



HAL
open science

Sewage workers with low antibody responses may be colonized successively by several *Tropheryma whipplei* strains

Florence Fenollar, Thomas Marth, Jean-Christophe J.-C. Lagier, Emmanouil Angelakis, Didier Raoult

► To cite this version:

Florence Fenollar, Thomas Marth, Jean-Christophe J.-C. Lagier, Emmanouil Angelakis, Didier Raoult. Sewage workers with low antibody responses may be colonized successively by several *Tropheryma whipplei* strains. *International Journal of Infectious Diseases*, 2015, 35, pp.51-55. 10.1016/j.ijid.2015.04.009 . hal-01785415

HAL Id: hal-01785415

<https://amu.hal.science/hal-01785415>

Submitted on 4 May 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License



Sewage workers with low antibody responses may be colonized successively by several *Tropheryma whippelii* strains



Florence Fenollar^a, Thomas Marth^b, Jean-Christophe Lagier^a, Emmanouil Angelakis^a, Didier Raoult^{a,*}

^a Aix-Marseille Université, Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes (URMITE) UM63, CNRS 7278, IRD 198, Inserm 1095, 13005 Marseille, France

^b Abteilung Innere Medizin, Krankenhaus Maria Hilf, Daun, Germany

ARTICLE INFO

Article history:

Received 16 March 2015

Received in revised form 13 April 2015

Accepted 15 April 2015

Corresponding Editor: Eskild Petersen, Aarhus, Denmark.

Keywords:

Tropheryma whippelii

Sewage workers

Asymptomatic carriage

Whipple's disease

SUMMARY

Objectives: Asymptomatic faecal carriage of *Tropheryma whippelii*, the agent of Whipple's disease, is reported among sewage workers. However, the potential development of such carriage is unknown. A 7-year follow-up of *T. whippelii*-carrying sewage workers is reported.

Methods: Nineteen sewage workers previously detected as faecal carriers of *T. whippelii* were followed to ascertain the chronicity of their carriage. Faeces were tested by molecular assays using quantitative real-time PCR specifically targeting *T. whippelii*. Serological anti-*T. whippelii* Western blotting was also performed.

Results: Seventy-nine percent (15/19) of workers exhibited a strong immune response against *T. whippelii*. Among these, five were followed for more than 1 year. Four maintained a strong response, with three carrying the same strain and one becoming negative. The fifth exhibited a decreased immune response, a negative faeces result, and subsequent carriage of another strain. Three individuals with low immune responses were also followed. Two never developed a response, with one carrying the same strain and one becoming negative and then positive with another strain; the third developed a strong response and became negative.

Conclusions: Chronic *T. whippelii* carriers appear to be protected against reinfection, but those with low or decreasing antibody levels may be re-colonized by another strain.

© 2015 The Authors. Published by Elsevier Ltd on behalf of International Society for Infectious Diseases. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Tropheryma whippelii, the causative agent of Whipple's disease, can cause numerous clinical problems. Chronic infections include classic Whipple's disease, which is disseminated and is defined by small-bowel biopsy involvement, and localized infections such as endocarditis, encephalitis, uveitis, or arthritis.^{1–5} *T. whippelii* has also been associated with acute infections, including gastroenteritis, pneumonia, and bacteraemia, which likely correspond to a primary infection.^{3,6–8} The natural history of classic Whipple's disease remains poorly understood because *T. whippelii* is a common bacterium,^{9,10} and in contrast, Whipple's disease and *T. whippelii* infections are rarely reported.^{2,11}

Asymptomatic stool carriage of *T. whippelii* has been detected in humans, with varying prevalence depending on age, geographic area, and exposure.^{9,12} In France, the prevalence is higher among sewage workers (12–26%) and homeless people (13%) than in the general adult population (4%).^{12–14} In rural Senegal, the prevalence is 17% for adults and reaches 75% in children less than 5 years old;¹⁵ the prevalence in rural Gabon is 9.7% for adults and reaches 40% in children less than 5 years old.¹⁶ Furthermore, *T. whippelii* prevalence reaches 38% among relatives of French patients with Whipple's disease or chronic carriers.¹⁴ In addition, the seroprevalence of *T. whippelii* has been estimated at 50% in France, 73% in rural Senegal, and 77% among French relatives of patients or chronic carriers.^{14,15}

As Whipple's disease has not been reported, for example, in sewage workers or homeless people, and is very rarely reported in individuals of African descent, the group with the highest *T. whippelii* exposure rate, it has to be assumed that additional

* Corresponding author. Tel.: +33 4 91 32 43 75; fax: +33 4 91 38 77 72.
E-mail address: didier.raoult@gmail.com (D. Raoult).

factors contribute to the shift from asymptomatic carriage of the bacterium to systemic Whipple's disease.⁹ The pathogenetic factors are most likely host-related, and a rather weak HLA association and subtle defects of the mucosal immune system have been described in patients with classic Whipple's disease.^{17–19} However, it remains unclear why this should be sufficient to accelerate *T. whipplei* infection. Thus, as described in the recent literature, medical immunosuppression initiated in patients with unclear arthritis^{2,20,21} may exacerbate *T. whipplei* infection in some individuals. Western blot serology performed in patients with classic Whipple's disease generally shows that these patients have low immune reactivity, whereas asymptomatic carriers typically develop a strong immune response against *T. whipplei*.²² Finally, despite the apparent clearance of *T. whipplei* in Whipple's disease patients following antibiotic treatment, reinfections with a new genotype have been observed.^{3,23}

These epidemiological, clinical, and biological findings regarding *T. whipplei* suggest that host factors play a key role in the pathogenesis of Whipple's disease.^{1,9} Some recent evidence also suggests that it could have a genetic basis.²⁴ Furthermore, although most cases do not result in clinical involvement, these results explain why a unique bacterium can cause a potentially fatal disease. The clinical and biological follow-up of a French cohort of asymptomatic sewage worker carriers is reported here.

2. Patients and methods

2.1. Individuals

Nineteen sewage workers were detected as carriers of *T. whipplei* during a faecal survey among the staff working with sewage in Marseille, France.¹² When carriage was detected during the screening survey, the patients were invited to a specialized consultation with one of the authors (DR) to evaluate the chronicity of their carriage, the potential presence of clinical manifestations, and the presence of an immune response against *T. whipplei*. Depending on the individual, they were followed at two specialized consultations and underwent one clinical examination per year. It was not possible to take a duodenal biopsy from these workers. This study was approved by the local ethics committee. All participants gave written informed consent.

2.2. Molecular assays

DNA was extracted from stool or saliva samples using Qiagen columns (QIAamp DNA kit; Qiagen, Courtaboeuf, France) in accordance with the manufacturer's recommendations. The samples were handled under sterile conditions to avoid cross-contamination. A specific quantitative real-time PCR (qPCR) assay using specific oligonucleotide TaqMan probes to target *T. whipplei* repeated sequences was performed to detect DNA from this bacterium in the specimens, as described previously.²⁵ A case was defined as positive if two independent qPCR assays targeting different repeated sequences were positive, with cycle threshold (Ct) values of <35. The *T. whipplei* strain Marseille-Twist was used as a positive control and PCR mixes were used as a negative control. The human β -actin gene was detected systematically in parallel to check the quality of the extracted DNA.²⁶

2.3. Genotyping

Genotyping was performed on stool samples positive for *T. whipplei* using a multi-spacer system, as described previously.²⁷ Each of the four highly variable genomic sequences (HVGs) obtained from each specimen were compared to those available in

both the GenBank database and our internal laboratory database to determine the corresponding genotype.^{23,27}

2.4. Western blot serology

Serological assays were performed by Western blotting. Native and deglycosylated proteins were prepared, separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), and transferred onto nitrocellulose membranes, as described previously.²² The membranes were incubated with primary sera, washed, and then incubated with a peroxidase-conjugated goat anti-human antibody (Southern Biotech, Birmingham, AL, USA), as described previously. Detection was performed using chemiluminescence (Enhanced Chemiluminescence Western Blotting Analysis System; Amersham Biosciences, Uppsala, Sweden) with an automated film processor (Hyperprocessor; GE Healthcare, Vélizy-Villacoublay, France). The films were scanned with Image Scanner III (GE Healthcare). Image analysis was performed with GelEval 1.21b FrogDance software and ImageJ 1.39 v software (Wayne Rasband, National Institutes of Health) in order to quantify the signal of the bands on the Western blots, and interpretation was performed as reported previously.²²

3. Results

3.1. Individuals

All 19 sewage carriers were seen for their first specialized consultation and were also seen by a physician at each of their follow-up consultations. None of the sewage workers presented clinical manifestations (arthralgia, diarrhoea, or weight loss) at the time of the first consultation or during the follow-up period.

3.2. First consultation

The data from the first consultation are summarized in Table 1. Western blot serology showed that 15 of 19 (79%) sewage workers exhibited a strong immune response against *T. whipplei*, whereas four (21%) had a low or absent immune response. Among the 15 individuals with a strong immune response, PCR for *T. whipplei* in faeces and saliva was negative in four individuals (26.7%). Eleven of the 15 (73.3%) patients with a strong response were still positive for *T. whipplei* in faeces, including two (13.3%) who were also carriers of *T. whipplei* in saliva. Among the four patients with a low immune response, three were positive for *T. whipplei* in faeces but not saliva. One was negative in both saliva and faeces.

3.3. Follow-up period

The data from the follow-up consultations are summarized in Table 1. Among the four individuals with a low immune response, one individual (SW1) never developed an immune response against *T. whipplei*; he always carried the same *T. whipplei* strain, genotype 36, in his faeces for the entire 6-year follow-up and was also a saliva carrier. Another individual (SW2) never developed an immune response against *T. whipplei* during the 2-year follow-up and was alternately faeces positive and negative for *T. whipplei*. Two different *T. whipplei* strains were detected; he first carried genotype 119 and then carried genotype 11 after PCR had become negative. Another individual (SW3) developed protective immunity against this *T. whipplei* strain, as his faeces became negative for *T. whipplei*; during the second year of follow-up, the faeces were still negative for *T. whipplei*, but a decreased immune response was observed. The last individual (SW4) was lost to follow-up after 1 year.

Table 1
Summary of the follow-up of 19 sewage worker carriers of *Tropheryma whippelii*

19 SW	2006		2006		2007	2008	2009	2010	2011	2012	2013
	First WB	Samples	1 st visit	2 nd visit							
SW 1	Low IR	Faeces	Pos (36) ^a	Pos	Pos (36)	Pos (36)	Pos (36)	Pos	Pos (36)	Pos (36)	
		Saliva	Neg	Pos	Pos	Neg	Neg	Neg	Neg	Neg	
SW 2	Absent	Faeces	Pos (119)	Neg	Pos (11)	Neg					
		Saliva	Neg	Neg	Neg	Neg					
SW 3	Low IR	Faeces	Pos		Neg	Neg					
		Saliva	Neg		Neg	Neg					
SW 4	Low IR	Faeces	Neg	Neg							
		Saliva	Neg	Neg							
SW 5	Strong IR	Faeces	Pos (29)	Neg	Pos (90)						
		Saliva	Neg	Neg	Neg						
SW 6	Strong IR	Faeces	Pos (82)	Pos (82)	Pos (82)	Pos (82)	Pos (82)	Pos (82)	Pos (82)	Pos (82)	Pos (82)
		Saliva	Neg	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg
SW 7	Strong IR	Faeces	Pos (39)	Pos	Pos (39)	Pos (39)	Neg				
		Saliva	Neg	Neg	Neg	Pos	Neg				
SW 8	Strong IR	Faeces	Pos (39)	Pos	Pos (39)	Pos (39)	Pos	Pos (39)			
		Saliva	Neg	Neg	Neg	Pos	Neg	Neg			
SW 9	Strong IR	Faeces	Pos	Pos	Pos	Neg					
		Saliva	Pos	Pos	Pos	Neg					
SW 10	Strong IR	Faeces	Pos								
		Saliva	Pos								
SW 11	Strong IR	Faeces	Pos	Pos							
		Saliva	Neg	Neg							
SW 12	Strong IR	Faeces	Pos	Pos							
		Saliva	Neg	Neg							
SW 13	Strong IR	Faeces	Pos								
		Saliva	Neg								
SW 14	Strong IR	Faeces	Pos		Neg						
		Saliva	Neg		Neg						
SW 15	Strong IR	Faeces	Neg								
		Saliva	Neg								
SW 16	Strong IR	Faeces	Neg								
		Saliva	Neg								
SW 17	Strong IR	Faeces	Neg								
		Saliva	Neg								
SW 18	Strong IR	Faeces	Neg								
		Saliva	Neg								
SW 19	Strong IR	Faeces	Neg								
		Saliva	Neg								

SW, sewage worker; WB, Western blot, IR, immune response; Pos, positive; Neg, negative.

^aThe genotype, when available, is indicated in brackets.

Key:

Absence or low immune response against <i>T. whippelii</i>
Presence of a strong immune response against <i>T. whippelii</i>
Analysis not performed

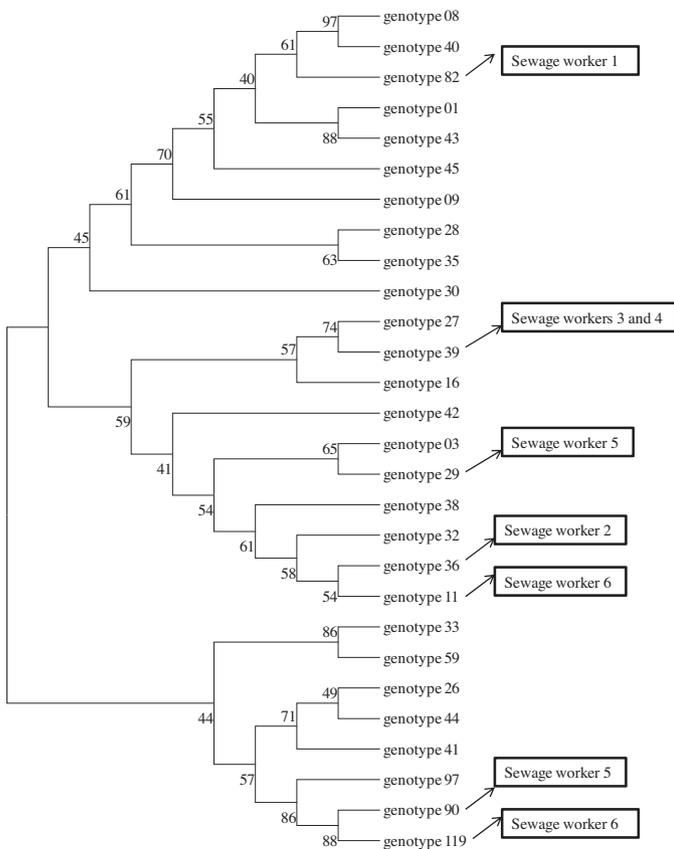


Figure 1. Neighbour-joining tree of *Tropheryma whipplei* genotypes; arrows indicate genotypes found among sewage workers.

Among the 15 individuals with a strong immune response, five were followed for more than 1 year. Among these, four individuals exhibited a strong immune response during the entire follow-up. At 2 years of follow-up, one individual became faeces-negative, whereas three were always positive for *T. whipplei*. They all carried the same strain as that present at the time of the first detection (genotype 82 for SW6 and genotype 39 for SW7 and SW8). In particular, SW6 carried genotype 82 for 7 years. For the last individual (SW5), a decreased immune response and negativity of the faeces were observed after the first year. The second year, the faeces were again positive for *T. whipplei*, but with a different strain (genotype 90) to that initially detected (genotype 29). Unfortunately, contact with this patient was lost and a serum sample was not obtained for testing by Western blotting when he was a carrier of the new genotype. A dendrogram showing the phylogenetic organization of the genotypes is presented in Figure 1.

4. Discussion

A high prevalence of *T. whipplei* among sewage workers in Marseille, France has been found previously.¹² The study presented here confirms that sewage workers can be asymptomatic carriers of *T. whipplei* for years. Indeed, none of the *T. whipplei*-positive workers tested had classic clinical manifestations of Whipple's disease, such as arthralgia or chronic diarrhea.² We believe that these findings are reliable because each positive result was confirmed by the amplification of a second *T. whipplei*-specific sequence, and the DNA extraction quality, including the presence of inhibitors, was examined for all samples analyzed.²⁵ Moreover, the systematic analysis of negative controls yielded the expected results.

Although a high prevalence of asymptomatic carriage of *T. whipplei* has been observed previously in sewage workers, homeless people, people living in rural Africa, and relatives of patients or *T. whipplei* carriers, this follow-up analysis provides the first evidence that asymptomatic carriage of *T. whipplei* can be chronic.^{16,28} The longest carriage of the same strain was 7 years. This study also confirmed that all the asymptomatic carriers with PCR-positive saliva specimens also harboured *T. whipplei* in their faeces.

In this study, it was found for the first time that chronic *T. whipplei* carriers can be colonized by different *T. whipplei* strains over time. Overall, genotyping has shown a high genetic diversity of *T. whipplei*; in contrast, clinical signs are not related to specific *T. whipplei* strains.^{9,12,27,29} A high genetic diversity among the *T. whipplei* isolates recovered from sewage workers was also observed. Thus, no clonal *T. whipplei* strain was detected among sewage workers, although this has been reported for homeless people (a potential epidemic clone was detected) and family members (intrafamilial circulation of a single clone has been observed).²⁸ This may be linked to the mode of contamination, including direct human contact for homeless people or relatives and indirect human contact through faeces and sewage for sewage workers (epidemic versus endemic).

None of the chronic carriers followed for more than 1 year and who exhibited a strong immune response to *T. whipplei* throughout the follow-up period was reinfected by another *T. whipplei* genotype. In contrast, among three carriers who presented a low immune response, two were carriers of two different *T. whipplei* strains. Each of these individuals was PCR-negative for *T. whipplei* in their faeces prior to the detection of a new strain. These results indicate the significance of different immune responses among asymptomatic carriers.^{14,22} The low immune reactivity of the two carriers likely allowed reinfection by another *T. whipplei* strain, as already reported for Whipple's disease patients who were cured prior to being reinfected with another strain.^{23,30} Another previously suggested hypothesis is that chronic *T. whipplei* carriers are colonized by different *T. whipplei* strains, with one strain becoming dominant.¹² Another hypothesis is that such individuals are only able to produce a strain-specific immune response, which is comparable to what has already been observed for giardiasis, with partial protective immunity.^{31,32}

Finally, *T. whipplei* is a bacterium that is associated with an underestimated number of acute clinical manifestations. The working conditions of sewage workers directly in contact with stool may explain the high prevalence of *T. whipplei* among this population. We also found that chronic asymptomatic carriers of *T. whipplei* can present different immune responses against *T. whipplei*.

In conclusion, chronic carriers of *T. whipplei* are apparently protected against reinfection, but those with low or decreasing antibody levels may be re-colonized by another *T. whipplei* strain.

Acknowledgements

We thank the IHU Méditerranée Infection for financial support.
Conflict of interest: No conflict of interest to declare.

References

1. Fenollar F, Puéchal X, Raoult D. Whipple's disease. *N Engl J Med* 2007;**356**: 55–66.
2. Lagier JC, Lepidi H, Raoult D, Fenollar F. Systemic *Tropheryma whipplei*: clinical presentation of 142 patients with infections diagnosed or confirmed in a reference center. *Medicine (Baltimore)* 2010;**89**:337–45.
3. Fenollar F, Lagier JC, Raoult D. *Tropheryma whipplei* and Whipple's disease. *J Infect* 2014;**69**:103–12.

4. Fenollar F, Nicoli F, Paquet C, Lepidi H, Cozzone P, Antoine JC, et al. Progressive dementia associated with ataxia or obesity in patients with *Tropheryma whipplei* encephalitis. *BMC Infect Dis* 2011;**11**:171.
5. Fenollar F, Celard M, Lagier JC, Lepidi H, Fournier PE, Raoult D. *Tropheryma whipplei* endocarditis: a 28 patient series and review. *Emerg Infect Dis* 2013;**19**:1721–30.
6. 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.
7. Bousbia S, Papazian L, Auffray JP, Fenollar F, Martin C, Li W, et al. *Tropheryma whipplei* in patients with pneumonia. *Emerg Infect Dis* 2010;**16**:258–63.
8. 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.
9. Keita AK, Raoult D, Fenollar F. *Tropheryma whipplei* as a commensal bacterium. *Future Microbiol* 2013;**8**:57–71.
10. Keita AK, Mediannikov O, Ratmanov P, Diatta G, Bassene H, Roucher C, et al. Looking for *Tropheryma whipplei* source and reservoir in rural Senegal. *Am J Trop Med Hyg* 2013;**88**:339–43.
11. Dobbins III WO. Whipple's disease. Springfield, IL: Thomas Books; 1987.
12. Fenollar F, Trani M, Davoust B, Salle B, Birg ML, Rolain JM, et al. Prevalence of asymptomatic *Tropheryma whipplei* carriage among humans and nonhuman primates. *J Infect Dis* 2008;**197**:880–7.
13. Schöniger-Hekele M, Petermann D, Weber B, Müller C. *Tropheryma whipplei* in the environment: survey of sewage plant influxes and sewage plant workers. *Appl Environ Microbiol* 2007;**73**:2033–5.
14. Fenollar F, Keita AK, Buffet S, Raoult D. Intrafamilial circulation of *Tropheryma whipplei*, France. *Emerg Infect Dis* 2012;**18**:949–55.
15. Keita AK, Bassene H, Tall A, Sokhna C, Ratmanov P, Trape JF, et al. *Tropheryma whipplei*: a common bacterium in rural Senegal. *PLoS Negl Trop Dis* 2011;**5**:e1403.
16. Ramharter M, Harrison N, Buhler T, Herold B, Lagler H, Lotsch F, et al. Prevalence and risk factor assessment of *Tropheryma whipplei* in a rural community in Gabon: a community based cross-sectional study. *Clin Microbiol Infect* 2014;**20**:1189–94.
17. Martinetti M, Biagi F, Badulli C, Feurle GE, Muller C, Moos V, et al. The HLA alleles DRB1*13 and DQB1*06 are associated to Whipple's disease. *Gastroenterology* 2009;**136**:2289–94.
18. Moos V, Kunkel D, Marth T, Feurle GE, LaScola B, Ignatius R, et al. Reduced peripheral and mucosal *Tropheryma whipplei*-specific Th1 response in patients with Whipple's disease. *J Immunol* 2006;**177**:2015–22.
19. Marth T, Kleen N, Stallmach A, Ring S, Aziz S, Schmidt C, et al. Dysregulated peripheral and mucosal Th1/Th2 response in Whipple's disease. *Gastroenterology* 2002;**123**:1468–77.
20. Mahnel R, Kalt A, Ring S, Stallmach A, Strober W, Marth T. Immunosuppressive therapy in Whipple's disease patients is associated with the appearance of gastrointestinal manifestations. *Am J Gastroenterol* 2005;**100**:1167–73.
21. Marth T. Increased risk of complicated Whipple's disease and endocarditis following tumor necrosis factor alpha inhibitors: a case control study. *World J Gastroenterol* 2014;**6**:1278–84.
22. Fenollar F, Amphoux B, Raoult D. A paradoxical *Tropheryma whipplei* Western blot differentiates patients with Whipple's disease from asymptomatic carriers. *Clin Infect Dis* 2009;**49**:717–23.
23. Lagier JC, Fenollar F, Lepidi H, Raoult D. Evidence of lifetime susceptibility to *Tropheryma whipplei* in patients with Whipple's disease. *J Antimicrob Chemother* 2011;**66**:1188–9.
24. Biagi F, Badulli C, Feurle GE, Muller C, Moos V, Schneider T, et al. Cytokine genetic profile in Whipple's disease. *Eur J Clin Microbiol Infect Dis* 2012;**31**:3145–60.
25. Fenollar F, Laouira S, Lepidi H, Rolain JM, Raoult D. Value of *Tropheryma whipplei* quantitative PCR assay for the diagnosis of Whipple's disease: usefulness of saliva and stool specimens for first-line screening. *Clin Infect Dis* 2008;**47**:659–67.
26. Mediannikov O, Fenollar F, Socolovschi C, Diatta G, Sokhna C, Bassene H, et al. *Coxiella burnetii* in humans and ticks in rural Senegal. *PLoS Negl Trop Dis* 2010;**6**:e654.
27. Li W, Fenollar F, Rolain JM, Fournier PE, Feurle GE, Müller C, et al. Genotyping reveals a wide heterogeneity of *Tropheryma whipplei*. *Microbiology* 2008;**154**:521–7.
28. 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* 2013;**17**:e67–8.
29. Wetzstein N, Fenollar F, Buffet S, Moos V, Schneider T, Raoult D. *Tropheryma whipplei* genotypes 1 and 3, in central Europe. *Emerg Infect Dis* 2013;**19**:341–2.
30. Lagier JC, Fenollar F, Lepidi H, Giorgi R, Million M, Raoult D. Treatment of classic Whipple's disease: from in vitro results to clinical outcome. *J Antimicrob Chemother* 2014;**69**:219–27.
31. Istre GR, Dunlop TS, Gaspard GB, Hopkins RS. Waterborne giardiasis at a mountain resort: evidence for acquired immunity. *Am J Public Health* 1984;**74**:602–4.
32. Isaac-Renton JL, Lewis LF, Ong CS, Nulsen MF. A second community outbreak of waterborne giardiasis in Canada and serological investigation of patients. *Trans R Soc Trop Med Hyg* 1994;**88**:395–9.