than the heart in this worker cannot be ruled out.

Our results indicate high seroprevalence of Q fever among workers at the scouring factory studied. Continuous exposure to the Q fever agent was the likely cause of atypical antibody responses evoking a chronic or relapsing disease in the absence of any clinical symptom. These results indicated the need to analyze paired serum samples and to rely on medical follow-up before establishing a definitive diagnosis.

Given the continuous occupational risk to which these workers are exposed, hiring of pregnant women or persons with underlying medical conditions, such as valvulopathy or immunologic depression, should be avoided. Moreover, annual serologic testing should be conducted on all exposed persons to detect any evolution toward the chronic form of the disease, which can be life-threatening. Although less dangerous than anthrax, Q fever is still a highly prevalent occupational disease that affects persons working with animal hairs in industrial environments and commonly referred to as woolsorters (*10*).

Acknowledgments

We thank the factory workers for participating in the study and S. Malbrecq for assistance with real-time PCRs.

This study was supported by the Occupational Medicine group Provikmo, the Veterinary and Agro-chemical Research Centre, the Slovak Ministry of Education (grant no. 2/0127/10), and the Slovak Academy of Sciences.

Pierre Wattiau, Eva Boldisova, Rudolf Toman, Marjan Van Esbroeck, Sophie Quoilin, Samia Hammadi, Hervé Tissot-Dupont, Didier Raoult, Jean-Marie Henkinbrant, Mieke Van Hessche, and David Fretin

Author affiliations: Veterinary and Agrochemical Research Centre, Brussels, Belgium (P. Wattiau, M. Van Hessche, D. Fretin); Slovak Academy of Sciences, Bratislava, Slovakia (E. Boldisova, R. Toman); Institute of Tropical Medicine, Antwerp, Belgium (M. Van Esbroeck); Institute of Public Health, Brussels (S. Quoilin, S. Hammadi); Université de la Méditerranée, Marseille, France (H. Tissot-Dupont, D. Raoult); and Occupational Health Medicine Group Provikmo, Verviers, Belgium (J.-M. Henkinbrant)

DOI: http://dx.doi.org/10.3201/eid1712.101786

References

1. Maurin M, Raoult D. Q fever. Clin Microbiol Rev. 1999;12:518–53.
2. Schimmer B, Morroy G, Dijkstra F, Schneeberger PM, Weers-Pothoff G, Timen A, et al. Large ongoing Q fever outbreak in the south of the Netherlands, 2008. Euro Surveill. 2008;13:pii:18939.
3. Wallensten A, Moore P, Webster H, Johnson C, van der Burgt G, Pritchard G, et al. Q fever outbreak in Cheltenham, United Kingdom, in 2007 and the use of dispersion modelling to investigate the possibility of airborne spread. Euro Surveill. 2010;15:pii:19521.
4. Wilson LE, Couper S, Prempeh H, Young D, Pollock KG, Stewart WC, et al. Investigation of a Q fever outbreak in a Scottish co-located slaughterhouse and cutting plant. Zoonoses Public Health. 2010;57:493–8. doi:10.1111/j.1863-2378.2009.01251.x
5. European Centre for Disease Prevention and Control. Risk assessment on Q fever, 2010 [cited 2011 Jun 16]. http://www.ecdc.europa.eu/en/publications/Publications/1005_TER_Risk_Assessment_Qfever.pdf
6. Working Group on Foodborne Infections and Intoxications. Trends and sources. Report on zoonotic agents in Belgium, 2011 [cited 2011 Jun 16]. http://www.afsca.be/publicationsthematiques/_documents/2008-2009_Report-on-zoonotic-agents_en.pdf
7. Schimmer B, Notermans DW, Harms MG, Reimerink JH, Bakker J, Schneeberger P, et al. Low seroprevalence of Q fever in the Netherlands prior to a series of large outbreaks. Epidemiol Infect. 2011; Feb 16:1–9. 10.1017/S0950268811000136.
8. Frankel D, Richet H, Renvoisé E, Raoult D. Q fever in France, 1985–2009. Emerg Infect Dis. 2011;17:350–6.
9. Wattiau P, Klee SR, Fretin D, Van Hessche M, Menart M, Franz T, et al. Occurrence and genetic diversity of *Bacillus anthracis* strains isolated in an active wool-cleaning factory. Appl Environ Microbiol. 2008;74:4005–11. doi:10.1128/AEM.00417-08
10. Wattiau P, Govaerts M, Frangoulidis D, Fretin D, Kissling E, Van Hessche M, et al. Immunologic response of unvaccinated workers exposed to anthrax, Belgium. Emerg Infect Dis. 2009;15:1637–40.

Address for correspondence: Pierre Wattiau, Veterinary and Agrochemical Research Centre, Bacterial Diseases, Groeselenbergstr. 99, Brussels B-1180, Belgium; email: piwat@var.fgov.be

Coxiella burnetii Infection in Roe Deer during Q Fever Epidemic, the Netherlands

To the Editor: A Q fever epidemic among humans started in the Netherlands in 2007 and peaked in 2009 (*1*). Epidemiologic evidence linked the epidemic to abortions and deliveries among *Coxiella burnetii*–infected dairy goats and dairy sheep (*1*,*2*). However, questions arose about whether *C. burnetii* infection in free-living wildlife might be another source of Q fever in humans. *C. burnetii* has a wide host range (*3*), but to our knowledge no studies had addressed its occurrence in nondomestic animals in the Netherlands (*4*).

The main objective of this study was to look for evidence of *C. burnetii* infection in carcasses of free-living roe deer (*Capreolus capreolus*) in the Netherlands, where *C. capreolus* is the most common species of wild ruminant. Additional objectives were to 1) analyze characteristics, location, and time of death of case-animals for

more information on the infection in roe deer and 2) determine the genotype of *C. burnetii* strains from roe deer and compare them with the genotype of strains from domestic animals and humans for evidence of spillover.

The sample consisted of 79 roe deer that were euthanized or found dead in 9 of the 12 provinces in the Netherlands during January 2008–May 2010. All animals had undergone postmortem examination, and tissue samples were frozen until testing. Tissues tested were lung (n = 46), spleen (n = 50), bone marrow (n = 50), liver (n = 74), and kidney (n = 75), as available. We extracted DNA by using the DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany). A duplex quantitative PCR targeting the IS*1111a* element was used with an internal control gene, as described (*2*). Tissues with cycle threshold (C_t) values <34 (1/case) were typed by using multilocus variable-number tandem-repeat analyses (MLVA) for 11 loci, as described (*2*,*5*); results were compared with known MLVA typing data from the Netherlands.

Of the 79 roe deer examined, 18 (23%) had positive PCR results for *C. burnetii* DNA in multiple (5/18, 28%) or single (13/18, 72%) tissues. The average C_t value was 36.30 (range 32.07–39.47). Among 29 roe deer for which all 5 tissues were tested, no single tissue was more frequently positive than others for *C. burnetii* (χ^2 = 1.07, df = 4, p = 0.9) or had lower C_t values (single factor analysis of variance, p = 0.58). These findings indicate that testing multiple tissues per individual enhances case detection.

No specific sex, age, or health effects were observed. Of 48 male deer, 10 (21%) had positive results, compared with 8 (27%) of 30 female deer (1 missing value; χ^2 = 0.35, df = 1, p = 0.55). Of 50 deer ≥1 year of age, 15 (30%) had positive results, compared with 2 (15%) of 13 deer <1 year of age (16 missing values; 2-tailed Fisher exact test, p = 0.49). Postmortem findings varied for *C. burnetii*–positive deer.

C. burnetii cases occurred in most provinces studied (6/9, 66%) and in all 3 study years. Significantly more *C. burnetii*–positive deer were observed in 2010 (13/30, 43%) than in 2008 (2/18, 11%) and 2009 (3/31, 10%) (χ^2 = 11.62, df = 2, p < 0.01). This finding might represent sample bias or indicate spatial or temporal clustering in 2010.

The *C. burnetii* genetic material found in roe deer may indicate past or ongoing infection (*6*). Although positive cases occurred in all seasons, those more likely to represent ongoing infection (multiple infected tissues and C_t values <36; n = 4) occurred in March, April, and June. Clinical Q fever in roe deer might occur more frequently in late gestation and around parturition, as in domestic ruminants (*7*,*8*). Furthermore, Q fever in wildlife might have its own sylvatic cycle (*4*,*9*). However, analogous to human cases in 2007–2010 (*1*), the pattern could also include spillover events from domestic livestock.

Tissues of 2 springtime case-animals had C_t values <34. MVLA typing of these strains yielded partial genotypes (Figure). Comparison with those of strains from domestic dairy animals or humans during 2007–2010 showed that these 2 strains from roe deer differed from the main goat- and sheep-derived strain involved in the Q fever epidemic (genotype CbNL01 [*2*]) and from other strains found (inconclusive for CbN108; Figure).

Our study confirmed that *C. burnetii* infection occurs in free-living roe deer in the Netherlands. *C. burnetii* DNA was detected in roe deer of both sexes and age groups with no particular health effect, and it was detected in animals in different provinces and in all years studied; the highest *C. burnetii* DNA loads occurred in spring and early summer. Detection of genetic material by PCR does not always imply viable infective bacteria (*6*). However, because the infectious dose of *C. burnetii* is

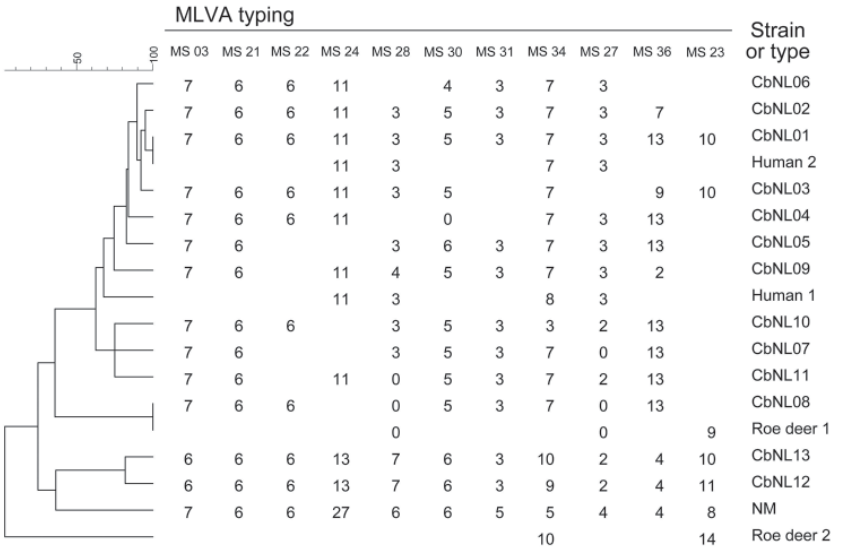

Figure. Phylogenetic tree with genotypes of *Coxiella burnetii* from goat, human, and roe deer samples from the Netherlands. Genotypes were determined on the basis of 11 multilocus variable-number tandem-repeat analyses (MLVA). The number of repeats per locus is shown; open spots indicate missing values. Roe deer 1 was an adult female found dead on March 30, 2010, in Friesland Province. Roe deer 2 was a young female deer involved in a traffic accident on April 6, 2010, in Utrecht Province. The goat and human samples have been described (*2*). Scale bar indicates genetic relatedness. Human 1, QKP 1; Human 2, QKP 2; NM, Nine Mile reference strain; MS, MiniSatellite.

low (*10*), our findings support the use of preventive hygiene measures (*4*) to minimize zoonotic risk when handling roe deer. The 2 MLVA-typed strains provided no evidence for spillover of the predominant strain involved in the Q fever epidemic in the Netherlands. More studies are required to adequately understand Q fever cycles in wildlife and their relationship with Q fever in domestic animals and humans.

Acknowledgments

We thank Natashja Beusekom-Buijs, Zorica Zivkovic, Ruby Wagensveld-van den Dikkenberg, Louis van den Boom, and Rob Buijs for logistical support and Albert de Boer for making the phylogenetic tree.

This study received financial support from the Dutch Ministry of Economic Affairs, Agriculture and Innovation; the Dutch Ministry of Health, Welfare and Sport; and the Faculty of Veterinary Medicine, Utrecht University.

Jolianne M. Rijks, Hendrik I.J. Roost, Peter W. van Tulden, Marja J.L. Kik, Jooske IJzer, and Andrea Gröne

Author affiliations: Dutch Wildlife Health Centre, Utrecht, the Netherlands (J. M. Rijks, M.J.L. Kik, J. IJzer, A. Gröne); and Central Veterinary Institute, part of Wageningen University and Research Center, Lelystad, the Netherlands (H.I.J. Roost, P.W. van Tulden).

DOI: http://dx.doi.org/10.3201/eid1712.110580

References

1. Roest HIJ, Tilburg JJHC, van der Hoek W, Vellema P, van Zijderveld FG, Klaassen CHW, et al. The Q fever epidemic in the Netherlands: history, onset, response and reflection. Epidemiol Infect. 2011;139:1–12. doi:10.1017/S0950268810002268
2. Roest HIJ, Ruuls RC, Tilburg JJHC, Nabuurs-Franssen MH, Klaassen CHW, Vellema P, et al. Molecular epidemiology of *Coxiella burnetii* from ruminants in Q fever outbreak, the Netherlands. Emerg Infect Dis. 2011;17:668–75.
3. Astobiza I, Barral M, Ruiz-Fons F, Barandika JF, Gerrikagoitia X, Hurtado A, et al. Molecular investigation of the occurrence of *Coxiella burnetii* in wildlife and ticks in an endemic area. Vet Microbiol. 2011;147:190–4. doi:10.1016/j.vetmic.2010.05.046
4. van Rotterdam B, Langelaar M, van der Giessen J, Roest HJ, Gröne A. Q fever in wild animals in Europe, attention to hunters [in Dutch]. Tijdschr Diergeneeskd. 2010;135:420–2.
5. 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</jrn>
6. Sukocheva OA, Marmion BP, Storm PA, Lockhart M, Turra M, Graves S. Long-term persistence after acute Q fever of non-infective *Coxiella burnetii* cell components, including antigens. QJM. 2010;103:847–63.
7. Aitken RJ. Delayed implantation in roe deer (*Capreolus capreolus*). J Reprod Fertil. 1974;39:225–33. doi:10.1530/jrf.0.0390225
8. Woldehiwet Z. Q fever (coxiellosis): epidemiology and pathogenesis. Res Vet Sci. 2004;77:93–100. doi:10.1016/j.rvsc.2003.09.001
9. Angelakis E, Raoult D. Q fever. Vet Microbiol. 2010;140:297–309. doi:10.1016/j.vetmic.2009.07.016
10. Jones RM, Hertwig S, Pitman J, Vipond R, Aspán A, Bölske G, et al. Interlaboratory comparison of real-time polymerase chain reaction methods to detect *Coxiella burnetii*, the causative agent of Q fever. J Vet Diagn Invest. 2011;23:108–11. doi:10.1177/104063871102300118

Address for correspondence: Jolianne M. Rijks, Dutch Wildlife Health Centre, Yalelaan 1, 3584 CL, Utrecht, the Netherlands; email: j.m.rijks@uu.nl

Ranavirosis in Invasive Bullfrogs, Belgium

To the Editor: Massive global declines in amphibians have been attributed to various causes, including infectious diseases such as chytridiomycosis and ranavirosis. Chytridiomycosis and ranaviral disease are international notifiable diseases because they have been listed by the World Organisation for Animal Health in its Animal Health Code.

Ranavirosis is caused by icosahedral cytoplasmic DNA viruses that belong to the family *Iridoviridae*, in particular by 4 species of *Ranavirus*: Frog Virus 3 (FV3), Bohle iridovirus, *Ambystoma tigrinum* virus, and a possible species *Rana catesbeiana* virus Z. In Europe, FV3 has been identified in several outbreaks of ranavirosis, characterized by mass deaths, notably in green frogs (*Pelophylax* sp.) in Denmark, Croatia, and the Netherlands (*1*,*2*); *Rana temporaria* and *Bufo bufo* in the United Kingdom (*3*,*4*); and *Alytes obstetricans* and *Ichthyosaura alpestris* in Spain (*5*). The invasive exotic bullfrog (*Lithobates catesbeianus*) has been introduced in several European countries and has established large breeding populations in France, Italy, Germany, Greece, and Belgium (*6*).

In addition to their direct effect on native amphibians through competition and predation, bullfrogs are thought to be carriers of chytridiomycosis (*7*,*8*) and, possibly, ranaviruses. Although mass deaths of *L. catesbeianus* tadpoles has been reported in aquaculture facilities, *L. catesbeianus* tadpoles are generally considered a subclinical reservoir of ranaviruses in the United States (*9*).

To assess the role of bullfrogs as carriers of ranaviruses in Europe, we collected 400 clinically healthy tadpoles of *L. catesbeianus* from 3 invasive bullfrog populations at Hoogstraten, Belgium (51°47′N,