advisories and travel health precautions were subsequently released, including those from Canada and United States (*9*,*10*). These precautions recommended that preventative measures such as vaccination and safe food and water consumption practices be adhered to by residents and visitors to affected regions. Although the public health community anticipated that travel-associated cases would be diagnosed in Québec, this report of a documented case (supported by laboratory and epidemiologic data) emphasizes the domestic and international public health risk caused by the nationwide outbreak in Haiti. It also illustrates the need for an accurate travel history in clinical and laboratory diagnosis of cholera infections.

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Matthew W. Gilmour, Valérie Martel-Laferrière, Simon Lévesque, Christiane Gaudreau, Sadjia Bekal, Céline Nadon, and Anne-Marie Bourgault

Author affiliations: Public Health Agency of Canada, Winnipeg, Manitoba, Canada (M.W. Gilmour, C. Nadon); Centre Hospitalier de l'Université de Montréal, Montreal, Quebec, Canada (V. Martel-Laferrière, C. Gaudreau, A.-M. Bourgault); and Institut National de Santé Publique du Québec, Ste-Anne-de-Bellevue, Quebec (S. Lévesque, S. Bekal, A.-M. Bourgault)

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Address for correspondence: Matthew W. Gilmour, National Microbiology Laboratory, Public Health Agency of Canada, 1015 Arlington St, Winnipeg, Manitoba R3E 3R2, Canada; email: matthew.gilmour@phac-aspc.gc.ca

Easy Diagnosis of Invasive Pneumococcal Disease

To the Editor: Invasive pneumococcal disease (IPD) causes many cases of severe disease and death among children <5 years of age, mostly in developing countries (*1*,*2*). Before conjugate vaccines can be introduced in developing countries, information about disease epidemiology is urgently needed. The lack of laboratories equipped to perform pneumococcal serotyping leads to the need to send isolates to reference laboratories. Good sample preservation is necessary to prevent samples from arriving at the laboratory in poor condition. We evaluated the usefulness of multiplex real-time PCR from strains and blood samples kept at room temperature on dried blood spot (DBS) filter paper for detecting and serotyping *Streptococcus pneumoniae*. DBS screening is a reliable method that requires only a small amount of blood; it is used for the diagnosis of several human diseases (*3*,*4*).

To validate the technique, we selected 15 pneumococcus clinical isolates representing 15 serotypes (1, 5, 19A, 19F, 14, 3, 7F, 4, 6A, 6B, 8, 9N, 18C, 23A, 23F) obtained during 2009 from patients at Hospital Sant Joan de Déu, in Barcelona. These isolates, used as controls, had been serotyped by quellung reaction at the Instituto de Salud Carlos III, Majadahonda-Madrid, Spain. These strains were cultured overnight at 35°C in 5% carbon dioxide on Columbia agar plates with 5% sheep blood (bioMérieux SA, Marcy l'Etoile, France). A suspension of each strain was adjusted to match a 0.5 McFarland standard (equivalent to 10^8 colony-forming units (CFU)/mL). Stock solutions of pneumococcus culture for each previously identified serotype were injected into blood

previously extracted from 2 healthy volunteers. Serial dilutions of 100,000 CFU/mL to 1,000 CFU/mL (1,000 to 10 CFU equivalents/PCR) were performed. A total of 100 µL of blood was applied to DBS filter paper, and another 100 µL was used for DNA extraction from fresh blood. All DBS samples were air dried for 1 week. The procedure was also performed on negative control blood samples.

DNA was extracted from DBS and fresh blood samples by using the NucliSense easyMAG automated extraction platform (bioMérieux, Boxtel, the Netherlands) according to the manufacturer's instructions. DNA detection of the *pneumolysin* (*ply*) gene by real-time PCR was performed according to a published assay (*5*). In addition, we performed a multiplex real-time PCR for molecular serotype detection of serotypes 1, 3, 5, 4, 6A, 6B, 7FA, 8, 9VANL, 14, 15BC, 18CB, 19A, 19FBC, 23F, 23A and the conserved capsular gene *wzg* as described by Tarrago et al. (*6*). DNA extracts were amplified with the Applied Biosystems 7300 Real-time PCR System (Applied Biosystems, Foster City, CA, USA). Negative results were defined as those with cycle threshold >40.

To evaluate the reliability obtained with this in vivo approach, we performed identification and serotyping of *S. pneumoniae* in 25 DBS samples from 25 children at Saint John of God Hospital in Mabesseneh-Lunsar, Sierra Leone. This hospital does not perform blood cultures. IPD was confirmed when DNA of a *pneumolysin (ply)* gene and an additional capsular gene of *S. pneumoniae* were detected by multiplex real-time PCR of DBS samples.

Detection of *ply*, *wzg*, and the specific gene for molecular serotype showed that both fresh blood and DBS samples yielded correctly positive results from the 10-fold serial dilutions analyzed (Table). With respect to the 25 (11 female and 14 male) patients from Sierra Leone who had suspected IPD, the median age was 25.71 months (range 15 days to 96 months); all had a diagnosis of fever without apparent source, and 16 also had malaria. Of these 25 children, DBS samples from 15 (60%) yielded a positive result for the *ply* and *wzg* genes, so they were considered confirmed episodes of IPD. A serotype included in 13-valent conjugate vaccine was detected in 6 (40%) of 15 positive samples: serotypes 3, 7FA, 19A, 6A, 6B, and 9VNL (1 sample each). In the remaining 9 samples, the results for *ply* gene and *wzg* gene were positive, but none of the 24 tested serotypes was detected.

This preliminary study enabled us to demonstrate that DBS screening is a reliable and easy method for diagnosing IPD and also for epidemiologic surveillance of the more frequent serotypes. The main limitation of our study is the small number of DBS samples sent from Saint John of God Hospital in Sierra Leone.

In conclusion, the DBS technique enables reproducible transport of samples for identification and serotyping of *S. pneumoniae* by multiplex PCR. The use of DBS on filter paper is an attractive alternative method for storing samples at room temperature and easily transporting them. Additional studies, including evaluation of the relative sensitivity of this method compared to direct culture, are necessary.

Table. Sensitivity of real-time PCR for detecting *Streptococcus pneumoniae ply* or *wzg* genes or a specific gene for molecular serotype from fresh or dried blood spot samples*

	Serial dilutions correctly detected, CFU equivalent/PCR†	
Gene or serotype	Fresh blood	Dried blood spot
ply gene	1.10^1–1.10^3	1.10^1–1.10^3
wzg gene	1.10^1–1.10^3	1.10^1–1.10^3
Serotype 1	1.10^1–1.10^3	1.10^1–1.10^3
Serotype 5	1.10^1–1.10^3	1.10^1–1.10^3
Serotype 19A	1.10^1–1.10^3	1.10^1–1.10^3
Serotype 19F	1.10^1–1.10^3	1.10^1–1.10^3
Serotype 14	1.10^1–1.10^3	1.10^1–1.10^3
Serotype 3	**1.10^2–1.10^3**	1.10^1–1.10^3
Serotype 7F	1.10^1–1.10^3	1.10^1–1.10^3
Serotype 4	1.10^1–1.10^3	1.10^1–1.10^3
Serotype 6A	1.10^1–1.10^3	**1.10^2–1.10^3**
Serotype 6B	1.10^1–1.10^3	1.10^1–1.10^3
Serotype 8	1.10^1–1.10^3	1.10^2–1.10^3
Serotype 9N	1.10^1–1.10^3	1.10^1–1.10^3
Serotype 18C	1.10^1–1.10^3	1.10^1–1.10^3
Serotype 23A	1.10^1–1.10^3	1.10^1–1.10^3
Serotype 23F	1.10^1–1.10^3	1.10^1–1.10^3

*Serial dilutions (10–1,000 CFU equivalent/PCR) of 15 pneumococcus cultures mixed with 15 negative-control blood samples were analyzed. **Boldface** indicates results that differ from others.
†10 CFU equivalent/PCR = 1,000 CFU equivalents/mL blood.

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Laura Selva, Xavier Krauel, Roman Pallares, and Carmen Muñoz-Almagro

Author affiliations: University Hospital Sant Joan de Déu, Barcelona, Spain (L. Selva, X. Krauel, C. Muñoz-Almagro); Saint John of God Hospital, Lunsar, Sierra Leone (X. Krauel); Bellvitge Hospital, Barcelona (R. Pallares); and University of Barcelona, Barcelona (R. Pallares)

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Address for correspondence: Carmen Muñoz-Almagro, Molecular Microbiology Department, University Hospital Sant Joan de Déu, P° Sant Joan de Déu, nº 2, 08950 Esplugues, Barcelona, Spain; email: cma@hsjdbcn.org

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Mimivirus-like Particles in Acanthamoebae from Sewage Sludge

To the Editor: Mimivirus is a giant, double-stranded DNA virus. Its 650-nm diameter and 1.2-Mb genome make it the largest known virus (*1*). In 2003, mimivirus was isolated from a water cooling tower in Bradford, UK, after a pneumonia outbreak and was reported to infect *Acanthamoeba polyphaga* amebae (*2*). Subsequently, a small number of additional isolates have been reported (*3*).

Mimivirus has been associated with pneumonia, and this association was strengthened after antibodies to mimivirus were found in serum samples from patients with community- and hospital-acquired pneumonia and after mimivirus DNA was found in bronchoalveolar lavage specimens (*4*). More direct evidence of pathogenicity was illustrated when a pneumonia-like disease developed in a laboratory technician who worked with mimivirus and showed seroconversion to 23 mimivirus-specific proteins (*5*).

We report finding mimivirus-like particles during our molecular study of *Acanthamoeba* spp. abundance and diversity in final-stage conventionally treated sewage sludge from a wastewater treatment plant in the West Midlands, UK. Using metagenomic DNA extracted from the sludge (*6*), we estimated the abundance of *Acanthamoeba* spp. by using real-time PCR (*7*) and found it to be ≈1 × 10^2/g sludge. To assess species diversity, we amplified an *Acanthamoeba* spp.–specific 18S rRNA target, which resulted in products of ≈450 bp (*8*). PCR products were cloned and sequenced, revealing low *Acanthamoeba* spp. diversity with a predominance of clones most similar to *A. palestinensis* (22/25 clones), which fall within the T6 clade according to the classification of Stothard et al. (*9*). A small number (3/25) of clones showed closest similarity to acanthamoebae belonging to the T4 clade, which includes strains considered to be human pathogens, including some *A. polyphaga* strains.

Acanthamoebae were isolated from fully digested sewage sludge by inoculating diluted sludge onto cerophyl-Prescott infusion agar and subculturing onto nonnutrient agar plates streaked with heat-killed *Escherichia coli*. Cultures were incubated at 20°C and 30°C and examined under an Axioskop 2 microscope (Zeiss, Oberkochen, Germany) at 100× magnification; cells of interest were examined at 1,000× magnification. One clonal population of an *Acanthamoeba* sp. isolated at 20°C, which demonstrated typical trophozoite and cyst morphology, contained large numbers of particles either within vacuoles or within the cytoplasm (Figure). Vacuoles were densely packed with particles that appeared to be constantly moving; vacuole size varied from that typical of food vacuoles to large vacuoles that occupied most of the cell volume (expanded online Figure, panels B, D, and G, www.cdc.gov/EID/content/17/6/1127-F.htm). Because the particles were assumed to be bacterial pathogens, efforts were made to produce an axenic culture of the ameba isolate, and 16S rRNA PCR was performed to identify any intracellular bacteria. DNA was extracted by using a phenol chloroform method according to Griffiths et al. (*6*). However, no 16S rRNA PCR products were amplified.

Months later, an image review led to recognition of unusual arrangements of intracellular particles in a lattice-like structure in which each particle was surrounded by 6 others. Measurement of rows of particles, assuming tight packing, gave an average particle size of 620 nm. At this point, we realized that the particles were virus-like