MAJOR ARTICLE

Value of *Tropheryma whipplei* Quantitative Polymerase Chain Reaction Assay for the Diagnosis of Whipple Disease: Usefulness of Saliva and Stool Specimens for First-Line Screening

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Background. Whipple disease (WD) is a chronic infectious disease caused by *Tropheryma whipplei*. WD DNA has been found in stool and saliva specimens from patients and asymptomatic carriers.

Methods. A total of 4418 samples that were sent to our center for determination of WD were tested by a *T. whipplei*–specific quantitative polymerase chain reaction (PCR) based on repetitive sequences. Definite WD was diagnosed in 71 patients, including 55 patients with classic WD (defined by positive results of periodic acid-Schiff staining and/or specific immunohistochemistry of small-bowel biopsy specimens) and 16 patients with localized WD (including patients with endocarditis, neurologic infection, and uveitis).

Results. Of the persons without WD, 2.3% had stool specimens positive for *T. whipplei* by PCR and 0.2% had saliva specimens positive for *T. whipplei* by PCR. Diagnosis of WD was likely in patients with positive results of both PCR of saliva specimens and PCR of stool specimens (positive predictive value, 95.2%). When the bacterial load was $>10^4$ colony-forming units per g of stool, the positive predictive value was 100%. A negative result of PCR of a saliva or stool specimen had a negative predictive value of 99.2% for classic WD. For localized WD, positive results of both PCR of saliva specimens and PCR of stool specimens had a sensitivity of 58% (compared with 94% for classic WD). The positive predictive value of testing of blood, cerebrospinal fluid, and urine specimens was 100% for each, and the positive predictive value for testing of duodenal biopsy specimens was 97.5%.

Conclusions. *T. whipplei*–specific quantitative PCR of saliva and stool specimens should be performed as first-line noninvasive screening for WD. When the results for both types of specimens are positive, diagnosis of classic WD should be highly suspected, especially if a high bacterial load is detected. Because PCR of saliva and stool specimens lacks sensitivity for determination of localized WD, invasive samples should be tested on the basis of clinical manifestations.

Whipple disease (WD) is a chronic infectious disease [1–5]. The causative bacterium *Tropheryma whipplei* has only recently been successfully cultivated [6, 7]. Culture for *T. whipplei* has allowed complete sequencing of the genome, providing a rational choice of DNA targets for PCR assays [8–10].

The clinical spectrum of WD has broadened greatly since the first detection of the bacterium in small-bowel specimens with use of 16S rRNA PCR [11, 12]. In addition to causing classic WD, which is characterized by histological lesions in the gastrointestinal tract, *T. whipplei* has been observed in asymptomatic carriers and in the context of several localized diseases not involving histological digestive lesions, such as neurological infection, blood culture–negative endocarditis, uveitis, adenopathy, arthritis, and spondylodiscitis [3]. Thus, the diagnostic strategy had become more difficult.

Since 2000, our center has been a reference laboratory for the diagnosis of WD [6]. The PCR techniques that we have used have evolved with improvement in

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Table 1. Diagnostic criteria for *Tropheryma whipplei* infection and asymptomatic carriage that were applied in our study.

Condition	Criteria
Definite classic Whipple disease	Positive results of PAS staining and/or specific IHC of an SBB specimen
Definite localized infection	Negative results of PAS staining of SBB specimens, in addition to 1 or 2 positive results of 2 PCR assays targeting 2 different sequences in SBB specimens
Definite localized neurologic infection	2 positive results of 2 PCR assays targeting 2 different sequences in 2 different CSF specimens
Definite infective endocarditis	Positive results of PAS staining and/or specific IHC and 2 positive results of PCR assays targeting 2 different sequences in a cardiac valve specimen
Definite uveitis	2 positive results of PCR assays targeting 2 different sequences in an aqueous humor specimen
Definite localized adenopathy	Positive results of PAS staining and/or specific IHC and 2 positive results of PCR assays targeting 2 different sequences in an adenopathy specimen
Definite pulmonary infection	2 positive results of PCR assays targeting 2 different sequences in a pulmonary biopsy specimen
Definite articular infection	2 positive results of PCR assays targeting 2 different sequences in an articular fluid specimen
Possible localized neurologic infection	1 positive result of 2 PCR assays targeting 2 different sequences in 1 or 2 different CSF specimens
Possible uveitis	1 positive result of 2 PCR assays targeting 2 different sequences in an aqueous humor specimen
Asymptomatic carriage	Negative results of PAS staining and/or specific IHC of SBB specimens, in addition to 1 or 2 positive results of 2 PCR assays targeting 2 different sequences in SBB specimens and a positive result of *T. whipplei*–specific PCR targeting 2 different genes in saliva or/and stool specimens

NOTE. IHC, immunohistochemistry; PAS, periodic acid-Schiff; SBB, small-bowel biopsy.

technologies and in knowledge about *T. whipplei*. Early techniques involved PCR assays targeting the 16S rRNA gene and the 16S–23S intergenic regions of *T. whipplei* [5, 12]. Later, a quantitative real-time PCR (qPCR) targeting this intergenic region was developed [13]. Most recently, a new qPCR that is based on genome analysis and that targets repeated sequences of *T. whipplei* has substantially enhanced sensitivity, compared with the previous qPCR assay, but has similar specificity [10]. For all of these assays, when an amplified product is detected, the identification of *T. whipplei* is confirmed by sequencing. More recently, specific oligonucleotide Taqman probes have been used for identification of *T. whipplei* [14–16]. Asymptomatic carriage, mainly in stool and saliva, has been reported; thus, it is a priori difficult to use these samples for diagnosis [3, 13, 16–23]. However, we recently found that the bacterial load, as determined by qPCR, was higher in patients than in carriers [15]. Here, we report our 4-year experience with molecular diagnosis of WD by targeting repeated sequences to evaluate the role of molecular diagnosis of the disease.

PATIENTS, MATERIALS, AND METHODS

Patient recruitment. Our laboratory (Marseille, France) receives samples from many locations in France. For patients from Marseille, the samples are transported at room temperature. For patients outside Marseille, the samples are frozen at −80°C and transported on dry ice. When a result is consistent with WD, the physicians are asked to provide additional samples and data. This study is based on the analysis of samples tested from October 2003 through August 2007. The local ethics committee approved the study.

Case definitions. The diagnostic criteria used for the case definitions are detailed in table 1. Our criteria for establishing a definite diagnosis of classic WD were the presence of positive results of periodic acid-Schiff staining and/or specific immunohistochemistry of a small-bowel biopsy specimen [3, 24]. The hallmark of localized extra-intestinal infection due to *T. whipplei* is the lack of histological lesions in the small-bowel biopsy specimens [3, 25]. On the basis of our results, patients were classified as having definite, excluded, or possible WD.

PCR assays. From October 2003 through March 2004, all specimens were tested by qPCR targeting repeated sequences of *T. whipplei;* when an amplified product was detected, sequencing was performed. Since April 2004, all specimens have been tested by qPCR targeting repeated sequences of *T. whipplei* with use of specific oligonucleotide Taqman probes for *T. whipplei* identification. A 10-fold dilution of a standard suspension of 10^6 *T. whipplei* strain Marseille-Twist was used as a positive control and for quantification, as reported elsewhere [15]. For each assay, at intervals of 5 samples, *T. whipplei*–negative water, PCR mix, and human samples were also evaluated. In cases of discrepancies between the 2 qPCR assays (mean number of instances per year, 8) or of incorrect controls (mean number of instances per year, 4), samples were submitted to additional DNA extraction and/or qPCR assays. A 1-g stool sample, a 200-μL saliva sample, 1 biopsy sample, or a 200-μL sample of body fluid was submitted for DNA extraction with the QIAamp DNA

Table 2. Evaluation of diagnosis of *Tropheryma whipplei* infection by quantitative real-time PCR from October 2003 through August 2007.

Specimen type	Result				Sensitivity, %	Specificity, %	PPV, %	NPV, %	Total no. of specimens tested
	True positive	False positive	True negative	False negative					
Duodenal biopsy	40	1	380	12	72.7	99.7	97.6	96.9	433
Gastric biopsy	3	0	12	2	60.0	100	100	85.7	17
Colonic biopsy	1	1	21	2	33.0	95.4	50.0	91.3	25
Blood	17	0	913	29	36.9	100	100	96.9	959
Adenopathy	4	0	621	1	80.0	100	100	99.8	626
CSF	17	0	578	19	47.2	100	100	96.8	614
Articular fluid	3	0	28	0	100	100	100	100	31
Cardiac valve	5	0	16	0	100	100	100	100	21
Aqueous humor	2	0	781	0	100	100	100	100	783
Urine	3	0	57	5	37.5	100	100	91.9	65
Pulmonary biopsy	1	0	1	0	NA	NA	NA	NA	2
Brain biopsy	1	0	17	0	NA	NA	NA	NA	18
Skeletal muscle biopsy	2	0	1	0	NA	NA	NA	NA	3
Saliva	26	1	431	19	57.7	99.9	96.0	95.7	450
Stool	30	7	292	5	81.0	97.6	81.0	98.3	334
Saliva or stool	38	7	733	6	84.4	99.0	84.4	99.2	784
Saliva and stool	20	1	214	12	62.5	99.5	95.2	94.6	247

NOTE. A true-positive result was defined as a positive saliva or stool specimen from a patient with Whipple disease (WD), and a false-positive result was defined as a positive saliva or stool specimen from a person without WD. A true-negative result was defined as a negative saliva or stool specimen from a person without WD, and a false-negative result was defined as positive saliva and stool specimens and a negative stool or saliva specimen from patients with WD. Sensitivity was defined as the number of true-positive results divided by the sum of true-positive and false-negative results. Specificity was defined as the number of true-negative results divided by the sum of true-negative and false-positive results. NA, not applicable; NPV, negative predictive value (defined as the number of true-negative results divided by the sum of true-negative and false-negative results); PPV, positive predictive value (defined as the number of true-positive results divided by the sum of true-positive and false-positive results).

MiniKit (Qiagen), which was used in accordance with the manufacturer's recommendations.

From October 2003 through March 2004, the *T. whipplei*–specific qPCR assay targeting a 164–base pair sequence of the bacterium incorporated the primer pairs in the reaction mix: 53.3 forward (5′-AGAGAGATGGGGTGCAGGAC-3′) and 53.3 reverse (5′-AGCCTTTGCCAGACAGACAC-3′). PCR mixes were prepared with use of a Fast-Start DNA Master SYBR Green kit (Roche), according to the manufacturer's instructions. qPCR was performed in a LightCycler thermocycler (Roche). All PCR products were sequenced as described elsewhere [7]. If the result of this first assay was positive, the result was systematically confirmed by a second qPCR using a second set of primer pairs: 342 forward (5′-AGATGATGGATCTGCTTTCTTATCTG-3′) and 492 reverse (5′-AACCCTGTCCTGCACCCC-3′). These pairs targeted a different DNA sequence with use of the same qPCR and sequencing protocol as described above.

Since April 2004, the *T. whipplei*–specific qPCR targeting a 155–base pair sequence of the bacterium incorporated the primer pair TW27 forward (5′-TGTTTTGTACTGCTTGTAACAGGATCT-3′) and TW182 reverse (5′-TCCTGCTCTATCCCTCCTATCATC-3′), and a Taqman probe (27 forward–182 reverse, 6-FAM-AGAGATACATTTGTGTTAGTTGTTACA-TAMRA) was used in the reaction mix. qPCR was performed in a LightCycler, as described elsewhere [14–16]. If the result of this first assay was positive, the result was systematically confirmed by a second PCR using a second set of primer pairs: TW13 forward (5′-TGAGTGATGGTAGTCTGAGAGATATGT) and TW163 reverse (5′-TCCATAACAAAGACAACAACCAATC). This second PCR used a Taqman probe (13 forward–163 reverse, 6-FAM-AGAAGAAGATGTTACGGGTTG-TAMRA) that targeted a different 150–base pair sequence, as described elsewhere [14–16].

Statistical methods. Sensitivity, specificity, negative predictive value, and positive predictive value (PPV) were calculated on the basis of the number of definite and excluded diagnoses. Statistical analysis was performed with use of EpiInfo, version 6.04a (Centers for Disease Control and Prevention). A statistically significant differential expression was considered when $P < .05$.

RESULTS

Description of the population. From October 2003 through August 2007, a total of 4418 samples from French patients were tested. A definite diagnosis of WD was determined for 71 pa-

Table 3. Data for saliva and stool specimens obtained from patients with classic Whipple disease (WD), patients with localized infection due to *Tropheryma whipplei,* and patients who received an excluded diagnosis of WD.

Clinical context	Specimen type, no. of positive specimens/no. of tested specimens (%)		
	Saliva	Stool	Saliva or stool
Classic WD	22/34 (65)	23/25 (92)	30/32 (94)
Localized infection	4/11 (36)	7/11 (64)	7/12 (58)
Endocarditis	1/4 (25)	1/3 (33)	1/4 (25)
Neurologic infection	1/2 (50)	2/3 (67)	2/3 (67)
Uveitis	1/2 (50)	2/2 (100)	2/2 (100)
Adenopathy	0/1 (0)	0/1 (0)	0/1 (0)
Pulmonary infection	1/1 (100)	1/1 (100)	1/1 (100)
Knee prosthesis infection	0/1 (0)	1/1 (100)	1/1 (100)
Asymptomatic carriers	1/432 (0.2)	7/299 (2.3)	7/740 (0.9)

tients, and a definite diagnosis of classic WD was determined for 55 patients, 7 of whom had experienced relapse or therapeutic failure. A definite diagnosis of localized neurologic infection was made for 6 patients, and 4 patients were classified as having possible localized neurologic infection. A definite diagnosis of endocarditis was established for 5 patients. A definite diagnosis of uveitis was made for 2 patients, and 8 patients were considered to have possible uveitis. Definite diagnoses of localized adenopathy, pulmonary infection, and knee prosthesis infection were determined for 1 patient each. Seven patients were considered to be asymptomatic carriers (*T. whipplei* was found in their stool specimens) (tables 2–4).

Saliva and stool specimens. Results for saliva and stool specimens are summarized in tables 2–4. Both saliva and stool specimens were available for 23 patients with classic WD; both samples were positive for 15 patients (65%), and both samples were negative for 1 patient (4.3%). Only stool specimens were positive for 7 patients. No patient presented with only a positive saliva specimen. For 6 patients with classic WD, only saliva specimens were available, and all were positive. For 1 patient with classic WD, only a stool specimen was available, and it was positive. For 4 of 12 patients with localized infection, qPCR results were positive for both saliva and stool specimens: 1 patient had neurologic infection, 1 had uveitis, 1 had blood culture–negative endocarditis (marked by positive results of PCR of a small-bowel biopsy specimen), and 1 had pulmonary infection. PCR results were negative for both saliva and stool specimens from 4 patients (1 had neurologic infection, and 3 had endocarditis). PCR results were positive for stool specimens and negative for saliva specimens from 3 patients (1 had neurologic infection, 1 had uveitis, and 1 had knee prosthesis infection). Finally, for 1 patient with adenopathy, PCR results were negative for saliva specimens, but PCR of a stool specimen was not performed. Seven (2.3%) of 299 patients were asymptomatic carriers with carriage in stool. All asymptomatic carriers had their saliva specimens tested, and only 1 specimen was positive. Thus, only 1 (0.2%) of 432 patients was considered to be an asymptomatic carrier with carriage in saliva. The mean bacterial load ($\pm$SD) in stool specimens was $5.7 \times 10^5 \pm 8 \times 10^5$ cfu/g (range, 85 to 2.5×10^6 cfu/g) for patients, compared with $7.4 \times 10^2 \pm 10^3$ cfu/g (range, 170–3×10^3 cfu/g) for asymptomatic carriers. Thus, the amounts of *T. whipplei* in stool samples from patients were significantly higher than those in stool samples from asymptomatic carriers ($P = .02$). A cutoff *T. whipplei* load of 10^3 cfu/g of stool had a PPV of 96% but failed to provide a diagnosis for 4 patients (2 with classic WD and 2 with localized infection). A *T. whipplei* load of 10^4 cfu/g of stool had a PPV of 100% but failed to provide a diagnosis for 6 additional patients with classic WD.

Digestive biopsy specimens. Twelve (23%) of the 52 patients who had definite infection had a small-bowel biopsy specimen negative for *T. whipplei* by qPCR, periodic acid-Schiff staining, and specific immunohistochemistry. All of these patients had no digestive symptoms, but 4 had localized neurologic infection, 3 had endocarditis, 2 had uveitis, 1 had adenitis, 1 had knee prosthesis infection, and 1 had pulmonary infection. One (0.26%) of the 380 patients who received a diagnosis of excluded WD was considered to be an asymptomatic carrier on the basis of testing of a duodenal biopsy specimen (table 4). Three gastric biopsy specimens from patients with classic WD were tested in parallel with small-bowel biopsy specimens, and both types of specimens were positive for *T. whipplei* by qPCR. Only 1 of 3 colonic biopsy specimens from patients with classic WD was positive by qPCR. In addition, 1 patient was an asymptomatic carrier (*T. whipplei* was found in a colic biopsy specimen) (table 4).

Other specimens. Seventeen (37%) of 46 blood specimens tested for patients with WD were qPCR positive. Blood specimens were negative for all patients who had specimens tested and who had localized neurologic infection, uveitis, adenopathy, or knee prosthesis infection. A positive blood qPCR result was determined for 1 of the 3 patients with endocarditis who had samples obtained. Positive CSF qPCR results were observed in several circumstances. Of the 17 patients with qPCR–positive CSF specimens, 6 presented with localized neurologic infection due to *T. whipplei,* 4 had classic WD without neurologic manifestations, 6 presented with classic WD with neurologic manifestations, and 1 had classic WD associated with uveitis. Of the 19 patients with negative specimens, 16 had classic WD without neurologic involvement, 2 had localized uveitis, and 1 had endocarditis. Four patients presented with possible localized neurologic infection due to *T. whipplei.* In our series, on the basis of testing of aqueous humor samples, 2 patients presented with definite localized uveitis due to *T. whipplei.* Eight patients were considered to have possible uveitis due to *T.*

whipplei. Four patients presented with positive results of qPCR of adenopathy samples (3 had classic WD with mesenteric adenopathies, and 1 had localized mediastinal adenopathy). One patient with localized pulmonary infection also had mediastinal adenopathy, but the specimen was qPCR negative. Three of 8 patients with classic WD had a positive urine qPCR result. For patients with localized infection, only 1 urine sample was tested, and it was qPCR negative. No asymptomatic carriage was found among 57 tested urine specimens.

DISCUSSION

To our knowledge, this is the first large case series to investigate the value of qPCR for the diagnosis of WD. The predictive value of PCR for WD is a major question because of the existence of false-positive and false-negative results and of asymptomatic carriers. Indeed, the main problem with PCR techniques is the risk of laboratory contamination. The risk is present during several steps of the PCR procedure, including during the obtainment of the samples, the isolation of the DNA, and the actual performance of the PCR assays [26, 27]. Semi-nested and nested PCR, which are associated with a high risk of contamination, should be avoided [28]. The validity of the data reported here is based on strict experimental procedures and controls, including rigorous positive and negative controls to validate the test. In addition, each positive PCR result was confirmed by the successful amplification of an additional DNA sequence. Thus, we believe that our results are valid, because they were cautiously checked, and we systematically tested the positive samples with use of an additional DNA target and performed an additional DNA extraction for each doubtful specimen.

There is also the problem of false-negative results attributable to the presence of inhibitors or low amounts of bacteria in the specimen. In this case, either all of the PCR assay results are negative, or only the result of the most sensitive PCR assay is positive. The latter situation may lead to a false-negative diagnosis. If the diagnosis of WD is ruled out, consequences may be dramatic, as suggested by the death of a patient for whom results of 1 of 3 PCR assays of joint fluid samples and duodenal biopsy samples were positive but for whom the result of periodic acid-Schiff staining of a duodenal biopsy sample was negative [29]. The positive PCR results were neglected, and a systemic clinical disorder of unknown origin was assumed, leading to treatment with indomethacin and prednisone. The patient died of multiorgan failure, but the autopsy revealed WD. Therefore, when only 1 of the 2 results of PCR of a CSF or aqueous humor specimen was confirmed to be positive, we preferred to consider the diagnosis of a localized infection linked to *T. whipplei* instead of excluding the diagnosis.

In our study, asymptomatic carriage of *T. whipplei* DNA in saliva and stool in the general population was less frequent than was reported elsewhere [3, 15, 18, 19]. In our study, only 2.3% of patients without WD were positive for *T. whipplei*. However, this carriage has been more frequently reported in sewer workers (12%–25%) and patients with hepatitis or cirrhosis than in the general population [15, 30]. Carriage in saliva also occurs less frequently (0.2%) in the general population than in sewer workers (2.2%) and has been observed only in those with carriage in stool [3, 15]. Presumptive diagnosis of WD should be highly considered when patients present with both saliva and stool specimens positive for *T. whipplei* by qPCR, which has a PPV of 95.2%. In addition, if the bacterial load in stool is $>10^4$ cfu/g, the PPV is 100%, and *T. whipplei*–specific PCR of blood, gastric, and small-bowel biopsy specimens should be performed. Conversely, classic WD is unlikely in patients with saliva or stool specimens negative for *T. whipplei* by qPCR, with a negative predictive value of 99.2%, and additional specific investigations for classic WD could be stopped. A diagnostic strategy for classic WD, depending on results of qPCR of saliva and stool specimens, is proposed in figure 1. Finally, the fact that bacterial loads in stool specimens from carriers are low could explain the divergences of prevalence estimations between studies.

Classic WD is often suspected but is rarely diagnosed. A screening test by noninvasive qPCR of saliva and stool specimens may help to select the patients for whom testing of digestive biopsy specimens should be performed to confirm the diagnosis. The PPV for testing of duodenal biopsy specimens was 97.5% in our series. Asymptomatic carriage of *T. whipplei* DNA in small-bowel specimens was reported in 5% of patients in 1 study [19], but these data have not been reproduced among other large series [13, 22]. In our series, the percentage of patients with asymptomatic carriage of *T. whipplei* in duodenal biopsy specimens was estimated to be 0.26%. Gastric biopsy specimens should be tested in parallel with small-bowel biopsy specimens. Testing of gastric biopsy specimens presents highly specific results. Even if our data show that such testing may lack sensitivity, it is important to underline that 1 recent report revealed that it may be helpful for the diagnosis when testing of small-bowel biopsy specimens failed [31]. Our data confirm that colonic biopsy specimens are not useful samples for the diagnosis of classic WD, because testing of such samples lacks both sensitivity and specificity.

Since October 2003, of the 71 WD diagnoses made, 16 (22.5%) corresponded to localized infection due to *T. whipplei*. The diagnosis of such cases without specific histological involvement is difficult and may be delayed. In this series, 1 of the 5 patients with *T. whipplei* endocarditis presented with a positive result of PCR of a duodenal biopsy specimen but negative results of periodic acid-Schiff staining and specific immunohistochemistry. It is uncertain whether these patients with localized infections will never have digestive involvement or

Table 4. PCR results for 71 patients with definite Whipple disease (WD) and 7 asymptomatic carriers.

Patient(s), sample type	PCR result		Total no. of samples tested
	Positive	Negative	
55 Patients with definite classic WD[a]			
Duodenal biopsy	39	0	39
Gastric biopsy	3	0	3
Colonic biopsy	1	2	3
Blood	16	19	35
Adenopathy	3	0	3
CSF	11[b]	15	26
Articular fluid	1	0	1
Saliva	22	12	34
Stool	23	2	25
Saliva or stool	30	2	32
Saliva and stool	16	8	24
Urine	3	5	8
Skeletal muscle biopsy	2	0	2
6 Male patients with definite localized neurologic infection due to *Tropheryma whipplei*[c]			
Duodenal biopsy	0	4	4
Blood	0	3	3
CSF	6	0	6
Brain biopsy	1	0	1
Saliva	1	1	2
Stool	2	1	3
Saliva or stool	2	1	3
Saliva and stool	1	1	2
5 Male patients with definite infective endocarditis due to *T. whipplei*[d]			
Duodenal biopsy	1	4	5
Gastric biopsy	0	1	1
Jejuno-ileum biopsy	0	1	1
Blood	1	2	3
Cardiac valve	5	0	5
CSF	0	2	2
Saliva	1	3	4
Stool	1	2	3
Saliva or stool	1	3	4
Saliva and stool	1	2	3
Urine	0	1	1
2 Patients with definite uveitis due to *T. whipplei*[e]			
Duodenal biopsy	0	2	2
Gastric biopsy	0	1	1
Blood	0	2	2
CSF	0	2	2
Aqueous humor	2	0	2
Saliva	1	1	2
Stool	2	0	2
Saliva or stool	2	0	2
Saliva and stool	1	0	2
A 39-year-old man with definite adenopathy due to *T. whipplei*			
Duodenal biopsy	0	1	1
Adenopathy	1	0	1
Saliva	0	1	1
Saliva or stool	0	1	1

(continued)

Table 4. ***(Continued.)***

Patient(s), sample type	PCR result		Total no. of samples tested
	Positive	Negative	
Saliva and stool	NA	NA	NA
A 54-year-old woman with definite pulmonary infection due to *T. whipplei*			
Duodenal biopsy	0	1	1
Blood	0	1	1
CSF	0	1	1
Mediastinal adenopathy	0	1	1
Saliva	1	0	1
Stool	1	0	1
Saliva or stool	1	0	1
Saliva and stool	1	0	1
A 73-year-old man with definite knee prosthesis infection due to *T. whipplei*			
Duodenal biopsy	0	1	1
Blood	0	1	1
Articular fluid	1	0	1
Saliva	0	1	1
Stool	1	0	1
Saliva or stool	1	0	1
Saliva and stool	0	1	1
7 Asymptomatic carriers of *T. whipplei*[f]			
Duodenal biopsy	1[g]	4	5
Colic biopsy	1[h]	0	1
Blood	0	3	3
CSF	0	2	2
Saliva	1	6	7
Stool	7	0	7
Saliva or stool	7	0	7
Saliva and stool	1	6	7

[a] Fifteen female patients and 40 male patients (mean age ± SD, 57 ± 13 years; range, 32–79 years).
[b] Four samples from patients with classic WD who did not have clinical neurologic manifestations were positive.
[c] Mean age ± SD, 47 ± 8.7 years (range, 36–59 years).
[d] Mean age ± SD, 66 ± 5.4 years (range, 57–70 years).
[e] A 78-year-old female patient and a 80-year-old male patient.
[f] Four male and 3 female patients (mean age ± SD, 50 ± 14 years; range, 30–67 years). None of the asymptomatic carriers developed WD later.
[g] The sample was obtained from a 67-year-old woman with myeloma who had positive quantitative real-time PCR results but negative results of periodic acid-Schiff staining and specific immunohistochemistry of a duodenal biopsy sample. A stool specimen obtained from the woman was also PCR positive, but her saliva specimen was negative.
[h] The sample was obtained from a 47-year-old man infected with HIV and hepatitis C virus who presented with chronic diarrhea. A stool specimen obtained from the man was positive for *T. whipplei* by quantitative real-time PCR, but his saliva, duodenal biopsy, and gastric biopsy specimens were negative. Histological analysis of his colonic biopsy specimen revealed chronic colitis, but results of periodic acid-Schiff staining and specific immunohistochemistry were negative. No other potential pathogens (parasites, viruses, or bacteria) were found.

whether they will develop it later. Indeed, it is possible that, at the beginning of WD onset, digestive involvement is sparse and focal, leading to diagnostic difficulties. Later, when the digestive form has developed, the diagnosis is more easily determined.

Although qPCR of saliva and stool specimens is useful for diagnosis of classic WD, it is not efficient for diagnosis of localized infection, for which the samples tested will depend on the clinical manifestations. For localized endocarditis, a blood sample should be tested. One case of asymptomatic carriage in the blood was reported, but it was never confirmed [20]. In our study, the PPV of PCR of blood samples was 100%, and the main limitation of testing of blood samples is the lack of sensitivity [32]. Low amounts of *T. whipplei* DNA in blood and the presence of PCR inhibitors may explain this low sensitivity. Only 1 of 3 blood specimens from patients with localized endocarditis was positive for *T. whipplei,* underlining that it is still difficult to establish the diagnosis of this clinical entity. Currently, diagnosis is usually obtained with use of cardiac valve samples, removed during surgical procedures, that are tested mainly by broad-spectrum PCR targeting the 16S rRNA sequence, followed by sequencing.

Diagnosis of localized neurologic infection is still difficult. Clinical manifestations lack specificity and encompass a large spectrum of neurology [3, 33, 34]. PCR of a CSF specimen is

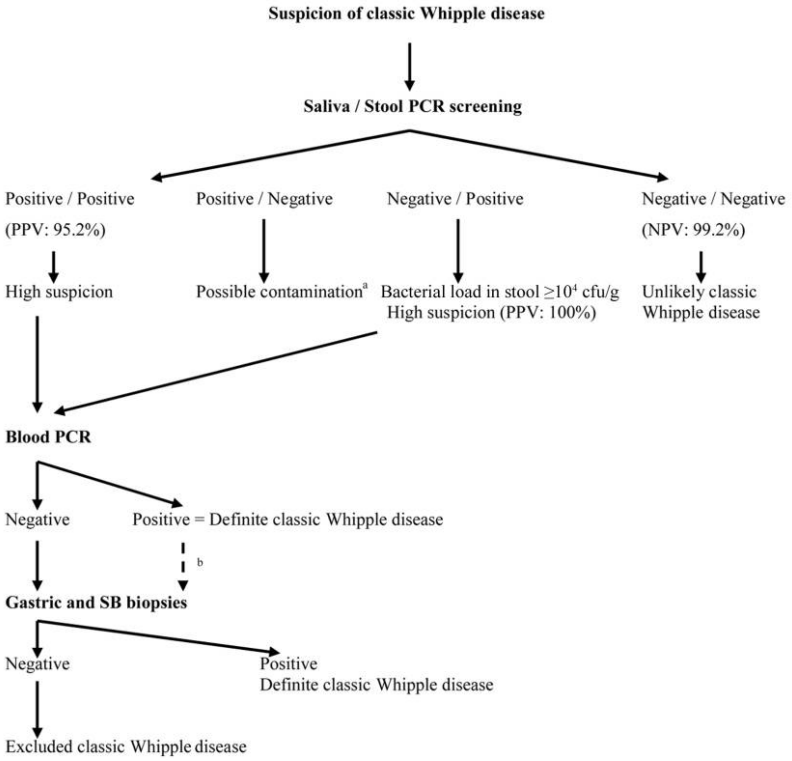

Figure 1. Diagnostic strategy for classic Whipple disease, depending on results of quantitative real-time PCR of saliva and stool specimens. PCR results were considered to be positive when results of 2 PCR assays using 2 different DNA targets were positive. NPV, negative predictive value; PPV, positive predictive value; SB, small bowel. [a]Check for laboratory contamination and test another time. [b]Gastric and SB biopsies should be performed to see the extent of the lesions and to have a point of comparison for the follow-up.

highly specific, but it may lack sensitivity, as suggested by the discrepant results of qPCR of CSF specimens from 4 patients. When the result of CSF PCR is negative but a brain lesion is observed on MRI or CT, a brain biopsy can be performed to obtain a specimen for specific PCR analysis and immunohistochemistry. 16S rRNA PCR assay of CSF and brain biopsy specimens could also be useful, but this technique is less sensitive than qPCR [10]. Diagnosis of *T. whipplei* uveitis is difficult, because the amount of aqueous humor specimen is low, and testing of such specimens may lack sensitivity, as suggested by the discrepant results of our qPCR assays for 8 patients. In addition, after the first obtainment of samples, additional aqueous humor samples cannot be obtained for additional testing. Analysis of CSF samples did not help with the diagnosis of *T. whipplei* uveitis in our series. It is important to emphasize that 1 of the 2 diagnoses of definite *T. whipplei* uveitis was determined by molecular screening by 16S rRNA PCR. This diagnostic strategy by broad-spectrum PCR also allowed the diagnosis of localized pulmonary and knee prosthesis infection. Thus, in the context of a positive 16S rRNA PCR result, specific *T. whipplei* qPCR is useful to confirm the diagnosis.

When classic WD is suspected, qPCR screening of saliva and stool specimens should be performed first. When results of both tests are positive, diagnosis of WD is highly suspected, especially if bacterial load in stool is $>10^4$ cfu/g. When the results of the tests are negative, classic WD is unlikely. Localized disease, which accounts for nearly one-quarter of cases, can be suspected in a broad spectrum of clinical manifestations. In this case, qPCR of saliva and stool specimens lacks sensitivity. Broad-spectrum and specific qPCR assays, with use of specimens obtained on the basis of clinical manifestations, are necessary.

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References

1. Whipple GH. A hitherto undescribed disease characterized anatomically by deposits of fat and fatty acids in the intestinal and mesenteric lymphatic tissues. Bull Johns Hopkins Hosp **1907**; 18:382–91.
2. Marth T, Raoult D. Whipple's disease. Lancet **2003**; 361:239–46.
3. Fenollar F, Puéchal X, Raoult D. Whipple's disease. New Engl J Med **2007**; 356:55–66.

4. Fenollar F, Raoult D. Whipple's disease. Clin Diagn Lab Immunol **2001**; 8:1–8.
5. Dutly F, Altwegg M. Whipple's disease and "*Tropheryma whippelii.*" Clin Microbiol Rev **2001**; 14:561–83.
6. Raoult D, Birg M, La Scola B, et al. Cultivation of the bacillus of Whipple's disease. N Engl J Med **2000**; 342:620–5.
7. La Scola B, Fenollar F, Fournier PE, Altwegg M, Mallet MN, Raoult D. Description of *Tropheryma whipplei* gen. nov., sp. nov., the Whipple's disease bacillus. Int J Syst Evol Microbiol **2001**; 51:1471–9.
8. Raoult D, Ogata H, Audic S, et al. *Tropheryma whipplei* Twist: a human pathogenic Actinobacteria with a reduced genome. Genome Research **2003**; 13:1800–9.
9. Bentley SD, Maiwald M, Murphy LD, et al. Sequencing and analysis of the genome of the Whipple's disease bacterium *Tropheryma whipplei.* Lancet **2003**; 361:637–44.
10. Fenollar F, Fournier PE, Robert C, Raoult D. Use of genome selected repeated sequences increases the sensitivity of PCR detection of *Tropheryma whipplei.* J Clin Microbiol **2004**; 42:401–3.
11. Wilson KH, Blitchington R, Frothingham R, Wilson JA. Phylogeny of the Whipple's disease–associated bacterium. Lancet **1991**; 338:474–5.
12. Relman DA, Schmidt TM, MacDermott RP, Falkow S. Identification of the uncultured bacillus of Whipple's disease. N Engl J Med **1992**; 327:293–301.
13. Fenollar F, Fournier PE, Raoult D, Gerolami R, Lepidi H, Poyart C. Quantitative detection of *Tropheryma whipplei* DNA by real-time PCR. J Clin Microbiol **2002**; 40:1119–20.
14. Raoult D, Fenollar F, Birg ML. Culture of *Tropheryma whipplei* from the stool of a patient with Whipple's disease. N Engl J Med **2006**; 355: 1503–5.
15. Fenollar F, Trani M, Davoust B, et al. Carriage of *Tropheryma whipplei* in stools of sewer workers and human controls, but not in monkeys and apes. J Infect Dis **2008**; 197:880–7.
16. Rolain JM, Fenollar F, Raoult D. False positive PCR detection of *Tropheryma whipplei* in the saliva of healthy people. BMC Microbiol **2007**; 7:48.
17. Dutly F, Hinrikson HP, Seidel T, Morgenegg S, Altwegg M, Bauerfeind P. *Tropheryma whippelii* DNA in saliva of patients without Whipple's disease. Infection **2000**; 28:219–22.
18. Street S, Donoghue HD, Neild GH. *Tropheryma whippelii* DNA in saliva of healthy people. Lancet **1999**; 354:1178–9.
19. Ehrbar HU, Bauerfeind P, Dutly F, Koelz HR, Altwegg M. PCR-positive tests for *Tropheryma whippelii* in patients without Whipple's disease. Lancet **1999**; 353:2214.
20. Misbah SA, Stirzaker D, Ozols B, Franks A, Mapstone NP. Anonymous survey of blood donors by polymerase chain reaction for *Tropheryma whippelii.* QJM **1999**; 92:61.
21. Maibach RC, Dutly F, Altwegg M. Detection of *Tropheryma whipplei* DNA in feces by PCR using a target capture method. J Clin Microbiol **2002**; 40:2466–71.
22. Maiwald M, von Herbay A, Persing DH, et al. *Tropheryma whippelii* DNA is rare in the intestinal mucosa of patients without other evidence of Whipple disease. Ann Intern Med **2001**; 134:115–9.
23. Amsler L, Bauernfeind P, Nigg C, Maibach RC, Steffen R, Altwegg M. Prevalence of *Tropheryma whipplei* DNA in patients with various gastrointestinal disease and in healthy controls. Infection **2003**; 31:81–5.
24. Lepidi H, Fenollar F, Gerolami R, et al. Whipple's disease: immunospecific and quantitative immunohistochemical study of intestinal biopsy specimens. Hum Pathol **2003**; 34:589–96.
25. Lepidi H, Fenollar F, Dumler JS, et al. Cardiac valves in patients with Whipple endocarditis: microbiological, molecular, quantitative histologic, and immunohistochemical studies of 5 patients. J Infect Dis **2004**; 190:935–45.
26. Peters RP, van Agtmael MA, Danner SA, Savelkoul PH, Vandenbroucke-Grauls CM. New developments in the diagnosis of bloodstream infections. Lancet Infect Dis **2004**; 4:751–60.
27. Fenollar F, Raoult D. Molecular genetic methods for the diagnosis of fastidious microorganisms. APMIS **2004**; 112:785–807.
28. Apfalter P, Reischl U, Hammerschlag MR. In-house nucleic acid amplification assays in research: how much quality control is needed before one can rely upon the results? J Clin Microbiol **2005**; 43:5835–41.
29. Müller SA, Vogt P, Altwegg M, Seebach JD. Deadly carousel or difficult interpretation of new diagnostic tools for Whipple's disease: case report and review of the literature. Infection **2005**; 33:39–42.
30. Schoniger-Hekele M, Petermann D, Weber B, Muller C. *Tropheryma whipplei* in the environment—survey of sewage plant influxes and sewage plant workers. Appl Environ Microbiol **2007**; 73:2033–5.
31. Pruss H, Katchanov J, Zschenderlein R, Loddenkemper C, Schneider T, Moos V. A patient with cerebral Whipple's disease with gastric involvement but no gastrointestinal symptoms—a consequence of local protective immunity? J Neurol Neurosurg Psychiatry **2007**; 78:896–8.
32. Marth T, Fredericks D, Strober W, Relman D. Limited role for PCR-based diagnosis of Whipple's disease from peripheral blood mononuclear cells. Lancet **1996**; 348:66–7.
33. Louis ED, Lynch T, Kaufmann P, Fahn S, Odel J. Diagnostic guidelines in central nervous system Whipple's disease. Ann Neurol **1996**; 40: 561–8.
34. Gerard A, Sarrot-Reynauld F, Liozon E, et al. Neurologic presentation of Whipple disease: report of 12 cases and review of the literature. Medicine (Baltimore) **2002**; 81:443–57.