MAJOR ARTICLE

An Experimental Mouse Model to Establish *Tropheryma whipplei* as a Diarrheal Agent

Khatoun Al Moussawi,* Nada Malou,* Jean-Louis Mege, Didier Raoult, and Benoit Desnues

URMITE, Unité Mixte de Recherche 6236, Centre National de Recherche Scientifique, Université de la Méditerranée, Marseille, France

*Tropheryma whipplei* has long been considered as a rare bacterium causing a rare disease, Whipple’s disease. However, recent advances now suggest that *T. whipplei* is a ubiquitous environmental bacterium that may cause gastroenteritis, commonly associated with viral pathogens. We developed an animal model to support this hypothesis. We found that orally given *T. whipplei* induced diarrhea in mice, without spreading into the intestines. Aggravating factors, such as damage to the intestinal mucosa, favored bacterial spreading. Indeed, bacterial presence was prolonged in stools of dextran sulfate-treated mice, and bacteria were detected in the colon. This resulted in an immune response, with *T. whipplei*-specific serum IgM and IgG and fecal IgA, as measured by newly introduced immuno–polymerase chain reaction technique. Our results confirm that *T. whipplei* is an agent causing gastroenteritis and suggest that existing mucosal damage may favor bacterial invasion of tissues.

(See the editorial commentary by Wilson et al, on pages 4–5.)

*Tropheryma whipplei* was first identified as the etiologic agent of Whipple’s disease, a rare disease characterized by weight loss, weakness, anorexia, chronic diarrhea, and abdominal pain [1]. However, recent findings have shown that, although Whipple’s disease is rare, *T. whipplei* is a very common and ubiquitous bacterium. Indeed, *T. whipplei* has been detected in sewage samples [2] and in the stool of healthy persons without Whipple’s disease [3, 4], although asymptomatic carriers have lower bacterial loads in the stool than do patients with Whipple’s disease [4, 5]. Recent studies now suggest that acute *T. whipplei* infection might result in common clinical presentations [6], such as febrile bacteremia and cough [7] or pneumonia [8]. In addition, strong evidence has suggested that *T. whipplei* causes mild gastroenteritis in children, associated with seropositivity and high bacterial loads in the stool, comparable to those in patients with Whipple’s disease [9]. Of interest, in these children, recovery from diarrhea is associated with the disappearance of *T. whipplei* DNA in stool samples. Another interesting finding in that study was that, in 33% of cases, *T. whipplei* was found in association with other pathogens transmitted through the fecal-oral route (*Salmonella* species, *Giardia duodenalis*, *Campylobacter jejuni*, rotavirus, adenovirus, and calicivirus) [9]. Collectively, it appears that primary infection with *T. whipplei* results in gastroenteritis, as suggested by the detection of *T. whipplei* in feces for the duration of diarrhea. *T. whipplei* may interact in conjunction with other enteric pathogens to cause diarrhea [10].

In this study, we aimed to confirm the role of *T. whipplei* as an agent of gastroenteritis during primary infection by developing a murine model. To test our hypothesis that existing damage to the intestinal mucosa favors intestinal colonization by *T. whipplei*, we used an experimental model of colonic inflammation, introduced by Okayasu et al [11]. Dextran sodium sulfate (DSS)–induced colitis displays several features that are found in humans, including inflammation that originates in the distal colon and progresses to involve the entire colon [12]. We found that oral administration of *T. whipplei* to healthy mice resulted in mild diarrhea at
day 4 after infection without any signs of tissue invasion. In mice with DSS-induced intestinal injury, *T. whipplei* induced an immune response and could be retrieved from colonic samples and triggered an immune response.

Together, these results confirm our hypothesis and demonstrate that *T. whipplei* is a causative agent of diarrhea.

**METHODS**

**Bacteria and Mice**

The *T. whipplei* strain Twist-Marseille (CNCM I-2202) was cultured in HEL cells and purified as described elsewhere [13]. Female C57BL/6 mice were purchased from Charles River Laboratories at 4–6 weeks of age. Colitis was induced in a group of 10 mice by treatment with 2.5% DSS (MW, 30,000–50,000; MP Biomedicals) in their drinking water. After 7 days, DSS treatment ended, and mice were infected per os with $5 \times 10^6$ *T. whipplei* organisms. Other animals (10 per group) received 106 *T. whipplei* in their drinking water only, 2.5% DSS only, or water only.

**Histology and Immunohistochemistry**

Sections of paraffin-embedded tissues (5 μm) were stained with hematoxylin-eosin. Histological changes were quantified using a scoring system, as described elsewhere [15]. For immunohistologic detection of *T. whipplei*, tissue sections were deparaffinized in xylene, rehydrated in graded ethanol, and incubated with rabbit antibodies against *T. whipplei* ([16], 1:2,000 dilution). Bacteria were visualized using the Immunostain-Plus kit (Zymed) according to the manufacturer’s recommendations.

**Real-Time PCR**

DNA was extracted from intestine, stool, liver, spleen, and blood samples with use of the QIAamp DNA MiniKit (Qiagen) according to the manufacturer’s recommendations. *T. whipplei*-specific quantitative PCR was conducted by targeting a 135-bp specific sequence using the primer pair WT7745 forward (5′-CCCTTGGATTGCCTACCTT-3′) and WT7745 reverse (5′-AGCCCTCGGAAAAGAGG-3′) and a Taqman probe (5′-6-FAM-CCGGTTATCCCGGCAGGGA-3′). PCR was conducted in an ABI PRISM 7900 HT (Applied Biosystems) using the 2x Quantitect SYBR Green PCR Master Mix (Qiagen).

**Quantitative Real-Time Reverse-Transcription (RT)–PCR**

cDNA was synthesized from 1 μg of total colonic RNA with use of SuperScript II RNase H reverse transcriptase (Invitrogen). Specific primers for TNF, IL-6, IFNγ and β-actin were designed using Primer3Plus, available online at http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi (TFN: forward, 5′-AGAAACACAAATGCTGGGACA-3′, reverse, 5′-TCTGGAAAGGCTCTGAAGTAGGA-3′; IL-6: forward, 5′-GTTTCTGGAATATCTGGTA-3′, reverse, 5′-CTCTGAAGGACTCTGGGCTTG-3′; IFNγ: forward, 5′-GACTGTGGGTGTTGACCT-3′, reverse, 5′-GAGAAGCTGGCAAAAAAGGA-3′; and β-actin: forward, 5′-TGGAATCTCTGTTGGGATCCCATGAAAC-3′, reverse, 5′-TAAAAACGCAGCTCACAGACCTGGC-3′. Quantitative RT-PCR was performed using LightCycler-Fastart DNA Master SYBR Green (Roche Diagnostics) as described elsewhere [17]. The amount of target mRNA was expressed relative to the β-actin internal control by $2^{-\Delta Ct}$, where ΔCt = Ct_target - Ct_β-actin.

**Antibody Determination**

Circulating *T. whipplei*-specific antibodies were determined as described elsewhere [18], using fluorescein-conjugated goat antibodies directed against mouse IgG (Beckman Coulter) and rat antibodies against mouse IgM (BD Pharmingen) at 1:400 dilution. Titers of samples were determined by end point dilution.

**Quantitative Immuno-PCR**

Stool samples were collected from mice and mixed in phosphate-buffered saline (pH, 7.2) containing soybean trypsin inhibitor (0.5 mg/mL; Sigma), phenylmethylsulfonyl fluoride (0.25 mg/mL; Sigma), 0.05 M EDTA, and 0.05% Tween 20 (Sigma), before centrifugation at 20,000 × g for 5 min. Supernatants were stored at −20°C until use. *T. whipplei* antigen extract was prepared by disruption via sonication. Proteins were precipitated by using PlusOne 2-D Clean-Up Kit (Amersham Biosciences) and were suspended in rehydration solution (7 M urea, 2 M thiourea, and 4% v/v CHAPS). One microgram of *T. whipplei* protein extract in coating buffer (Chimera Biotec GmbH) was coated on Nunc TopYield microtiter modules (VWR). Washing buffers A and B, coating buffer, blocking solution, conjugate dilution buffer (CDB), biotin-free CDB (CDB-b), anti–biotin-DNA conjugate antibody (CHI-biotin), and mastermix were provided in the Imperacer CHI biotin Kit (Chimera Biotec). Unbound *T. whipplei* proteins were removed by washing with buffer A and the modules were blocked with blocking solution overnight, before being washed with buffer B.

For *T. whipplei*-specific IgA detection, stool sample was incubated in each well for 1 hour under orbital shaking. The unbound components were eliminated by washes in buffer.
B. Biotinylated goat anti-mouse IgA (1:1,000 in CDB) were added for 1 h. The antibiotin antibody–DNA conjugate (1:200 in CDB-b) was then incubated for 45 min. To eliminate contamination and unbound components, 3 washes with buffer B were performed, followed by 2 washes with buffer A.

For quantitative PCR, 30 µL of mastermix were added to each well, and the modules were sealed with an optical adhesive. PCR was performed in the ABI PRISM 7900 HT with FAM as fluorophore, according to conditions provided by Chimera Biotec. The background fluorescence threshold that differentiates negative from positive reactions was set immediately above the negative controls.

Statistics
Statistical analysis was performed using GraphPad Prism software with the Mann-Whitney U test. For multiple comparisons, 1-way analysis of variance, followed by a Newman-Keuls multiple comparison test was applied. P < .05 was considered to be statistically significant.

RESULTS
*T. whipplei* Induces Mild and Transient Diarrhea But Does Not Invade Intestine
On the basis of epidemiological studies and human data, we hypothesized that primary *T. whipplei* infection would trigger diarrhea in mice. To examine this possibility, we first infected C57BL/6 mice orally with 5 *×* 10^6 bacteria to mimic the suspected mode of contamination. All mice remained clinically healthy, with no mortality and no statistically significant differences in weight change between the 2 groups (infected and control) over the 14-day observation period (Figure 1A). In a first set of experiments, stool samples were collected daily from the cages. *T. whipplei* DNA was detected after 1 day (Ct, 33.25 ± 0.84) but not thereafter, suggesting that bacteria did not colonize mice. Mice were then sacrificed, and the intestine, liver, spleen, and blood were collected for detection of *T. whipplei* DNA. As suggested by the transient detection of bacteria in stool samples, we were not able to detect *T. whipplei* DNA in any tissue tested. In accordance with macroscopic examination, histological analysis revealed that oral infection of mice with *T. whipplei* did not result in pathological abnormalities of the colon, compared with uninfected mice. This finding was also confirmed by measuring the levels of TNF and IL-6 mRNA in the colon, which were similar in control and *T. whipplei*–infected mice (Figure 1B).

Serological testing was performed on days 8 and 14 after infection. Neither circulating immunoglobulin (Ig) M nor IgG against *T. whipplei* was detected in the blood. We also measured *T. whipplei*–specific IgA levels in stool samples by quantitative immuno-PCR, which enables very sensitive detection of antigen by means of specific antibody–DNA conjugates [19]. To evaluate the sensitivity and specificity of quantitative immuno-PCR, we performed 10-fold dilutions in double of human isotypic IgA diluted in mouse stool ranging from 0.1 µg to 0.1 ng. Immuno-detection was performed with a biotinylated antihuman IgA antibody that did not cross-react with mouse IgA. Background fluorescence was determined using stool samples without human IgA added. The Ct values obtained with stool samples collected from *T. whipplei*–infected mice (34.855 ± 0.375) were not significantly different from the background fluorescence (33.7 ± 2.121). Finally, stool samples were obtained from the distal colon on days 4, 8, and 14 after infection. Diarrhea was monitored by measuring the ratio of wet weight to dry weight of feces. Of interest, we found that *T. whipplei* infection significantly increased this ratio on day 4 (1.938 ± .3195 and 2.564 ± .6458, for the control and *T. whipplei*–infected group, respectively; P = .0283) (Figure 1C). However, this increase was transient, and by days 8 and 14 after infection, no statistically significant differences in stool water content were observed between infected and uninfected mice. We conclude that oral administration of *T. whipplei* to healthy mice results in transient diarrhea and excretion of *T. whipplei* but does not cause local or systemic infection.
DSS-Induced Colitis Predisposes Mice to Tissue Infection With T. whipplei

We hypothesized that damage to the intestinal mucosa favors T. whipplei colonization of mice. Colitis was induced by exposing mice to 2.5% DSS in drinking water for 7 days. DSS-treated mice exhibited clinical signs of intestinal inflammation (including diarrhea, occult or perianal bleeding, and weight loss), which started on day 6 after treatment initiation and was most prominent on day 15 (Figures 2A and 2B). Thereafter, symptoms subsided and mice recovered their body weight gradually beginning 16 days after initiation of DSS treatment (Figure 2A). Over the observation period, no mortality was observed. Histological examination and histological scores of colon samples taken at the end of DSS treatment confirmed these findings and revealed a destruction of the bowel wall architecture and dense cellular inflammation (Figures 3A and 3B). One day after DSS treatment ended, mice were orally infected with $5 \times 10^6$ T. whipplei organisms. Infection did not result in a significant body weight change among DSS-treated mice (Figure 2A). Stool samples were then collected and analyzed for the presence of T. whipplei with use of PCR. In contrast to untreated mice, T. whipplei was detected during the first 4 days after infection in mice treated with DSS (Ct, 31.80 ± 0.12, 32.41 ± 0.40, and 34.31 ± 0.19, on days 1, 2, and 4, respectively), suggesting that mucosal damage prolonged T. whipplei carriage. Stool samples from the cecum and distal colon were collected on days 4, 8, and 14 after infection and were processed for diarrheal evaluation. We found that T. whipplei infection resulted in a slight, but not significant, increase of the wet weight/dry weight ratio of feces (4.660 ± 1.147 and 5.900 ± 1.527 at day 4 after infection, for the DSS group and DSS plus T. whipplei, respectively) (Figure 2B). In accordance with macroscopic data (weight loss, diarrhea, and

![Figure 2](image-url)

**Figure 2.** Body weight changes and assessment of diarrhea following DSS treatment and T. whipplei infection. Mice were administered either 2.5% DSS or water for 6 days before per os infection with T. whipplei. (A) Weight was monitored daily from the beginning of DSS treatment to the infection (day -6 to day 0) and for 14 days after infection (n = 10 per group). (B) On day 4 postinfection, stool samples were collected from the distal colon and evaluated for diarrhea as the ratio between wet weight and dry weight of the feces (n = 10 per group). ns, non significant; *, P < .05; ***, P < .005.

![Figure 3](image-url)

**Figure 3.** Colitis induced by DSS and immune response. Mice were administered 2.5% DSS or water for 6 days before per os infection with T. whipplei. (A) At the end of DSS treatment, colon tissue from control (untreated) and DSS-treated mice was collected and processed for histopathology by hematoxylin-eosin staining, revealing mucosal destruction and dense cellular infiltration. Representative histopathology is shown (magnification ×200). (B) Signs of colon inflammation (5 mice/group) were quantified in a blinded fashion using a score from 0–4 as described in the Experimental/Materials and Methods section. (C) Colons were harvested on day 4 postinfection. Total RNA was isolated and the expression of TNF, IL-6 and IFNγ mRNA was determined relative to β-actin mRNA by qRT-PCR. The results are expressed as the mean ratio ± SD from 3 mice.
perianal bleeding), histological analysis did not reveal significant differences in colitis severity between DSS-treated infected mice and mice treated with DSS only (Figure 3B). Finally, we measured the mRNA levels of the pro-inflammatory cytokines TNF and IL-6 in the colon from DSS-treated and DSS plus T. whipplei–infected mice at day 4. We found that DSS treatment alone resulted in an increase in the mRNA levels of TNF, IL-6, and IFNγ, compared with control mice (Figure 3C). In mice treated with DSS and infected with T. whipplei, TNF, IL-6, and IFNγ mRNA were increased, compared with DSS-treated mice, but not significantly (Figure 3C). Taken together, these results suggest that T. whipplei did not significantly exacerbate colitis induced by DSS, even if diarrhea and proinflammatory cytokine expression were slightly increased.

To determine whether intestinal mucosal damage contributes to T. whipplei invasion, we performed PCR on colon, spleen, liver, and blood from DSS-treated mice infected with T. whipplei on days 4, 8, and 14. Colon samples were positive for T. whipplei on day 4 after infection, whereas the spleen, liver, and the blood remained negative. These data were further confirmed by immunohistochemistry of colonic samples taken on day 4 after infection, which revealed the presence of T. whipplei both intracellularly and extracellularly (Figure 4A). DSS-treated mice progressively cleared organisms that were undetectable by PCR and immunohistochemistry by days 8 and 14 after infection. The spleen, liver, and blood from these mice remained negative for T. whipplei over the course of the experiment. Finally, T. whipplei–specific antibodies were measured in serum samples from DSS-treated mice orally infected with T. whipplei (Figure 4B). T. whipplei–specific IgM were detected on day 8 after infection in 3 of 5 mice, with titers of 1:50. T. whipplei–specific IgG was detected at titers of at least 1:200 at 14 days after infection in all mice (5 of 5). Two of those 5 mice had titers of 1:400. Stool samples collected on day 14 after infection in DSS-treated mice were positive for T. whipplei–specific IgA, with a mean Ct value of 27.29 ± 0.35, which corresponds to 0.9 ± 0.1 ng of T. whipplei–specific IgA, whereas for the negative controls (uninfected untreated mice and uninfected DSS-treated mice), Ct values were 34.85 ± 0.37 and 36.54 ± 0.96, respectively. We conclude that preexisting tissue damage facilitates T. whipplei colonization and invasion of the mucosa, thereby prolonging bacterial excretion and triggering an immune response.

DISCUSSION

T. whipplei has been detected in stool samples from healthy persons [4]. Although the natural reservoir or source of T. whipplei is still unknown, data suggest that infection results from fecal-oral or oral-oral transmission [1, 4]. Recently, Raoult et al [9] provided evidence that T. whipplei may cause mild gastroenteritis in children after primary contact with T. whipplei. Indeed, of 241 children with gastroenteritis, 15% were positive for T. whipplei. Of interest, T. whipplei DNA was not detected in patients recovering from a diarrheal episode [9], highlighting the association between T. whipplei and gastroenteritis. To establish a causal link between gastroenteritis and T. whipplei according to Koch’s postulate, we developed an in vivo murine model. Mice were orally infected with T. whipplei, to mimic the suspected mode of contamination. First, we showed that T. whipplei was transiently excreted after infection, even if it may be an enteral passage. Second, we found that T. whipplei induced transient diarrhea. In agreement with the previous observation that T. whipplei DNA was not detected in stool samples from patients recovering from diarrheal illness [9], we were not able to detect T. whipplei DNA in mouse stool samples after the diarrheal episode. Therefore, we fulfilled Koch’s postulate for the causative role of T. whipplei as an agent of acute diarrhea (Table 1). Overall, we demonstrated that primary infection with T. whipplei is symptomatic and results in a mild, self-limiting diarrhea.

After oral administration of T. whipplei, the intestine, spleen, liver, and blood remained negative for bacterial infiltration. This suggests that T. whipplei by itself is not able to invade tissues. This conclusion was supported by the absence of T. whipplei–specific antibodies. However, among patients with gastroenteritis, those infected with T. whipplei are more often coinfected with an associated pathogen, compared with patients with diarrhea in the absence of T. whipplei infection [9]. Recently, a convincing study in voles highlighted the importance of interactive pathogen communities in defining disease, by showing that infection with one microorganism definitely modulates susceptibility to others [10]. Such interactions may be negative

Figure 4. Bacterial invasion and antibody response Mice were administered 2.5% DSS or water for 6 days before per os infection with T. whipplei. (A) Immunohistochemistry was performed on colons from DSS-treated, T. whipplei–infected mice on day 4 postinfection and revealed the presence of bacteria (arrows, magnification ×400). (B) Blood from DSS-treated, T. whipplei–infected mice was collected on days 8 and 14 post-infection and analyzed for T. whipplei–specific IgM and IgG by immunofluorescence using inactivated T. whipplei. The results are expressed as titer means ± SD from 5 mice.
for at least one microbe, through competition for resource or cross-reaction of efficient immune response, but they can also be beneficial for $\geq 1$ microbe as a consequence of pathogen-induced immunodulation of the host. Therefore, we hypothesize that the physiological state of the host or cofactors, such as existing mucosal damage (mimicking the effects of potential coinfection), may facilitate T. whipplei colonization of tissues, thereby exacerbating the disease. To promote intestinal lesions, we used DSS, which causes epithelial injury, resulting in acute colitis [20–22]. Mice treated with DSS exhibited signs of colitis, such as weight loss, diarrhea, and profound histological colonic lesions. When DSS-treated mice were infected with T. whipplei, we observed several differences, compared with infection in untreated mice (Table 2). Bacteria were detected for a longer period in stool samples of treated mice, compared with untreated mice, suggesting that mucosal damage increases T. whipplei carriage. The severity of diarrhea and intestinal damages (as measured by clinical scores and proinflammatory cytokines) were slightly increased, but not significantly. This could be attributable to our DSS-based model, which may be too aggressive to detect differences between groups of mice. Furthermore, we cannot rule out the possibility that T. whipplei worsens existing mild gastroenteritis. More strikingly, we found that damage to epithelial intestinal cells promoted bacterial invasion into tissues. T. whipplei infection was confined to the intestines and did not reach the liver, spleen, or blood. Bacteria were transiently detected 4 days after infection, but not at 8 days or thereafter, suggesting that despite mucosal lesions, mice were able to control the infection. The acute stage of DSS-induced colitis is characterized by recruitment of neutrophils and T cells to lesion sites [20, 23]. In addition, mice with severe combined immunodeficiency eliminate T. whipplei, probably through NK cell activity [24]. Therefore, on the basis of the low pathogenic potential of T. whipplei, we hypothesize that protective immunity towards T. whipplei is, at least in part, mediated by innate immune cells. Finally, we found that only mice treated with DSS mounted an antibody response, as revealed by the presence of T. whipplei–specific IgM and IgG in the blood.

To determine whether T. whipplei–specific IgA was present, we developed a novel, highly sensitive and specific tool based on quantitative immune-PCR of stool samples, which enables the detection of very low amounts of antigen-specific IgA. We found that only stool samples from mice treated with DSS were positive for T. whipplei–specific IgA. This finding strengthens the aforementioned results and shows that mice treated with DSS exhibit an immune response against T. whipplei. Indeed, massive recruitment of leukocytes to the lesion sites before infection may favor subsequent bacterial recognition by immune cells and, therefore, support the immune response. This hypothesis is strengthened by the fact that, in vitro, dendritic cells are unable to mature after T. whipplei stimulation (B. Desnues, L. Gorvel and J. L. Mege, unpublished data) and that IgM and IgG responses are lacking or impaired in patients with Whipple’s disease, compared with healthy carriers [25–27].

In asymptomatic children (age, 2–4 years), the prevalence of T. whipplei–specific antibodies is $\sim$20% (mainly IgM, suggesting recent seroconversion) [9], although $\sim$50% of the general adult population has T. whipplei–specific antibodies [8, 9]. Therefore, it is tempting to speculate that primary infection with T. whipplei occurs during childhood and results in mild and transient diarrhea, as well as seroconversion in cases of co-infection with other enteric pathogens. This immunity acquired during childhood may protect adults against T. whipplei, explaining why most healthy carriers of T. whipplei do not have diarrheal episodes.

In conclusion, we demonstrate that T. whipplei is a causative agent of acute gastroenteritis and that preexisting damage to intestinal mucosa favors intestinal invasion.

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**References**


