrum β-lactamase (ESBL)–producing bacteria (*1*). We agree with the author that patient-to-patient transmission is a potentially important causal factor in the emergence of resistance for ESBL-producing bacteria as well as for other antimicrobial drug–resistant bacteria, such as vancomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus*, and drug-resistant *Pseudomonas* and *Acinetobacter* spp. For each of these resistant organisms, a complicated interplay likely exists between the causal importance of antibiotic selective pressure and patient-to-patient transmission by healthcare workers (*2–4*). The relative importance of these 2 causal mechanisms needs to be determined for each individual antimicrobial-resistant bacterium. The relative causal importance may be different for different outcomes: colonization on admission, colonization acquisition, and progression from colonization to infection. Understanding the relative importance for each of these outcomes is needed before determining whether infection control interventions or antimicrobial

drug stewardship policies will be effective. For example, we have published work that demonstrates that patient-to-patient transmission is important for colonization acquisition of ESBL-producing *Klebsiella* spp. and *Escherichia coli* (*5*).

We believe that a couple of points require clarification. In our article, we looked at risk factors for colonization with ESBL-producing bacteria on admission to an intensive care unit. We did not examine risk factors for selecting ESBL-producing bacteria as the letter implies. For the antimicrobial drugs identified as potential risk factors, we clearly stated in the discussion that the risk factors identified may be causally related to the outcome of ESBL-colonization or may only be statistically associated. We argue that even risk factors identified are not causal; they may be important because they can help determine which patients may need empiric antimicrobial drug therapy targeted to the ESBL-producing bacteria. Future research work is still needed to assess the relative importance of patient-to-patient transmission versus antimicrobial selective pressure.

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Aftermath

George Held

It's not the storm itself—wind and rain lashing shore,
uprooting trees, toppling poles and dousing lights,
flooding cellars and roads, capsizing boats—
but the aftermath—the bright calm, the pair
of drowned cats crumpled against the picket fence,
the parlor of Izzy's shack open for inspection,
the walls fallen flat on all sides, your own
roof filling the front yard, covering your car,
and your own twin daughters dazed by Nature's
petulance—that makes you reconsider
your life and weigh your possessions and the cost
of putting down stakes too near the coast
as the globe warms, and storms grow worse.

Fred Machetanz (1908–2002). Quest for Avuk (1973). Oil on board (81.3 cm × 130.8 cm). Anchorage Museum at Rasmuson Center, Anchorage, Alaska. 1974.047.001. Gift of Mr. and Mrs. Elmer E. Rasmuson

"I Am but Mad North-northwest: When the Wind is Southerly I Know a Hawk from a Handsaw"

—William Shakespeare, Hamlet

Polyxeni Potter*

"The true north strong and choked with ice," wrote Canadian poet Al Purdy, about the Arctic (*1*). "The sea ... was like the concentrated essence of all the blue that ever was; I could feel that blue seep into me and all my innards change colour. And the icebergs! They were shimmery lace and white brocade, and they became my standard for the word *beauty*" (*2*). Purdy's thrill at drifting with "the tides on Cumberland Sound and its blue fiord, [where] bergs and growlers are always in sight, even at the height of summer," (*1*) echoes the experience of many who visit the North; among them, Fred Machetanz, painter of iconic Alaska.

A native of Kenton, Ohio, educated at Ohio State University, the American Academy of Art in Chicago, and the Arts Students League in New York, he ventured to Unalakleet, a tiny fishing village on the Bering Sea, in 1935 to visit his uncle. Captivated by the landscape, he moved there to celebrate it in his work for years to come. "I was just nuts about Alaska" (*3*).

Artists have long traveled to the icy North. During the 18th and 19th centuries, as part of explorations, they documented discoveries and sought adventure and new cultures. John Webber, official painter for Captain James Cook's voyage (1776–1780), produced countless expertly painted records. By the end of European exploration and after the purchase of Alaska in 1867 from the Russians, travel increased. Naturalists and conservationists, John Burroughs, John Muir, and others, reported on their visits to the glaciers. North American artists, among them Eustace Ziegler, Ted Lambert, Sidney Laurence, Jules Dahlager, migrated to paint their romantic vision of "the last frontier" with its pristine wilderness and sparse inhabitants close to the land. Some of these visitors became the best landscape painters of the day. The art of Alaska's own populations, a long and rich tradition, was influenced by the onslaught of imported forms (*4*).

Much history of the Eskimo culture of North America in early 20th century comes to us from the work of Danish anthropologist Knud Rasmussen, whose expedition crossed North America from east of Baffin Land to Alaska and across the Bering Straight to Siberia. He lived to report conditions more inclement and dangerous for humans than nearly anywhere else in the world. "Cold and mosquitoes, / these two pests / come never together," goes the Iglulik song, "I lay me down on the ice, / Lay me down on the snow and the ice, / Till my teeth fall chattering" (*5*).

The relationship between humans and the physical world is widely explored in Inuit poetry. "We fear the weather spirit of earth, which we must fight against to wrest our food from land and sea. We fear Sila [the weather]" (*6*). Locals and sourdoughs of a bygone era under these extreme conditions, their rules for life and survival from snowstorm to snowstorm, the majestic surroundings and wildlife became Machetanz' work; during the early years, in books,

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photographs, movies, and lectures; then exclusively in paintings. He set up his easel opposite the windows of his tiny cabin near Palmer and started to recreate the surroundings. Over seven decades, he exhibited widely, built a reputation, and became one of Alaska's most beloved artists. "If anyone viewing my work has felt the beauty, the thrills and the fascination I have known in Alaska, then I have succeeded in what I set out to do" (*3*).

Though close to the artists of Alaska's romantic era, Machetanz lived the life he painted. He embraced the wilderness, "Why that land that they want back there ain't fit for nobody but goats, writers and artists" was the official opinion on the space staked out for his cabin (*3*). He joined a whaling crew, paddled his own umiak, drove dog sleds. If he painted an Athabascan woman with a birch bark baby carrier, he commissioned a carrier. "That's why we have these beautiful artifacts we've collected, which are made to scale, and made by experts, the natives who know them." He could "take a model and rotate it in the sunlight and get the light and shade on it" (*3*).

The art editor of Scribner's once joked about a Machetanz painting, "You've put a cherry colored head on that Eskimo." The painter corrected him, "If you see an Eskimo under a golden pink sun, you're going to see a red exactly like that….People don't realize the colors that we get here. And then we have a longer chance to look at those colors" because of the long hours of daylight in the summer and late spring (*3*).

As a young man, Machetanz visited Maxfield Parrish, then probably the most famous American artist; "…hardly a home in America existed that didn't have a Maxfield Parrish print" (*7*). He drove to Cornish, New Hampshire, to meet him, and they became friends for life. "I have always admired the art of Maxfield Parrish and a lot of the early painters of the Renaissance ... Vermeer and Titian and those. They used a technique…where they first, on the canvas or board ... painted the entire painting in one color—white ... then ... layers of transparent color, which you could look through and eventually get the final result. It's like putting a blue glass and a red glass over a white surface, and you could look through the blue and the red and you could see a purple, but it would be a transparent purple and quite different from an opaque purple of pigment" (*3*). This laborious technique is credited for the chill northern intensity of Machetanz' paintings: "... each layer has to be dry before I put on another layer, and my paintings contain six to eight layers of paint and varnish, and the only way I could dry them was by the sun or the stove." (*3*).

Quest for Avuk on this month's cover captures a theme of everyday life. Eskimo men paddle an umiak, a lightweight skin boat of the Arctic, searching for Avuk, likely a walrus (ayvuq [Central Siberian Yupik], aiviq [Inupiaq]). The men in camouflage kuspuks of cotton canvas over their parkas wear a look of intense concentration. A rifle, a toggling harpoon, and a sealskin float are visible from the side. The lithe vessel gliding noiselessly on the frigid waters allows immediate access to the hunt beneath the surface: seals, walruses, whales; in back, ice always in the invisible horizon.

"When I get home / With a catch that does not suffice, / I usually say / It was the fish / That failed— / Up the stream" (*6*). A hard stormy winter, when the caribou left and the seals were hard to find, could spell starvation for Machetanz' subjects, early Eskimo communities, isolated, completely dependent on traditional sources of sustenance, lashed by weather. "Life is so with us that we are never surprised…that someone has starved to death. We are so used to it ... They cannot help it, it is not their fault, it is either sila [the weather] or persaq [blizzard] or to'nraq [evil spirit, i.e., sickness]" (*6*).

"I have only my song, / Though it too is slipping from me" (*6*). Arctic populations in the United States and Canada now live largely in settled communities no longer completely dependent on walrus and fish. Long adapted to isolation and affected by infections linked to climate and culture, they are now also vulnerable to emerging plagues (*8*). Back in the 1980s, in his "Trees at the Arctic Circle," Al Purdy contemplated the strength of these trees: "And you know it occurs to me / about 2 feet under / those roots must touch permafrost / ice that remains ice forever / and they use it for their nourishment / use death to remain alive."

Now permafrost is melting. Heavily geared for ice, Arctic populations are facing yet another bout of rough weather, a warming trend. And unlike Shakespeare's hero, they have no need to feign madness.

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EMERGING INFECTIOUS DISEASES

Upcoming Issue

Streptococcus pneumoniae Serotype 19A in Korean Children

Effectiveness of Personal Protective Measures to Prevent Lyme Disease

Human Bocavirus Infections in Hospitalized Children and Adults

Genetic Determinants of Virulence of Pathogenic Lineage 2 Strains of West Nile Virus

Emergence of New Norovirus Variants on Spring Cruise Ships and Prediction of Winter Epidemics

Unexpected Occurrence of Plasmid-Mediated Quinolone Resistance Determinants in Environmental *Aeromonas* spp.

Cost-effectiveness of Human Papillomavirus Vaccination in the United States

Antiviral Stockpiling and Near Patient Testing for a Potential Influenza Pandemic

Molecular Typing of *Scedosporium* Isolates, Australia

Dissemination of *Escherichia coli* β-Lactamase CTX-M-15

Diagnosis of Cystic Echinococcosis in 9 Peruvian Highland Communities

Severe *Streptococcus pyogenes* Infections, United Kingdom, 2003–2004

Genetic Characterization of Feline Leukemia Virus in Florida Panthers

The Emergence of Polycystic Neotropical Echinococcosis

Atypical Bovine Spongiform Encephalopathies, France, 2001–2007

Burkholderia pseudomallei, Cambodia

Enteric Disease Surveillance in 6 US States

Methicillin-Resistant *Staphylococcus aureus*, Geneva, Switzerland, 1993–2005

Experimental Infection and Natural Contact Exposure of Dogs with Avian Influenza Virus (H5N1)

Complete list of articles in the February issue at http://www.cdc.gov/eid/upcoming.htm

Upcoming Infectious Disease Activities

January 23–25, 2008
International Symposium on Avian Influenza: Integration from Knowledge to Control
Bangkok, Thailand
http://www.biotec.or.th/AIconf2008

February 3–6, 2008
15th Conference on Retroviruses and Opportunistic Infections
Hynes Convention Center
Boston, MA, USA
http://www.retroconference.org

March 16–19, 2008
International Conference on Emerging Infectious Diseases
Hyatt Regency Atlanta
Atlanta, GA, USA
http://www.iceid.org

March 28–30, 2008
Clinical Infectious Disease Update Course 2008–Eleventh Annual Management Review for the Practicing Physician
The Grand Hyatt
New York, NY, USA
http://www.cbcbiomed.com

April 5–8, 2008
Society for Healthcare Epidemiology of America (SHEA) 18th Annual Scientific Meeting
Buena Vista Palace
Orlando, FL, USA
Abstract submission deadline: January 4, 2008
http://www.shea-online.org

April 8–11, 2008
Genomes 2008 - Functional Genomics of Microorganisms
Institut Pasteur
Paris, France
http://www.pasteur.fr/infosci/conf/sb/genomes_2008

Announcements

To submit an announcement, send an email message to EIDEditor (eideditor@cdc.gov). In 50–150 words, describe timely events of interest to our readers. Include the date of the event, the location, the sponsoring organization(s), and a website that readers may visit or a telephone number or email address that readers may contact for more information.

Emerging Infectious Diseases is a peer-reviewed journal established expressly to promote the recognition of new and reemerging infectious diseases around the world and improve the understanding of factors involved in disease emergence, prevention, and elimination.

The journal is intended for professionals in infectious diseases and related sciences. We welcome contributions from infectious disease specialists in academia, industry, clinical practice, and public health, as well as from specialists in economics, social sciences, and other disciplines. Manuscripts in all categories should explain the contents in public health terms. For information on manuscript categories and suitability of proposed articles see below and visit www.cdc.gov/eid/ncidod/ EID/instruct.htm.

Emerging Infectious Diseases is published in English. To expedite publication, we post articles online ahead of print. Partial translations of the journal are available in Japanese (print only), Chinese, French, and Spanish (www.cdc. gov/ncidod/EID/trans.htm).

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Manuscript Preparation. For word processing, use MS Word. List the following information in this order: title page, article summary line, keywords, abstract, text, acknowledgments, biographical sketch, references, tables, figure legends, appendixes, and figures. Each figure should be in a separate file.

Title Page. Give complete information about each author (i.e., full name, graduate degree(s), affiliation, and the name of the institution in which the work was done). Clearly identify the corresponding author and provide that author's mailing address (include phone number, fax number, and email address). Include separate word counts for abstract and text.

Keywords. Include up to 10 keywords; use terms listed in Medical Subject Headings Index Medicus.

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Types of Articles

Perspectives. Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), a one-sentence summary of the conclusions, and a brief biographical sketch. Articles in this section should provide insightful analysis and commentary about new and reemerging infectious diseases and related issues. Perspectives may also address factors known to influence the emergence of diseases, including microbial adaptation and change, human demographics and behavior, technology and industry, economic development and land use, international travel and commerce, and the breakdown of public health measures. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text.

Synopses. Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), a one-sentence summary of the conclusions, and a brief biographical sketch. This section comprises concise reviews of infectious diseases or closely related topics. Preference is given to reviews of new and emerging diseases; however, timely updates of other diseases or topics are also welcome. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text.

Research Studies. Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), a one-sentence summary, and a brief biographical sketch. Report laboratory and epidemiologic results within a public health perspective. Explain the value of the research in public health terms and place the findings in a larger perspective (i.e., "Here is what we found, and here is what the findings mean").

Policy and Historical Reviews. Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), a one-sentence summary of the conclusions, and a brief biographical sketch. Articles in this section include public health policy or historical reports that are based on research and analysis of emerging disease issues.

Dispatches. Articles should be no more than 1,200 words and need not be divided into sections. If subheadings are used, they should be general, e.g., "The Study" and "Conclusions." Provide a brief abstract (50 words); references (not to exceed 15); figures or illustrations (not to exceed 2); tables (not to exceed 2); and a brief biographical sketch. Dispatches are updates on infectious disease trends and research. The articles include descriptions of new methods for detecting, characterizing, or subtyping new or reemerging pathogens. Developments in antimicrobial drugs, vaccines, or infectious disease prevention or elimination programs are appropriate. Case reports are also welcome.

Commentaries. Thoughtful discussions (500–1,000 words) of current topics. Commentaries may contain references but no figures or tables.

Another Dimension. Thoughtful essays, short stories, or poems on philosophical issues related to science, medical practice, and human health. Topics may include science and the human condition, the unanticipated side of epidemic investigations, or how people perceive and cope with infection and illness. This section is intended to evoke compassion for human suffering and to expand the science reader's literary scope. Manuscripts are selected for publication as much for their content (the experiences they describe) as for their literary merit.

Letters. Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have 1 figure or table and should not be divided into sections. All letters should contain material not previously published and include a word count.

Books, Other Media. Reviews (250–500 words) of new books or other media on emerging disease issues are welcome. Name, publisher, number of pages, other pertinent details should be included.

Announcements. We welcome brief announcements (50–150 words) of timely events of interest to our readers. (Announcements may be posted online only, depending on the event date.)

Conference Summaries. Summaries of emerging infectious disease conference activities are published online only. Summaries, which should contain 500–1,000 words, should focus on content rather than process and may provide illustrations, references, and links to full reports of conference activities.