**Tropheryma whipplei**, a Potential Commensal Detected in Individuals Undergoing Routine Colonoscopy

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Mucosal biopsy samples from individuals not suspected of having Whipple’s disease were tested for the presence of *Tropheryma whipplei*. A sensitive and specific real-time PCR assay targeting a sequence present seven times in the *T. whipplei* genome was used. *T. whipplei* DNA was detected in 2.0 and 3.8% of the patients undergoing gastroduodenoscopy and colonoscopy, respectively, who were tested.

Whipple’s disease (WD) is a rare infectious disease with an annual incidence of <1/1,000,000 (1). The causative agent of this multisystemic syndrome is the Gram-positive bacterium *Tropheryma whipplei*, which belongs to the phylum Actinobacteria (2, 3). The major clinical features of gastrointestinal WD comprise weight loss and steatorrhea. Conventional analysis relies on periodic acid-Schiff (PAS) staining of mucosal biopsy specimens, in which bacteria captured in foamy macrophages may be recognized. However, PAS staining lacks both sensitivity and specificity (4). Culturing of *T. whipplei* is cumbersome, and its availability is limited to specialized laboratories (5). Following its culture, the entire genome of *T. whipplei* was sequenced, which paved the way for the design of specific primers and probes for real-time PCR analysis (6, 7). With only one copy of the rRNA operon in the entire *T. whipplei* genome, primers based on this region display suboptimal sensitivity (8). In addition, cross-reaction with closely related species has been described, which hampers specificity (9).

A repetitive sequence present in seven copies and unique to the *T. whipplei* genome (6–8) permitted the development of a real-time PCR assay with greater sensitivity and specificity than earlier methods (8, 9).

The aim of this prospective study was to determine the prevalence of *T. whipplei* in intestinal mucosal biopsy specimens in consecutive individuals undergoing routine colonoscopy or gastroduodenoscopy with a validated, highly sensitive molecular test. All individuals were recruited from the Outpatient Clinic of the Department of Gastroenterology of the VU University Medical Center, Amsterdam, The Netherlands. This study was approved by the institutional medical ethics board (NL21031.029.07). Written informed consent was obtained prior to enrollment. Regular exclusion criteria for the performance of endoscopy were applied. All of the individuals in the colonoscopy group underwent standard bowel cleansing with a polyethylene glycol solution (Klean-Prep; Norgine BV, Amsterdam, The Netherlands) 1 day prior to colonoscopy.

Patients’ heights, weights, and antibiotic and probiotic use in the 4 weeks prior to endoscopy were recorded, as well as symptoms associated with gastrointestinal WD, including unintentional weight loss, altered bowel habits, and fatty diarrhea. Samples were collected, handled, and stored as illustrated in Table S1 in the supplemental material. Briefly, biopsy specimens were harvested from the sigmoid colon and duodenal D2 during colonoscopy or gastroduodenoscopy. Specimens were analyzed for the presence of *T. whipplei* DNA as described in detail in the supplemental material.

Patient characteristics are summarized in Table S2 in the supplemental material. Of the 240 individuals included, 98 underwent a gastroduodenoscopy and 157 individuals underwent a colonoscopy, 15 of whom underwent both procedures successively on the same day.

*T. whipplei* DNA was detected in 6 (3.8%) of 157 colonic biopsy specimens and 2 (2.0%) of 98 duodenal biopsy specimens (Table 1). One of these patients underwent both endoscopic procedures with only positively tested colonic mucosa. No difference in the prevalence of *T. whipplei* in the proximal or distal gastrointestinal tract was observed (*P* = 0.489, two-sided Fisher exact test), although the number of individuals was low.

To the best of our knowledge, this is the first report of the prevalence of *T. whipplei* in colonic samples from individuals not suspected of having WD. In a large series of cases from a tertiary-care WD referral center, 22 colonic samples from patients, eventually without evidence of WD, were tested, 1 of which was positive. It is important to note that these patients were referred because they were suspected of having WD (10).

All of the patients in our study had a medical indication for endoscopy, with an increased chance of pathology, including WD (although most probably with a negligible incidence). While the results obtained may not be an accurate reflection of the preva-
lence of *T. whipplei* in the general population, its prevalence in our colonoscopy group was in agreement with its prevalence (2.3 to 10.7%) in fecal samples from healthy individuals (10–13).

The prevalence of *T. whipplei* in duodenal biopsy samples was in line with previously described percentages (0 to 4.8%) (13–16). Others, however, have reported a higher prevalence (7.24%) (17). These samples were sent from different countries to be analyzed in a tertiary center for WD, probably because of a suspicion of WD in symptomatic individuals, which may have contributed to this difference.

The cycle threshold ($C_T$) value is indicative of the bacterial load. The median $C_T$ value of colonic samples was 38.6 (range, 35.8 to 43.1). The $C_T$ values of the duodenal samples were 41.8 and 43.7 (see Table S3 in the supplemental material). A statistical trend between $C_T$ values of colonic and duodenal samples was observed ($P = 0.095$; Mann-Whitney test). In general, the $C_T$ values in our study were rather high, concordant with a low bacterial concentration and replication rate, albeit of influence in the development of WD symptoms. The $C_T$ values of jejunal biopsy specimens in WD patients in our validation cohort were all considerably lower (see Table S4 in the supplemental material). No difference between the $C_T$ values of patients and carriers was observed, while only low numbers of individuals could be included in this analysis ($P = 0.133$; Mann-Whitney test).

In the colonoscopy group, but not in the gastroduodenoscopy group, a statistical trend of increased prevalence of *T. whipplei* DNA in patients with self-reported altered bowel habits was observed ($P = 0.066$; two-sided Fisher exact test). Of these four colonoscopy patients, one suffered from constipation, which has been reported before in WD (18, 19). Antibiotic use, probiotic use, unintentional weight loss, and self-reported fatty diarrhea were not associated with the prevalence of *T. whipplei* in both groups (Table 1). Besides low concentrations of *T. whipplei*, it is also conceivable that various *T. whipplei* strains differ in pathogenetic potential in the clinical development of WD, although this has not been demonstrated before (20). The difference between carriage of *T. whipplei* and WD is as yet unexplained. Hence, the cultivation and subsequent whole-genome sequencing of additional *T. whipplei* strains from both patients and asymptomatic carriers remain important when elucidating the role of *T. whipplei* strains in (subclinical) WD.

In conclusion, we have shown that *T. whipplei* was present in the intestinal mucosa of patients without signs of WD, particularly in the colon. Since the prevalence of *T. whipplei* was much higher than the incidence of WD, the development of WD is likely to be dependent on host factors, the concentration of *T. whipplei* bacteria, and/or differences in the pathogenicity of *T. whipplei* strains.

**REFERENCES**


