

Table. Genotyping results for human and animal clinical samples, Q fever outbreak, the Netherlands

Patient/animal no.	Sample	Ms27*	Ms28*	Ms34*	Symptoms	Ct value†	Location
Patient 1	Plasma	3	3	8	Severe	34.4	1
Ewe 1	Vaginal swab	3	3	8	None	25.7	1
Ewe 2	Vaginal swab	3	3	8	None	16.3	1
Ewe 3	Vaginal swab	3	3	8	None	18.8	1
Lamb 1	Throat swab	3	3	8	None	27.9	1
Lamb 2	Throat swab	3	3	8	None	29.9	1
Lamb 3	Throat swab	3	3	8	None	28.9	1
Patient 2	Urine	3	3	7	None	31.7	2
	Throat swab	3	3	7		31.8	
Patient 3	Urine	NR‡	3	4	Mild	36.7	2
Patient 4	Sputum	4	3	7	Severe	34.2	2
Patient 5	Sputum	3	3	7	Severe	31.9	3
Nine Mile	Reference strain	4*	6*	5*			

\*The allele-calling convention used was as published (3), resulting in a 4, 6, 5 code assigned respectively to the 6-bp repeat unit loci Ms-27, Ms-28, and Ms-34 for the genome sequence of the Nine Mile RSA-493 strain (GenBank accession no. NC002971.1). Primers for these markers were redesigned to amplify significantly shorter PCR products and were combined into 1 multicolor multiplex PCR. Primer sequences for Ms-27 were 5'-HEX-TCTTTATTTTCAGGCCGGAGT-3' and 5'-GAACGACTCATTGAACACACG-3; for Ms-28, 5'-TMR-AGCAAAGAAATGTGAGGATCG-3 and 5'-GCCAAAGGGATATTTTGTCTTC-3; for Ms-34, 5'-FAM-TTCTTCGGTGAGTTGCTGTG-3' and 5'-GCAATGACTATCAGCGACTCGAA-3'.

†Cycle threshold (Ct) value obtained by using real-time PCR targeting the IS1111a element.

‡NR, no result obtained. A full genotype was obtained only in samples with the highest DNA loads (Ct value ≤35).

**Corné H.W. Klaassen,  
Marrigje H. Nabuurs-Franssen,  
Jeroen J.H.C. Tilburg,  
Maurice A.W.M. Hamans,  
and Alphons M. Horrevorts**

Author affiliations: Canisius Wilhelmina Hospital, Nijmegen, the Netherlands (C.H.W. Klaassen, M.H. Nabuurs-Franssen, J.J.H.C. Tilburg, A.M. Horrevorts); Radboud University Medical Centre, Nijmegen (M.H. Nabuurs-Franssen); and Food and Consumer Product Safety Authority, Eindhoven, the Netherlands (M.A.W.M. Hamans)

DOI: 10.3201/eid1504.081612

## References

1. Raoult D, Marrie TJ, Mege JL. Natural history and pathophysiology of Q fever. *Lancet Infect Dis*. 2005;5:219–26. DOI: 10.1016/S1473-3099(05)70052-9
2. Schimmer B, Morray G, Dijkstra F, Schneeberger PM, Weers-Pothoff G, Timer A, et al. Large ongoing Q fever outbreak in the south of the Netherlands, 2008. *Euro Surveill*. 2008;13:pii=18939 [cited 2009 Feb 18]. Available from <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18939>
3. Arricau-bouvery N, Hauck Y, Bejaoui A, Frangoulidis D, Bodier CC, Souriau A, et al. Molecular characterization of *Coxiella burnetii* isolates by infrequent restriction site-PCR and MLVA typing. *BMC Microbiol*. 2006;6:38. DOI: 10.1186/1471-2180-6-38

Address for correspondence: Corné H.W. Klaassen, Department of Medical Microbiology and Infectious Diseases, Canisius Wilhelmina Hospital, Weg door Jonkerbos 100, 6532 SZ Nijmegen, the Netherlands; email: c.klaassen@cwz.nl

## Correlation between Buruli Ulcer and Vector-borne Notifiable Diseases, Victoria, Australia

**To the Editor:** Buruli ulcer (BU) is a destructive skin disease caused by the toxin-producing environmental pathogen *Mycobacterium ulcerans*. Since the 1980s, BU has emerged as a major public health problem in rural West and Central Africa (1), where some researchers have suggested a role for aquatic insects as either reservoirs or vectors of *M. ulcerans* (2,3). However, this hypothesis remains unproven (4).

In contrast to the emerging BU–endemic areas in tropical rural West Africa, the climate of the Australian state of Victoria is temperate, yet locally acquired BU also has increased there in recent years (5). In addition, notifications have varied markedly from year to year for reasons not yet explained.

During the investigation of a new outbreak of BU in Victoria, we demonstrated that *M. ulcerans* is detectable by PCR in mosquitoes and that being bitten by mosquitoes increases the odds of being diagnosed with BU (6,7). However, *M. ulcerans*–positive mosquitoes might reflect only the presence of *M. ulcerans* in the local environment and play no role in transmission. To further investigate links between BU and mosquitoes, we compared patterns of notifications of BU with other notifiable diseases in Victoria. In particular, we were interested in any association between BU and the locally transmitted vector-borne alphaviruses Ross River virus (RRV) and Barmah Forest virus (BFV). Areas of BU and RRV/BFV endemicity overlap geographically, but areas with RRV and BFV are more extensive and include inland river systems where BU has not so far been reported.

Notification data for RRV, BFV, and other notifiable infections in Victoria are publicly available (8). Although BU was not made notifiable until January 2004 (before which notification was voluntary), since early 2000, most diagnoses were confirmed by culture or PCR at the Victorian Infectious Diseases Reference Laboratory, from which we obtained data for this report.

Our analysis showed that in the last 7 years (2002–2008), BU notifications correlated with combined RRV/BFV notifications ( $r^2 = 0.52$ ,  $p = 0.06$ ) (Figure). During the same period, no correlation was observed with tuberculosis, the other important mycobacterial disease in Victoria ( $r^2 = 0.12$ ,  $p = 0.43$ ); legionellosis, caused by a nonvectored water-associated pathogen ( $r^2 = 0.04$ ,  $p = 0.66$ ); or any other notifiable infectious disease (data not shown).

Although the environmental reservoir and mode of transmission of *M. ulcerans* remain unknown, mosquitoes are well known for transmitting RRV and BFV to humans, and year-to-year variation in incidence of these vector-borne viral infections is linked to changes in mosquito numbers (9,10). We are not implying that

*M. ulcerans*, RRV, and BFV are transmitted simultaneously from the same reservoir species to the same humans or by the same mosquitoes. Also, environmental conditions that promote outbreaks of RRV/BFV infection might promote BU outbreaks without any other connection. However, we believe the correlation we have identified between BU and other mosquito-borne diseases is striking and further strengthens the link between mosquitoes and the transmission of *M. ulcerans* in Victoria.

This study was performed with funding assistance from the Victorian Department of Human Services (Public Health Research Grant).

#### Paul D.R. Johnson and Caroline J. Lavender

Author affiliations: Austin Health, Melbourne, Victoria, Australia (P.D.R. Johnson); World Health Organization Collaborating Centre for *Mycobacterium ulcerans* (Western Pacific Region) and Victorian Infectious Diseases Reference Laboratory, North Melbourne, Victoria, Australia (P.D.R. Johnson, C.J. Lavender)

DOI: 10.3201/eid1504.081162

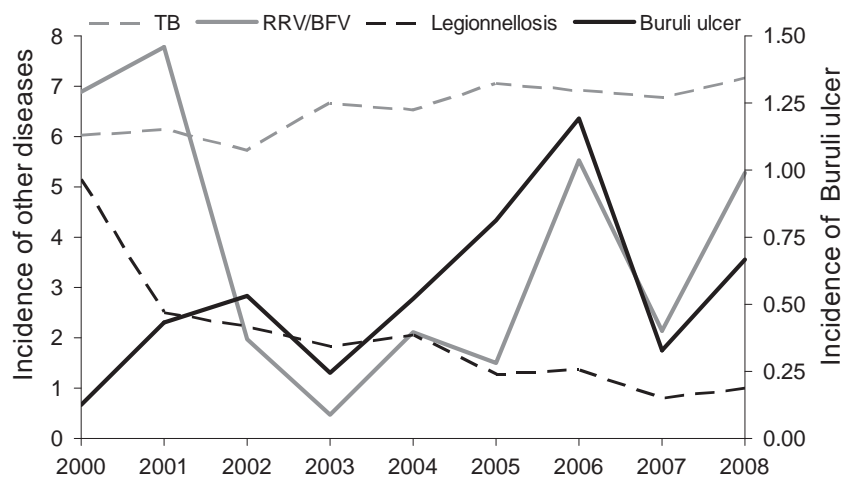


Figure. Numbers of cases per 100,000 inhabitants for selected notifiable diseases, Victoria, Australia, 2000–2008. Buruli ulcer is shown on the right y axis, other diseases on the left y axis: tuberculosis (TB), Ross River virus (RRV)/Barmah Forest virus (BFV), and legionellosis.

#### References

1. Johnson PD, Stinear T, Small PL, Pluschke G, Merritt RW, Portaels F, et al. Buruli ulcer (*M. ulcerans* infection): new insights, new hope for disease control. *PLoS Med.* 2005;2:e108 [erratum in *PLoS Med.* 2005;2:e173].
2. Marsollier L, Robert R, Aubry J, Saint Andre J, Kouakou H, Legras P, et al. Aquatic insects as a vector for *Mycobacterium ulcerans*. *Appl Environ Microbiol.* 2002;68:4623–8. DOI: 10.1128/AEM.68.9.4623-4628.2002
3. Portaels F, Elsen P, Guimaraes-Peres A, Fonteyne P, Meyers W. Insects in the transmission of *Mycobacterium ulcerans* infection. *Lancet.* 1999;353:986. DOI: 10.1016/S0140-6736(98)05177-0
4. Benbow ME, Williamson H, Kimbirauskas R, McIntosh MD, Kolar R, Quaye C, et al. Aquatic invertebrates as unlikely vectors of Buruli ulcer disease. *Emerg Infect Dis.* 2008;14:1247–54. DOI: 10.3201/eid1408.071503
5. Johnson PD, Hayman JA, Quek TY, Fyfe JA, Jenkin GA, Buntine JA, et al. Consensus recommendations for the diagnosis, treatment and control of *Mycobacterium ulcerans* infection (Bairnsdale or Buruli ulcer) in Victoria, Australia. *Med J Aust.* 2007;186:64–8.
6. Johnson PD, Azuolas J, Lavender CJ, Wishart E, Stinear TP, Hayman JA, et al. *Mycobacterium ulcerans* in mosquitoes captured during an outbreak of Buruli ulcer, southeastern Australia. *Emerg Infect Dis.* 2007;13:1653–60.
7. Quek TY, Athan E, Henry MJ, Pasco JA, Redden-Hoare J, Hughes A, et al. Risk factors for *Mycobacterium ulcerans* infection, southeastern Australia. *Emerg Infect Dis.* 2007;13:1661–6.
8. Department of Human Services. Notifications of infectious diseases [cited 2009 Jan 31]. Available from [http://www.health.vic.gov.au/ideas/downloads/daily\\_reports/statewide/rptVictorianSummary.pdf](http://www.health.vic.gov.au/ideas/downloads/daily_reports/statewide/rptVictorianSummary.pdf)
9. Dhileepan K. Mosquito seasonality and arboviral disease incidence in Murray Valley, southeast Australia. *Med Vet Entomol.* 1996;10:375–84. DOI: 10.1111/j.1365-2915.1996.tb00760.x
10. Passmore J, O'Grady KA, Moran R, Wishart E. An outbreak of Barmah Forest virus disease in Victoria. *Commun Dis Intell.* 2002;26:600–4.

Address for correspondence: Paul D.R. Johnson, Infectious Diseases Department, Austin Health, PO Box 5555, Heidelberg 3084, Melbourne, Victoria, Australia; email: [paul.johnson@austin.org.au](mailto:paul.johnson@austin.org.au)