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Looking for *Tropheryma whipplei* Source and Reservoir in Rural Senegal

Alpha Kabinet Keita, Oleg Mediannikov, Pavel Ratmanov, Georges Diatta, Hubert Bassene, Clémentine Roucher, Adama Tall, Cheikh Sokhna, Jean-François Trape, Didier Raoult, and Florence Fenollar*

Aix Marseille Université, Unité des Rickettsies, Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes (URMITE), UM63, CNRS 7278, IRD 198, INSERM 1095, 13005 Marseille, France; Campus Commun UCAD-IRD, Hann, Dakar, Sénégal; AP-HM, CHU Timone, Pôle Infectieux, 13005 Marseille, France; Unité d'Epidémiologie, BP 220, Institut Pasteur de Dakar, Dakar, Sénégal

Abstract. *Tropheryma whipplei*, the bacterium linked to Whipple's disease, is involved in acute infections and asymptomatic carriage. In rural Senegal, the prevalence of *T. whipplei* is generally high but is not homogeneous throughout households in the same village. We studied environmental samples collected in two Senegalese villages and conducted the survey to investigate the difference between households. Overall, the comparison between five households with very high *T. whipplei* prevalence and three households without any registered cases showed that the only difference was the presence of toilets in the latter (1/5 versus 3/3; $P = 0.01423$). Among the 1,002 environmental specimens (including domestic and synanthropic animals and dust sampled in households) tested for *T. whipplei* DNA, only four specimens were slightly positive. Humans are currently the predominant identified reservoir and source of *T. whipplei* in these populations. Limited access to toilets and exposure to human feces facilitate the fecal-oral transmission of *T. whipplei.*

INTRODUCTION

The natural history of *Tropheryma whipplei* continues to be clarified[1,2]; following contamination, patients develop acute infections including gastroenteritis, bacteremia, and pneumonia,[3–7] and they may develop specific antibodies. Depending on host factors, three methods of evolution are currently considered. The first corresponds to patients who can eradicate the bacterium and may harbor specific antibodies. The second includes people who carry the bacterium chronically for at least 5 years (Raoult D, unpublished data) while exhibiting strong immune responses. The third involves patients who suffer from sub-acute or chronic infections without developing sufficient antibody response. These infections include classic Whipple's disease, which is characterized by histological involvement of the small bowel, and localized infections without histological digestive involvement, such as endocarditis or encephalitis.[8]

The prevalence of *T. whipplei* carriage in stool and saliva specimens from healthy individuals depends predominantly on the geographic area, the occupation of the subjects, and their proximity to *T. whipplei* carriers.[9–12] In two Senegalese villages, the incidence of *T. whipplei* DNA in stool samples is ~17.4% among the healthy adult population, reaching 75% among healthy children younger than 5 years of age.[10]

Currently, two important, yet unsolved issues are the habitat of *T. whipplei* and its route of infectious transmission. In Europe, the first risk factor that has been shown is occupational: the rate of infection among underground sewer workers suggests the danger of exposure to human feces.[9,13] Another recent risk factor that has been identified is the living conditions of homeless people in shelters.[14] The poor hygiene and unsanitary conditions associated with shelters may explain the high prevalence of *T. whipplei* among homeless people. However, in Europe, the diseases linked to exposure to human feces (such as typhoid fever and shigellosis) are for the most part no longer a health problem.[15,16] In rural Senegal, the situation appears to be different, and the overall prevalence of these diseases is very high. However, there are families with very high prevalence of the diseases and others with very low or even negative prevalence.[10]

Our approach was to identify the factors associated with a high incidence of *T. whipplei* carriage in different households and to identify the possible environmental factors that play a role in the transmission of *T. whipplei*.

THE STUDY

Ethics statement and the populations of the two villages. We performed studies in Dielmo and Ndiop, which are two villages that are endemic for malaria in Senegal. Included in the Dielmo project was a study initiated in 1990 for the long-term investigation of host–parasite associations[10,17]; this cohort study was approved by the national ethics committee of Senegal, and written informed consent was obtained from all individuals.

Household study. On the basis of our previous studies,[3,10,18] we searched households in which *T. whipplei* had never been previously detected and households in which *T. whipplei* was highly prevalent. People were mainly *T. whipplei* carriers but there are also patients with *T. whipplei* bacteremia. An exhaustive on-site questionnaire was administered, including 1) the number of inhabitants of the household and their age and sex; 2) a description of the household (number and type of habitation, method of construction of walls, roof and floor [cement, straw, sheet metal, earth, or other materials]) and number of rooms; 3) household food (origin and type of food, storage of food, cooking location, the dishes used, consumption of raw food, manner of eating, either with cutlery or with hands); 4) the water (origin of the drinking water: well, open water source, other water sources), the number of canary systems to store the water, the number of glasses and type of glasses (metal, plastic, or other materials); 5) sanitary conditions (the presence, number, type, and equipment of showers or places to wash; the presence, number, type, and equipment of toilets); 6) hand washing (places to perform hand washing, whether hand washing is performed with or without soap, frequency of hand washing); 7) storage and disposal of waste; and 8) the environment (vegetation: presence, type, and number of animals). We have visited all of the households in the villages.

*Address correspondence to Florence Fenollar, Aix Marseille Université, Unité des Rickettsies, Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes (URMITE), UM63, CNRS 7278, IRD 198, INSERM 1095, Faculté de Médecine, 27 boulevard Jean Moulin, 13005 Marseille, France. E-mail: florence.fenollar@univ-amu.fr

Environmental study. We collected 795 environmental samples (Table 1) from both villages. Stool specimens from 118 domestic animals were collected (Figure 1), as were 677 arthropods. To predict the occurrence of *T. whipplei* in the household environment, 207 swab samples from the dust-accumulating surfaces of the main entrance, bed, and storehouse of each household were collected (Figure 1). The surfaces were swabbed with a moisturized (phosphate buffer saline) sterile cotton stick moistened with phosphate-buffered saline. After collection, the specimens were transferred at room temperature to Marseille (France).

Molecular analyses. The DNA was extracted from the arthropods, stool specimens, and suspensions from swabs using the BioRobot MDx Workstation (Qiagen, Courtaboeuf, France), following the manufacturer's instructions. The DNA was stored at 4°C until used. Quantitative real-time polymerase chain reaction (qPCR) was performed using a LightCycler instrument (Roche Diagnostics, Meylan, France), with the QuantiTect Probe PCR Kit, as described previously.[11] For *T. whipplei* detection, the specimen was first tested with the Twhi3F (5-TTG TGT ATT TGG TAT TAG ATG AAA CAG-3)/Twhi3R (5-CCC TAC AAT ATG AAA CAG CCT TTG-3) primer pair and the specific Twhi3 probe (6-FAM-GGG ATA GAG CAG GAG GTG TCT GTC TGG-TAMRA).[3] When a specimen was positive in this assay, the result was systematically confirmed by a second qPCR using the Twhi2F (5-TGA GGA TGT ATC TGT GTA TGG GAC A-3)/Twhi2R (5-TCC TGT TAC AAG CAG TAC AAA ACA AA-3) primer pair and the specific Twhi2 probe (6-FAM-GAG AGA TGG GGT GCA GGA CAG GG-TAMRA). We considered samples to be positive if both specific qPCRs were positive, i.e., the cycle number at the threshold level of log-based fluorescence (Ct) was lower than 40. To validate the test, we used positive and negative controls, as previously reported[9]; for positive specimens, genotyping was attempted, as previously described.[19]

TABLE 1

Data on the dust, domestic animals, and arthropod specimens tested for *Tropheryma whipplei* in Dielmo and Ndiop, Senegal

	Number of samples tested (number of positive)		
	Dielmo 13°43′N-16°24′W	Ndiop 13°41′N-16°22′W	Total analyzed (percentage of positive)
Dusts in household	93	114	207
Animal stool specimen	62 (2)	56 (1)	118 (2.5%)
Chicken	24 (1)	18 (1)	42 (4.8%)
Donkey	5	6	11
Goat	9 (1)	10	19 (5.2%)
Cattle	11	11	22
Duck	7	2	9
Domestic pigeon	4	2	6
Sheep	2	6	8
Dog	0	1	1
Ixodid species	61	256	317
Amblyomma variegatum	14	31	45
Hyalomma m. rufipes	2	43	45
Hyalomma truncatum	24	30	54
Rhipicephalus annulatus	1	2	3
Rhipicephalus ev. ev.	20	150	170
Fleas	194	45	239
Ctenocephalides felis	42	6	48
Echidnophaga gallinacean	144	6	150
Synosternus pallidus	8	33	41
Head lice (*Pediculus humanus*)	20	25	45
Mosquitoes	76	0	76
Anopheles gambiae	76	0	76
Total	506 (2)	496 (2)	1,002 (0.39%)

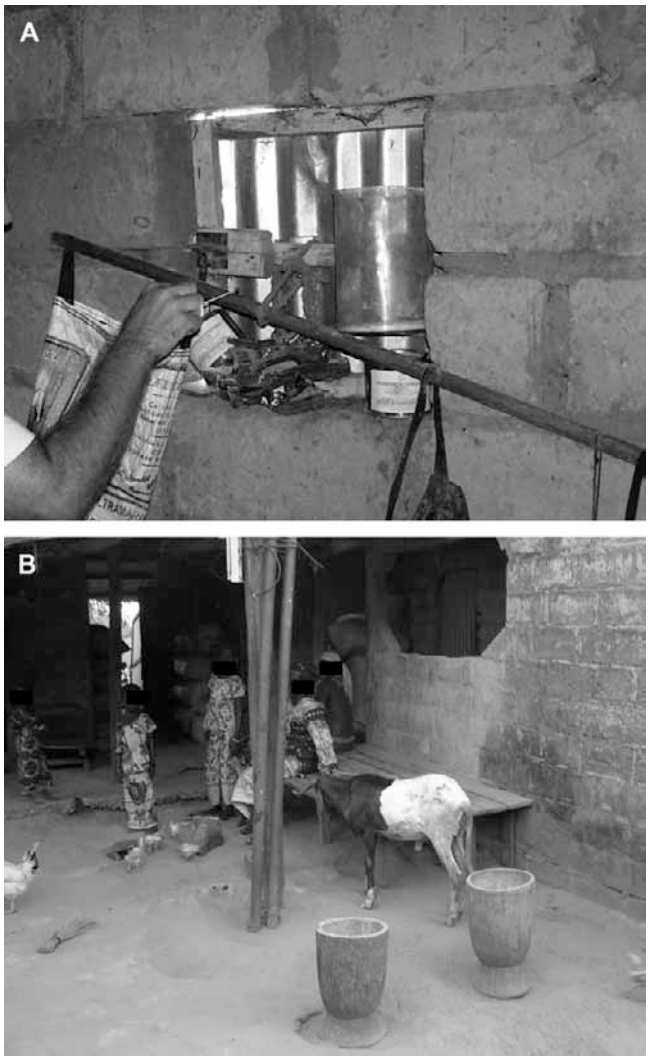

FIGURE 1. Households in Dielmo and Ndiop (Senegal). (**A**) Dust-accumulating surfaces in the storehouse of a household. (**B**) Close contact between the villagers and their domestic animals in households.

Statistical analysis. Statistical analyses were performed using the EpiInfo6 software (http://www.cdc.gov/epiinfo/Epi6/EI6dnjp.htm). The results were considered statistically significant when $P < 0.05$.

RESULTS

Household study. The first screening had identified in Dielmo two households without previous detection of *T. whipplei* (4 and 11) and two households in which both the prevalence of *T. whipplei* carriage and *T. whipplei* bacteremia were high (19 and 39) (Table 2). Only one significant difference ($P = 0.02275$) was observed between the four households. Households 4 and 11 had toilets (Figure 2), whereas households 19 and 39 did not have toilets. In household 4, the toilets were enclosed within a concrete wall, with a roof and door of sheet metal. In household 11, the toilets were surrounded by pieces of metal and wood, without a roof and with a fabric door.

TABLE 2

Prevalence of *Tropheryma whipplei* among inhabitants and the presence of toilets in their respective households

Households	Stool carriage Nb. Pos/Nb. tested (%)	Saliva carriage	Bacteremia	Sanitation/toilets
4	0/10 (0)	0/8 (0)	0/11 (0)	Closed toilet
11	0/3 (0)	0/6 (0)	0/13 (0)	Open toilet
19	2/9 (22.2)	0/10 (0)	6/32 (18.75)	No
39	5/6 (83.3)	0/1 (0)	4/21 (19)	No
29	0/3 (0)	0/6 (0)	0/3 (0)	Closed toilet
14	6/12 (50)	0/5 (0)	3/39 (7.69)	Open toilet
16	8/18 (44.4)	0/10 (0)	3/56 (5.35)	No
22	6/14 (42.85)	1/12 (8.33)	1/39 (2.56)	No

We have tried to enlarge the analyzed sample to confirm our first data. We identified another household (29) without previous *T. whipplei* detection and three other households (14, 16, and 22) in which *T. whipplei* carriage was high (Table 2). Households 29 and 14 had toilets, whereas there was not a toilet in household 16 or 22. Overall, the presence of toilets in households is significantly associated with the lack of *T. whipplei* among their inhabitants (3/3 versus 1/5, $P = 0.01423$). The toilets in household 29 were similar to those observed in household 4, and the toilets in household 14 were similar to those observed in household 11.

Environmental study. *Dust analysis.* In September 2011, 93 dust specimens from Dielmo and 114 from Ndiop were sampled. One specimen from a bed in household 1 in Ndiop presented bacterial DNA at very low concentrations (Ct for Twhi3 and Twhi2 of 36.68/36.82). No genotype was obtained because DNA loads were too low.[10,12]

Domestic animals and wildlife. In June 2011, stool specimens were collected from 42 chickens, 11 donkeys, 19 goats, 22 cattle, 9 ducks, 6 domestic pigeons, 8 sheep, and 9 dogs in Dielmo and Ndiop (Table 1). A chicken from Ndiop (belonging to household 10), a chicken from Dielmo (belonging to household 15), and a goat from Dielmo (belonging to household 11) were slightly positive for *T. whipplei* (Ct for Twi3 and Twhi2 of 35.89/34.83, 33.08/33.99 and 35.60/35.67, respectively). The positive specimens could not be genotyped because the DNA loads were too low.[10,12]

In November–December 2008, Ixodid ticks were collected from domestic animals (cows, goats, sheep, horses, and donkeys). Overall, 317 specimens, including 170 *Rhipicephalus evertsii evertsii*, 54 *Hyalomma truncatum*, 45 *Hyalomma marginatum rufipes*, 45 *Amblyomma variegatum*, and 3 *Rhipicephalus annulatus* were analyzed. Once a month during 2010, fleas were collected from human dwellings, dogs, and cats. A total of 239 fleas were analyzed, including 48 *Ctenocephalides felis*, 150 *Echidnophaga gallinacea* and 41 *Synosternus pallidus*. From October 2010 to January 2011, 110 head lice were collected from 45 healthy people and were analyzed. In January 2011, 76 *Anopheles gambiae* were sampled and analyzed. None of the arthropods were positive for *T. whipplei* (Table 1).

DISCUSSION

The high prevalence of *T. whipplei* among the population of these Senegalese villages may be explained by the presence of the bacterium in the environment.[10] However, *T. whipplei* has not previously been found in 105 water samples from sources in the villages, including canaries of households, wells of the villages, and open water sources from the area.[10] Entomological studies that have been previously conducted in these villages confirmed that Ixodid ticks, the vectors of spotted fevers, are highly prevalent in domestic animals and that close contact of humans with ticks is continual and permanent.[20,21] Other vectors of pathogenic bacteria, such as fleas and lice, are also highly prevalent.[22] We checked these arthropods for the presence of *T. whipplei* to verify the hypothesis of the role of the vectors in the epidemiology of *T. whipplei* infection. Finally,

FIGURE 2. Toilets in households in Dielmo and Ndiop (Senegal). (**A**) Household 4. A container with water and a cup are also present. (**B**) Household 11.

none of the tested arthropods were positive, showing that *T. whipplei* infection is not an arthropod-borne disease.

Villagers live in close contact with their animals, which live beside the households. *Tropheryma whipplei* was detected in very small amounts in the stools of two chickens and one goat. These data cannot support the hypothesis that domestic animals have a significant effect on the transmission of *T. whipplei*. We believe that these animals were transient carriers or accidental hosts of *T. whipplei* ingested with food contaminated by infected human feces.

Several bacteria, including *Coxiella burnetii* and *T. whipplei*, have been detected in dust[23]; the presence of *T. whipplei* DNA in the dust sample may be explained by the presence of small particles of human feces contaminating the dust in the village context. In addition to the effect of the dust inside the households, the village environment, where inhabitants spend much time, may be a direct source of infection. However, only one dust sample was slightly positive, excluding the role of dust as a reservoir of *T. whipplei*.

For several reasons, it has been speculated for a long time that *T. whipplei* has an environmental source: the presence of DNA of the bacterium in sewage samples[24]; the phylogenetic relatedness of *T. whipplei* to *Actinomycetes*, which are essentially environmental microorganisms, especially from soil but also from freshwater and seawater sediments[25]; and the high proportion of farmers among the first reported patients, which suggested exposure to soil as one possible route of infection.[25] The presence of *T. whipplei* in sewage plants may suggest a possible environmental reservoir of the bacterium, but may simply result from excretion of the bacterium from the stool specimens of patients and carriers.

We identified an endemic region in Senegal with a high incidence of *T. whipplei* in humans. We tried to perform an exhaustive collection of environmental samples from this region that may indicate a possible reservoir and/or the method of transmission. Previously published data,[10] and the results presented here, do not support the hypothesis of the presence of an environmental source of *T. whipplei* infection. Our environmental data cannot explain the high prevalence of *T. whipplei* infections and carriage in these Senegalese populations.

Several findings support the theory of inter-human transmission of *T. whipplei*,[14] including the existence of specific *T. whipplei* clones, not only among the population of Dielmo and Ndiop[10] but also among children during episodes of *T. whipplei* gastroenteritis,[4] among relatives of people positive for *T. whipplei*,[12] and among homeless people sleeping in the same shelter. Having analyzed the questionnaires, the only factor significantly associated with the circulation of *T. whipplei* within households is a lack of toilets in households. Thus, the relationship of the lack of toilet facilities to the detection of *T. whipplei* in households supports the theory of human-to-human transmission of the bacterium, with the inter-human transmission being from human feces through hand transmission.[14]

Tropheryma whipplei is known to be viable in human fecal and saliva samples, suggesting that the bacterium might be transmitted through both fecal-oral and oro-oral routes.[26,27] Depending on the living conditions of the subjects, either method of transmission may be prevalent.

The predominant reservoir of *T. whipplei* currently identified is found in humans. Humans comprise the only source of *T. whipplei* among these Senegalese populations in whom the bacterium is highly prevalent. Limited access to toilets and exposure to human feces may facilitate the fecal-oral transmission of *T. whipplei*.

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Authors' addresses: Alpha Kabinet Keita, Oleg Mediannikov, Pavel Ratmanov, Georges Diatta, Hubert Bassene, Clémentine Roucher, Cheikh Sokhna, Jean-François Trape, Didier Raoult, and Florence Fenollar, Aix Marseille Université, Unité des Rickettsies, Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes (URMITE), Marseille, France, and Campus Commun UCAD-IRD, Hann, Dakar, Sénégal, E-mails: kabinet1@hotmail.fr, olegusss1@gmail.com, ratmanov@gmail.com, georges.diatta@ird.fr, hubert.bassene@ird.fr, clementine.roucher@ird.fr, cheikh.sokhna@ird.fr, jean-francois.trape@ird.fr, didier.raoult@gmail.com, and Florence.fenollar@univ-amu.fr. Adama Tall, Unité d'Epidémiologie, Institut Pasteur de Dakar, Dakar, Sénégal, E-mail: tall@pasteur.sn.

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