of these singletons showed no correlation between clusters and geographic origin of samples.

Of the 191 samples, a total of 66 (34.6%) were from Marseille and represented 40 different genotypes. This finding underscores the broad heterogeneity in T. whipplei. Twelve (18.5%) of the 66 tested samples were genotype 3, which might be linked to the local outbreak among homeless persons mentioned above. Genotype 1, which is endemic to France, was found in only 2 (3.1%) persons in Marseille. The fact that Marseille is a metropolitan area with a high migration rate could play a role in the vast diversity of T. whipplei genotypes found there.

Questions regarding the epidemiologic character of Whipple disease remain unanswered, such as why the bacterium is highly prevalent but the disease is not. Persons with the putative immunologic defect probably responsible for classic Whipple disease (J) have the highest bacterial load in their stools. But these persons are unlikely to come into direct contact with one another. Thus, propagation of this bacterium on a large scale might be relatively limited, which could explain the high genetic diversity in the bacterial specimens assessed so far.

Two predominant genotypes seem to break out of this pattern: genotypes 1 and 3. Genotype 3 could be considered a genotype that causes small epidemics, whereas genotype 1 could be considered a genotype endemic to central Europe. Reasons for the success of these 2 genotypes remain unknown, but improvement of genotyping methods could provide the answers.

Author affiliations: Université Aix-Marseille Marseilles, France (N. Wetzstein, F. Fenollar, S. Buffet, D. Raoult); and Charité Universitätsmedizin Berlin, Berlin, Germany (N. Wetzstein, V. Moos, T. Schneider)

DOI: http://dx.doi.org/10.3201/eid1902.120709

References
1. Moos V, Schneider T. Changing paradigms in Whipple's disease and infection with Tropheryma whipplei. Eur J Clin Microbiol Infect Dis. 2011;30:1151–8. http://dx.doi.org/10.1007/s10096-011-1209-y
2. Fenollar F, Keita AK, Buffet S, Raoult D. Intrafamilial circulation of Tropheryma whipplei, France. Emerg Infect Dis. 2012;18:949–55. http://dx.doi.org/10.3201/eid1806.111038
3. Raoult D, Fenollar F, Rolain JM, Minodier P, Bosdure E, Li W, et al. Tropheryma whipplei in children with gastroenteritis. Emerg Infect Dis. 2010;16:776–82. http://dx.doi.org/10.3201/eid1605.091801
4. Fenollar F, Mediannikov O, Socolovschi C, Bassene H, Diatta G, Richet H, et al. Tropheryma whipplei bacteremia during fever in rural West Africa. Clin Infect Dis. 2010;51:515–21. http://dx.doi.org/10.1086/655677
5. Fenollar F, Ponge T, La SB, Lagier JC, Lefebvre M, Raoult D. First isolation of Tropheryma whipplei from bronchoalveolar fluid and clinical implications. J Infect. 2012;65:275–8. http://dx.doi.org/10.1016/j.jinf.2011.11.026
6. Keita AK, Brouqui P, Badiaga S, Benkouiten S, Ratmanov P, Raoult D, et al. Tropheryma whipplei prevalence strongly suggests human transmission in homeless shelters. Int J Infect Dis. Epub 2012 Jul 11. http://dx.doi.org/10.1016/j.ijid.2012.05.1033
7. Raoult D, Ogata H, Audic S, Robert C, Suhre K, Drancourt M, et al. Tropheryma whipplei Twist: a human pathogenic Actinobacteria with a reduced genome.

Yersinia pestis Plasminogen Activator Gene Homolog in Rat Tissues

To the Editor: Yersinia pestis causes plague, which primarily affects rodents, but is an invasive and virulent pathogen among humans. Y. pestis infection is endemic in small rodent populations in different parts of the world, and the bacterium is considered a potential bioweapon because it can be easily isolated, produced, dried, and dispersed as an aerosol. Antimicrobial drug treatment can be lifesaving during the early stages of illness; hence, rapid and sensitive methods for Y. pestis detection in environmental and clinical samples are required. Multiple PCR assays for Y. pestis detection that primarily detect markers located on plasmids have been developed (1–6). The plasminogen activator/coagulase (pla) gene, located on plasmid pPCP1, is incorporated into most Y. pestis PCR assays, and in several studies it was the prime or sole marker (1,2,5,7–9). Reasons for including pla in these assays are its occurrence in multiple copies, its absence from closely related Yersinia species, and its role in Y. pestis virulence (1,4,5).

Y. pestis

Plasminogen Activator Gene

Homolog in Rat Tissues

To the Editor: Yersinia pestis causes plague, which primarily affects rodents, but is an invasive and virulent pathogen among humans. Y. pestis infection is endemic in small rodent populations in different parts of the world, and the bacterium is considered a potential bioweapon because it can be easily isolated, produced, dried, and dispersed as an aerosol. Antimicrobial drug treatment can be lifesaving during the early stages of illness; hence, rapid and sensitive methods for Y. pestis detection in environmental and clinical samples are required. Multiple PCR assays for Y. pestis detection that primarily detect markers located on plasmids have been developed (1–6). The plasminogen activator/coagulase (pla) gene, located on plasmid pPCP1, is incorporated into most Y. pestis PCR assays, and in several studies it was the prime or sole marker (1,2,5,7–9). Reasons for including pla in these assays are its occurrence in multiple copies, its absence from closely related Yersinia species, and its role in Y. pestis virulence (1,4,5).
While validating the specificity of a multiplex qPCR assay for the detection of *Y. pestis* (6), we obtained DNA from the dissected peritoneum of a black laboratory rat (*Rattus rattus*), which tested positive for the *pla* gene. Two other *Y. pestis* signature sequences were not amplified. Additional samples were analyzed from black (*n = 11*) and brown (*Rattus norvegicus* [*n = 4*]) rats that had been caught on poultry and pig farms in the southeastern region of the Netherlands during 2008. Positive indicators for *pla* were found in samples from 8 of these black rats and in samples from 2 of the brown rats. Samples from 2 laboratory rats tested negative for *pla*. Inferences of the occurrence of *pla*-positive rats cannot be made because of low sample numbers and potential bias in capturing rats that had putative infections.

To exclude the possibility of contamination of host DNA with DNA from intestinal flora during isolation of the peritoneum, we examined the occurrence of *pla* in other tissues. Lung and liver samples were available from all 17 rats, and leg tissue samples were available from 7 rats, 5 of which had positive peritoneal tissue test results. The leg and lung tissues of 1 rat and the leg tissue of another rat tested positive, albeit at considerably lower quantities (higher quantification cycles) than *pla* values measured in peritoneal samples. These results did not support the likelihood of contamination during sampling or the occurrence of local infections; they did support the hypothesis of a systemic infection in the rats. To investigate whether the presence of the *pla* gene sequences indicated the presence of the carrier pPCP1 plasmid of *Y. pestis*, we designed PCR assays for the amplification of 3 conserved regions of this plasmid (online Technical Appendix, wwwnc.cdc.gov/EID/content1/12-0659-TechApp.pdf). Each assay produced PCR products from *Y. pestis*; only the transposase gene was amplified from *Y. pseudotuberculosis*. None of the PCR assays amplified DNA from samples collected from rats.

*Pla* genes obtained from 2 of the peritoneum samples collected from black rats were sequenced and appeared to be identical (GenBank accession no. HQ606074). Alignment with *Y. pestis pla* genes, which are highly conserved among *Y. pestis* isolates, revealed 11 nt differences in 880 bp (98.8% similarity). A BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) search retrieved such highly similar genes only from *Y. pestis* sequences; the next most similar sequences of other *Enterobacteriaceae* were at 78% similarity.

The general walking PCR procedure was used to explore sequences adjacent to the *pla* gene (online Technical Appendix). One PCR product was sequenced and appeared to be in part homologous to the *pla* gene, but the adjacent sequence displayed high homology to genes coding for replisome (rep) proteins in several bacterial genera in the family *Enterobacteriaceae*, e.g., *Escherichia*, *Shigella*, and *Salmonella*. The existence of a concatenated *pla*-rep sequence in rat tissue samples was confirmed by amplification of a PCR product from a primer targeting the *pla* gene, combined with a primer targeting the rep gene sequence that was acquired by using the general walking procedure. The resulting 223-bp PCR product (GenBank accession no. JQ756394) consisted of a 141-bp sequence identical to the *Y. pestis pla* gene, linked to a 72-bp sequence that was 97% similar to entero bacterial rep protein genes. Attempts to obtain more sequence information from rep sequences by using primers derived from conserved domains in entero bacterial rep genes were unsuccessful. This suggests that the *pla*-rep sequence is derived from uncharacterized bacteria. Rep proteins function as replication activators of their carrier plasmids.

The *rep* sequences identified in this study were most similar to those of plasmids involved in bacteriocin activity, a function that is also ascribed to the bacteriocin pesticin gene clusters of *Y. pestis* pPCP1 plasmids. The occurrence in unknown organisms that have *pla* genes that are similar to *Y. pestis pla* genes has consequences for the detection of *Y. pestis*. To prevent false positive results, detection protocols should include at least 1 supplemental target to confirm the presence of *Y. pestis* (6). In addition, investigators using *pla* gene analysis, for instance, while reconstructing ancient plague epidemics (10), should be aware of the occurrence of these homologs.

Ingmar Janse, Raditijo A. Hamidjaja, and Chantal Reusken
Author affiliation: National Institute for Public Health and the Environment, Bilthoven, the Netherlands
DOI: http://dx.doi.org/10.3201/eid1902.120659

References

1. Loïez C, Herwegh S, Wallet F, Armand S, Guinet F, Courcel RJ. Detection of *Yersinia pestis* in sputum by real-time PCR. J Clin Microbiol. 2003;41:4873–5. http://dx.doi.org/10.1128/JCM.41.10.4873-4875.2003
2. Skottman T, Piparin H, Hyytiäinen H, Myllysv SK, Skurnik M, Nikkari S. Simultaneous real-time PCR detection of *Bacillus anthracis*, *Francisella tularensis* and *Yersinia pestis*. Eur J Clin Microbiol Infect Dis. 2007;26:207–11. http://dx.doi.org/10.1007/s10096-007-0262-z
3. Stewart A, Satterfield B, Cohen M, O’Neill K, Robison R. A quadruplex real-time PCR assay for the detection of *Yersinia pestis* and its plasmids. J Med Microbiol. 2008;57:324–31. http://dx.doi.org/10.1099/jmm.0.47485-0
4. Tomaso H, Reisinger EC, Al Dahouk S, Frangoulidis D, Rakin A, Landt O, et al. Rapid detection of *Yersinia pestis* with multiplex real-time PCR assays using fluorescent hybridisation probes. FEMS Immunol Med Microbiol. 2003;38:117–26. http://dx.doi.org/10.1016/S0928-8244(03)00184-6
5. Higgins JA, Ezzell J, Hinnebusch BJ, Shipley M, Henchal EA, Ibrahim MS. 5′ nuclease PCR assay to detect *Yersinia pestis*. J Clin Microbiol. 1998;36:2284–8.
Coxiella burnetii in Ticks, Argentina

To the Editor: The Gamma-proteobacterium Coxiella burnetii is the causative agent of acute Q fever and chronic endocarditis in humans worldwide. It is transmitted primarily by aerosol route or by ingestion of fomites from infected animals, mostly from domestic ruminants (1). Although >40 tick species can be infected with C. burnetii, direct transmission of this agent to humans from infected ticks has never been properly documented. However, ticks may play a critical role in the transmission of C. burnetii among wild vertebrates (1). Only a few studies, mostly related to human clinical cases or seroepidemiologic evaluation of healthy animals, have reported C. burnetii in South America (2–4). However, to our knowledge, C. burnetii has never been reported in ticks in the continent.

During ecologic studies on Amblyomma parvum and A. tigrinum ticks in the Córdoba Province of Argentina, engorged nymphs were collected from the common yellow toothed cavy (the rodent Galea musteloides) (5,6). In the laboratory, engorged nymphs molted to adults (92 A. tigrinum, 13 A. parvum), which were individually submitted to the hemolymph test with Gimenez staining for detection of rickettsiae-like organisms (7). By the hemolymph test, 1 A. tigrinum female, and 2 A. parvum male ticks were found to contain red-stained rickettsiae-like structures. These 3 ticks were processed individually by the shell vial technique, with the purpose of isolating intracellular bacteria in Vero cell culture (7). Inoculated cells were always incubated at 28°C. Intracellular bacteria were successfully isolated from all 3 ticks and established in Vero cell culture, as demonstrated by Gimenez staining of infected cells from at least 10 subsequent passages, which all infected 100% of the cells (Figure, panel A). Infected Vero cells contained multiple vacuoles (Figure, panel B) that enclosed a seething mass of microorganisms (online Video; wwwnc.cdc.gov/EID/article/19/02/12-0362-F1.htm), compatible with Coxiella organisms. Such vacuoles were not seen in uninfected control Vero cells incubated under the same conditions as those of infected cells (Figure, panel C).

For molecular analyses, DNA from the infected cells of each of the 3 isolates was extracted by boiling at 100°C for 10 min; it yielded products of the expected size through PCR protocols selective for portions of 3 genes of the genus Coxiella: primers QR-F (5′-ATTGAAGAGTTT GATTCTGG-3′) and QR-R (5′-CG GCCCTCCCAAGGGTAG-3′) for the 16S rRNA gene (8); primers CAI844F (5′-ATT TTAGTG GGT TTCCG C-3′) and CAI844R (5′-CAT CGCATACGTTTCGGGAA-3′) for the cap gene (9); and primers Cox-F-pry2 (5′-TTATTCTCCAAGGCTCCGAGCGC-3′) and Cox-R-pry2 (5′ TTATCCGCCAGCAATCTCAATTATGG-3′) for the pyrG gene (9). PCR products underwent DNA sequencing in an automatic sequencer (Applied Biosystems/Perkin Elmer, Foster City, CA, USA) according to the manufacturer’s protocol. We sequenced 1,386, 557, and 545 nt of the genes 16S rRNA, cap, and pyrG, respectively, which were identical to each other for each gene amplified from the 3 tick isolates. By BLAST analyses (www.ncbi.nlm.nih.gov/blast), these sequences were 99.9% (1,384/1,386 nt), 99.6% (556/558 nt), and 99.6% (452/454 nt) identical to the corresponding GenBank sequences of the North American C. burnetii genes 16S rRNA, cap, and pyrG, respectively (HM208383, CP001020, CP001020). Partial sequences (16S rRNA, cap, pyrG) from C. burnetii generated in this study were deposited into GenBank and assigned nucleotide accession nos. JQ740886–JQ740888, respectively.

Infected Vero cell monolayers were fixed in a modified Karnovsky solution, stained with uranyl acetate and lead citrate, and examined in a transmission electron microscope according to standard procedures. Ultrastructurally, Coxiella organisms were identified by morphologic features within heavily infected Vero cells. The organisms possessed typical bacillary morphologic characteristics and were observed inside vacuoles (phagolysosomes) of different sizes, proportional to the...