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Epidemic Genotype of *Coxiella burnetii* among Goats, Sheep, and Humans in the Netherlands

To the Editor: The 2007–2010 Q fever epidemic among humans in the Netherlands was among the largest reported in magnitude and duration (*1*). The increase in human Q fever cases coincided with an increase in spontaneous abortions among dairy goats in the southeastern part of the Netherlands, an area that is densely populated with goat farms (*1*). Genotypic analyses of the involved isolates could confirm the possible link between the human and animal Q fever cases.

In previous studies, genotypic investigations of human and animal samples in the Netherlands were performed by using a 3-locus multilocus variable-number tandem repeats analysis (MLVA) panel and single-nucleotide polymorphism genotyping, respectively (*2,3*). The first study, performed on relatively few samples from a minor part of the affected area, showed that farm animals and humans in the Netherlands were infected by different but apparently closely related genotypes. More recently, genotyping by using a 10-locus MLVA panel provided additional information about the genotypic diversity of *Coxiella burnetii* among ruminants in the Netherlands: 1 dominant MLVA genotype was identified among goats and sheep throughout the entire affected Q fever area (*4*). A different panel of MLVA markers was applied to human samples (*5*). Four markers that are shared by both panels showed identical alleles in human and animal samples, again implicating goats and sheep as possible sources of the outbreak.

MLVA, which is based on relatively unstable repetitive DNA elements, is sometimes criticized for producing results that are too discriminatory or difficult to reproduce in different settings (*6*). Because of their instability, use of tandem repeats as genotyping targets can lead to problems with data interpretation and to overestimation of genotypic diversity by showing small variations in MLVA genotypes in isolates of otherwise identical background.

We used a more stable, sequence-based typing method, multispacer sequence typing (MST), on samples from humans and a group of ruminant animals (goats, sheep, and cattle) to establish a firmer correlation between Q fever cases in humans and animals (*7*). We identified MST genotypes using a Web-based MST database (http://ifr48.timone.univ-mrs.fr/MST_Coxiella/mst) containing genotypes from several countries in Europe. Ultimately, this study could answer the question of whether the current outbreak situation could have been caused by a specific *C. burnetii* strain in the ruminant population in the Netherlands.

Real-time PCR-positive specimens from 10 humans and 9 Q fever–positive specimens from goats and sheep collected from various locations throughout the affected area were used (*8*). We also included Q fever-positive specimens from cattle to rule out cattle as a possible source of Q fever infection. Five samples of cow's milk and 1 bovine vaginal swab sample were analyzed (online Appendix Table, wwwnc.cdc.gov/EID/article/18/5/11-1907-TA1.htm). MST33 was identified in 9 of 10 tested human samples and in the remaining 8 of 9 clinical samples from goats and sheep (online Appendix Table). MST33 has been isolated incidentally in nonoutbreak situations in human clinical samples obtained in France during 1996, 1998, and 1999 and from a placenta of an asymptomatic ewe in Germany during 1992. All samples from cattle in the Netherlands, 1 goat, and cow's milk contained genotype MST20. Genotype MST20 has also been identified in human clinical samples from France, in a cow's placenta from Germany isolated in 1992 and in rodents from the United States isolated in 1958. In 1 human bronchoalveolar lavage sample, a novel (partial) MST genotype was found. This may be an incidental Q fever case unrelated to the outbreak situation. Because no historical genotyping data for the period before the outbreak of Q fever in the Netherlands are available, this explanation needs further research.

MST genotyping shows the presence of genotype MST33 in clinical samples from humans, goats and sheep. These results confirm that goats and sheep are the source of human Q fever in the Netherlands. Few worldwide genotyping studies have been conducted, and therefore information about a possible global persistence of this genotype is lacking. This study also indicates that the outbreak among humans is not linked to *C. burnetii* in cattle, although the infection is widespread among dairy herds in the Netherlands (*10*), exemplifying that most outbreaks are related to goats and sheep rather than to cattle. In conclusion, the increase in the number of Q fever cases in the Netherlands among humans most likely results from MST33 in the goat population in the Netherlands and could have been facilitated by intensive goat farming in the affected area and its proximity to the human population.

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High Anti–Phenolic Glycolipid-I IgM Titers and Hidden Leprosy Cases, Amazon Region

To the Editor: Leprosy remains a serious public health issue. Although the World Health Organization elimination target was achieved in 2000, with a prevalence of <1 case/10,000 persons, despite progress since introduction of multidrug therapy (*1*), large pockets of poverty remain in which the disease is hyperendemic and underdiagnosed. In fact, in highly disease-endemic areas, the prevalence of previously undiagnosed leprosy cases in the general population has been reported to be 6× higher than the registered prevalence (*2*).

Most leprosy patients are in India and Brazil. In Brazil, new cases are concentrated in the Northeast, Midwest, and Amazon regions (from state capitals to the inner counties). Access to the health system is poor in these regions because of severe inequalities in the public health system of Brazil (*3*),

A total of 34,894 new cases were registered in Brazil during 2010 (*4*), corresponding to an incidence rate of 18.22 cases per 100,000 population. Pará State accounted for 10.2% of cases (3,562 cases), an incidence rate of 46.93 per 100,000 population. When only children <15 years of age were considered, Pará registered 389 new cases of leprosy in 2010, representing 10.9% of all cases, an incidence rate of 16.52 per 100,000 population. In Oriximiná, a county with 62,794 inhabitants in northwestern Pará, ≈800 km from Belém, Pará's capital, a mean of 13.8 cases per year were registered for the past 5 years.

In 2010, in Oriximiná, we collected plasma samples from 138 students 8–18 years of age, from 35 leprosy patients who received a diagnosis during 2004–2009, and from 126 contacts of these patients (Federal University of Pará Research Ethics Committee protocol no. 197/07). We tested all of these samples for anti–phenolic glycolipid-I (PGL-I) IgM; 42% of students, 54.3% of case-patients, and 45% of case-patient contacts were seropositive. In addition to collecting samples, we clinically examined the leprosy patients and their contacts, among whom we identified 3 new leprosy cases. We did not examine students at that time. Contacts were persons from the same household or neighborhood whom the index case-patient described as a person with whom he or she had a close relationship. Leprosy cases were diagnosed in the field on the basis of clinical signs, loss of sensation on the skin lesions, and presence of enlarged nerves. For operational reasons, skin smears were not performed. All cases were diagnosed by 2 leprologists. We used the Ridley-Jopling classification, associated with the indeterminate clinical type, as defined by the Madrid classification. The ELISA cutoff for positive results was arbitrarily established as an optical density of 0.295 based on the average plus 3× the SD of the test results from 14 healthy persons from the Amazon region (*5*).

Because studies of the seroprevalence among contacts have reported a proportion of seropositive persons ranging from ≈1.9% to 18.4% (*6*), we returned to Oriximiná 16 months after the first visit. We examined 2 groups of students and their contacts; 1 group was positive for anti–PGL-I, and the other group was negative for anti–PGL-I. We visited 44 households in 1 week. From the 35 leprosy patients encountered during the first visit, we selected 25 households to survey (14 with an anti–PGL-I–positive contact in the household and 11 without), and among students with results of anti–PGL-I serology, we selected 19 households (11 positive with an anti–PGL-I–positive contact in the household and 8 without). During our visits to all of these households, we examined 222 persons (Table).

When we arrived in Oriximiná, only 2 cases had been registered in the national notifiable diseases information system. By using our approach, 23 new cases were found after we investigated households that had a person positive for anti–PGL-I (15 multibacillary, 8 paucibacillary); we found only 7 new cases in households where residents were negative for anti–PGL-I (4 multibacillary, 3 paucibacillary) (Table). For comparison, during the last traditional leprosy campaign in Oriximiná in 2008, eight new cases were detected. Furthermore, by using our strategy, the local public health service detected 9 additional new cases during the 4 months after our departure from Oriximiná.

These data emphasize that contact examination is crucial for