

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 immunological defect probably responsible for classic Whipple disease (*I*) 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.

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## ***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).

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 incidence 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 in *Y. pestis*, we designed PCR assays for the amplification of 3 conserved regions of this plasmid (online Technical Appendix, [wwwnc.cdc.gov/EID/article1/12-0659-TechApp.pdf](http://wwwnc.cdc.gov/EID/article1/12-0659-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 genewalking 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 replicon (*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 genewalking 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 enterobacterial *rep* protein genes. Attempts to obtain more sequence information from *rep* sequences by using primers derived from conserved domains in enterobacterial *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 bactericidal 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.

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## ***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](http://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-FO (5'-ATTGAAGAGTTT GATTCTGG-3') and QR-RO (5'-CG GCCTCCCGAAGGTTAG-3') for the 16S rRNA gene (8); primers CAPI844F (5'-ATTTAGTG GGGTTTCGCG CAT-3') and CAPI844R (5'-CAT CAGCATACGTTTCGGGAA-3') for the *cap* gene (9); and primers Cox-F-pry2 (5'-TTATTTACCAAGTTCC GAGCCG-3') and Cox-R-pry2 (5'-TTTATCCCGAGCAAATTCA ATTATGG-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](http://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

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## Technical Appendix

### Primers Used in *Yersinia pestis* Plasminogen Activator Gene Homolog in Rat Tissues

Technical Appendix Table 1. Primers for amplification and sequencing of the plasminogen activator gene\*

Forward primer	Sequence (5'→3')	Reverse primer	Sequence (5'→3')	Position†
<i>plasq</i> -f	ATGAAAATCAATCTGAGTGGACAGATCAC	<i>plasq</i> -r	CAGAAGCGATATTGCAGAC	6990–7602
<i>plasq2</i> -f	TAACTATTCTGTCCGGGAGT	<i>plasq2</i> -r	ATTATCATGTGCCCGAAC	6696–7339

\**pla*, plasminogen activator/coagulase.

†Position in entry AF053945 from *Y. pestis* strain KIM5.

Technical Appendix Table 2. Primers for amplification and detection of conserved regions on plasmid pPCP1\*

Forward primer	Sequence (5'→3')	Reverse primer	Sequence (5'→3')	Position†	Present in
pPratTr21-f	TCTCGGTTCCCTCAGGAG	pPratTr21-r	TGCACGTCATACTCTTTTTTCT	397–1021	Ype + Yps
pPratPst-f	GAGATGGAGAAAGACAGTGA	pPratPst-r	ATTTTAACAATCCACTATCGATA	4817–5699	Ype
pPratR1-f	AGGCCATGAACGACT	pPratR1-r	GATGGGAAATACAACTACGAAAATTA	2100–2826	Ype

\*Ype, *Yersinia pestis*; Yps, *Yersinia pseudotuberculosis*.

†Position in entry AF053945 from *Y. pestis* strain KIM5.

Technical Appendix Table 3. Primers for genewalking

Primer	Sequence (5'→3')	Function
usPuTSP1	ATTTGGTATTAAGTGGATGAT	Upstream <i>Pla</i> TSP1 primer
us <i>Pla</i> TSP2	CTGCATTAGCACTCCCGGA	Upstream <i>Pla</i> TSP2 primer
us <i>Pla</i> TSP3	CCCGACAGAATAGTTATAATGG	Upstream <i>Pla</i> TSP3 primer
ds <i>Pla</i> TSP1	CCATTGATAAGAATAGTGGAGA	Downstream <i>Pla</i> TSP1 primer
ds <i>Pla</i> TSP2	TCTGTCTCTATTGGCGGAGA	Downstream <i>Pla</i> TSP2 primer
ds <i>Pla</i> TSP3	GCTGCCGGTATTTCCAATAA	Downstream <i>Pla</i> TSP3 primer

\*Following the instructions of the DNA Walking SpeedUp Premix Kit (Seegene Inc.), gene-specific nested primers targeting the flanking regions of the *pla* gene were designed. These were used in combination with supplied primers for 3 consecutive rounds of PCR amplification. *Pla*, plasminogen activator/coagulase.

Technical Appendix Table 4. Primers for amplification of the concatenated *pla*-*rep* sequence\*

Forward primer	Sequence (5'→3')	Reverse primer	Sequence (5'→3')	PCR product
us <i>Pla</i> TSP1	ATTTGGTATTAAGTGGATGAT	<i>Pla</i> Rep1	GGTAAATTTTCGTCGAAGTAT	Used for sequencing <i>pla</i> - <i>rep</i>
us <i>Pla</i> TSP1	ATTTGGTATTAAGTGGATGAT	<i>Pla</i> Rep2	TCATCCGTGTGATAGGGAC	Not formed
us <i>Pla</i> TSP1	ATTTGGTATTAAGTGGATGAT	<i>Pla</i> Rep3	GTCGCTCCAGTCAATCG	Not formed
us <i>Pla</i> TSP1	ATTTGGTATTAAGTGGATGAT	<i>Pla</i> Rep4	GGCGCGTTCTCACGG	Not formed
us <i>Pla</i> TSP1	ATTTGGTATTAAGTGGATGAT	<i>Pla</i> Rep5	ATCGCCCTGTAAGCCCATT	Not formed
us <i>Pla</i> TSP1	ATTTGGTATTAAGTGGATGAT	<i>Pla</i> Rep6	TCYTGCCAATAGCCC	Not formed

\*Primers *Pla*Rep 2–6 were based on an alignment of conserved domains in enterobacterial *rep* genes. *Pla*, plasminogen activator/coagulase; rep, replicon.