

# The Rise of *Tropheryma whippiei*: a 12-Year Retrospective Study of PCR Diagnoses in Our Reference Center

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*Tropheryma whippiei* is the causative agent of classic Whipple's disease (WD) and other clinical entities, such as localized infection. Asymptomatic carriers have also been reported, mainly based on the testing of fecal samples. Our objective was to undertake a retrospective analysis of molecular biology usage for the diagnosis of WD over a 12-year period in our reference center. We tested 27,923 samples from 15,473 patients. The number of patients tested and the number of patients with a positive PCR result for *T. whippiei* have increased significantly over the last 12 years ( $P < 0.0001$ ). Overall, *T. whippiei* was more frequently recovered from stools (43%), saliva (15%), duodenal biopsy samples (12.5%), blood (5%), and cerebrospinal fluid (CSF) (6%) and less commonly from cardiac valves (3%), urine (0.5%), skin biopsy samples (1%), lymph nodes (2.5%), aqueous humor (0.5%), and intra-articular fluid (1%). Among all the positive samples, we observed that stool samples and skin biopsy samples exhibited a higher prevalence of positivity by real-time quantitative PCR (qPCR) at 10.07% and 15.4%, respectively. The number of patients with a positive PCR result for *T. whippiei* has increased significantly over the last 12 years, although the positive ratio has not changed. Improvements in diagnostic tools have contributed greatly toward greater knowledge of WD and, consequently, the interest of physicians in this condition. In addition, we propose here an update of the diagnostic strategy for WD when qPCR is being used.

Whipple's disease (WD) is an infectious chronic disease caused by the bacterium *Tropheryma whippiei*. Classic Whipple's disease is characterized by histological lesions in the gastrointestinal tract, and diagnosis is made by a positive periodic acid-Schiff reaction in macrophages from a duodenal biopsy specimen (11). The clinical findings are principally arthralgia and digestive symptoms, such as weight loss, malabsorption, and chronic diarrhea. Other diverse clinical manifestations that could be associated with classic WD (2, 11, 14) are neurologic involvement (6 to 63% of cases), cardiac involvement (17 to 55% of cases), and pulmonary involvement (30 to 40% of cases) (9). We also observed lymphadenopathy, ocular signs, and cutaneous manifestations (13). In addition, recent studies have shown that the spectrum of infections due to *T. whippiei* is wide and can include localized infection occurring in the absence of small bowel involvement, such as neurological infection, pneumonia, endocarditis, uveitis, and spondylodiscitis (11). In addition, *T. whippiei* has also been observed in asymptomatic carriers, mainly in fecal and saliva samples (3, 10, 17). In Europe, the prevalence of this bacterium in fecal samples from the general healthy adult population is estimated to be 1% to 11% (8). *T. whippiei* has also been responsible for gastroenteritis in children (16). In one study, the bacterium was detected in 15% of fecal samples from children aged 2 to 4 years presenting with gastroenteritis (16). Moreover, this emerging pathogen has also been responsible for bacteremia in febrile patients from rural Senegal (8).

The emergence of this infectious disease is based on three main observations: the true incidence, the improvement of diagnostic tools, and the focus that physicians place on disease research. In 2000, *T. whippiei* was cultured for the first time (15), which allowed us to develop new tools for optimizing the molecular diagnosis of WD (5, 7). In particular, genome sequencing has aided in the identification of DNA targets for PCR assays (6).

Our objective was to undertake a retrospective analysis of PCR

usage over a 12-year period in our reference center, which has become a world reference laboratory for the diagnosis of WD.

## MATERIALS AND METHODS

The samples included in our study were received between January 2000 and December 2011 from France and other countries. The molecular detection of *T. whippiei* was performed using real-time quantitative PCR (qPCR) as described previously (7). Prior to October 2001, conventional PCR was used. The QIAamp DNA kit (Qiagen, Courtabouef, France) was used to extract DNA from the samples according to the manufacturer's recommendations. qPCR was performed with a LightCycler instrument (Roche Diagnostics, Meylan, France) with the QuantiTect Probe PCR kit. Since 2011, qPCR has been performed with the CFX96 Touch detection system (Bio-Rad, Marnes-la-Coquette, France). From October 2001 to September 2003, the specimens were analyzed by targeting the 16S to 23S rRNA gene intergenic spacer and the *rpoB* gene, as described elsewhere (5). When an amplified product was detected, sequencing was also systematically performed (12). From October 2003 through March 2004, the specimens were tested by targeting repeated sequences of *T. whippiei*, as reported previously (6). Since April 2004, these repeated sequences were detected using specific oligonucleotide TaqMan probes for *T. whippiei* identification (7). A case was defined by 2 positive qPCR results in assays targeting 2 different *T. whippiei* DNA sequences. The Twist-Marseille strain of *T. whippiei* was used as the positive control, and sterile water was used as the negative control. The human actin gene was also detected in parallel to verify the quality of the extracted DNA.

For data comparison, standard statistical software was used for statistical analyses, and the Student *t* test,  $\chi^2$  test, or Pearson coefficient correlation test was used when indicated; differences were considered statistically significant at a *P* value of  $<0.05$ .

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**RESULTS**

We tested 27,923 samples from 15,473 patients. A total of 1,241 samples (4.44% of all samples) from 717 patients (4.63% of all patients) were positive (Table 1), 571 patients were newly diagnosed with a positive PCR result for *T. whipplei*, and 103 patients were followed regularly in our laboratory. Since 2000, the number of samples received in our laboratory has significantly increased. We tested 230 samples in the year 2000 and 6,289 in 2011 ( $P < 0.0001$ ). The numbers of positive samples and diagnosed patients per year have significantly increased since 2000. We had 10 positive samples in 2000 versus 237 in 2011 ( $P < 0.0001$ ). Ten patients were diagnosed in 2000 versus 175 patients in 2011 ( $P < 0.0001$ ). During the 12-year period, the proportion of positive samples per year was 4.09%. The lower rates were 0.68% in 2001 and 0.78% in 2002, while the higher rates were 6.62% in 2005 and 6.07% in 2009. The proportion of positive patients per year also remained stable at 4.16% per year, with lower rates of 0.82% and 0.95% in 2001 and 2002, respectively, and a higher rate of 5.75% in 2005.

Among the 27,923 samples we received, 10,808 (38.71%) were from Marseille, 16,310 (58.41%) were from other parts of France, and 805 (2.88%) were from other countries, mainly Germany (32%), Belgium (16%), Italy (12.8%), Portugal (6.6%), Switzerland (6.2%), and the United States (5.3%). A significantly higher proportion of the samples from other countries were positive for *T. whipplei* at 9.1% (73/805) than from Marseille at 4.5% (483/10,808) and other parts of France at 4.2% (685/16,310) ( $P < 0.0001$ ).

Since 2004, the samples received in our laboratory have become more diverse. Until 2003, we received mainly duodenal biopsy samples, lymph nodes, cerebrospinal fluid (CSF), aqueous humor, and blood, but since 2004, we have received more saliva, fecal, urine, intra-articular fluid, and cardiac samples. Skin biopsy specimens were received from patients, mainly in 2008, in accordance with our recommendations (1). During the 12-year period, we increasingly received blood (21%), stool (19%), saliva (15%), duodenal biopsy samples (8%), and lymph nodes (9%). Overall, *T. whipplei* was more frequently recovered from stool (43%), saliva (15%), duodenal biopsy samples (12.5%), blood (5%), and CSF (6%) and less commonly from cardiac valves (3%), urine (0.5%), skin biopsy specimens (1%), lymph nodes (2.5%), aqueous humor (0.5%), and intra-articular fluid (1%).

We observed that stool and skin biopsy samples exhibited a higher prevalence of positivity by qPCR at 10.07% and 15.4%, respectively, than the other sample types, whereas the prevalences in duodenal biopsy samples, brain abscesses, intra-articular fluid, and cardiac valves were 7.24%, 5.9%, 5.9%, and 7.28%, respectively. The lower ratios may be explained by the samples' lack of sensitivity to qPCR detection or by a recruitment bias. The samples of saliva, blood, CSF, lymph nodes, aqueous humor, and urine had a low percentage of positivity at <5%.

**DISCUSSION**

qPCR is routinely performed in our laboratory, principally on stools and saliva, which are the noninvasive samples recommended for first-line screening when WD is suspected. The validity of the PCR results reported here is based on strict experimental protocols and controls, including positive and negative controls to validate the qPCR assay. In addition, each positive result was confirmed by the amplification of a second gene of *T. whipplei* to avoid false-positive results.

**TABLE 1** Diversity of samples received in our laboratory and positive samples per year between January 2000 and December 2011

Year	Stools <sup>a</sup>	Saliva <sup>a</sup>	Skin biopsy specimens <sup>a</sup>	Blood <sup>a</sup>	Duodenal biopsy specimens <sup>a</sup>	CSF <sup>a</sup>	Brain abscesses <sup>a</sup>	Lymph nodes <sup>a</sup>	Aqueous humor <sup>a</sup>	Intra-articular fluid <sup>a</sup>	Urine <sup>a</sup>	Cardiac valves <sup>a</sup>	Others <sup>a</sup>	Total <sup>b</sup>	Patients <sup>c</sup>
2000	0/0 (0)	0/0 (0)	0/1 (0)	1/7 (14.29)	5/54 (9.26)	0/14 (0)	0/4 (0)	1/108 (0.93)	0/21 (0)	0/0 (0)	0/0 (0)	0/3 (0)	3/18 (16.67)	10/230 (4.3)	10/208 (4.81)
2001	0/4 (0)	0/25 (0)	0/1 (0)	0/20 (0)	3/176 (1.70)	0/9 (0)	0/1 (0)	0/139 (0)	0/5 (0)	0/2 (0)	0/0 (0)	0/6 (0)	0/51 (0)	3/439 (0.68)	3/368 (0.82)
2002	0/1 (0)	0/5 (0)	0/1 (0)	0/49 (0)	1/61 (1.64)	0/15 (0)	0/0 (0)	0/140 (0)	0/233 (0)	0/4 (0)	0/0 (0)	(18.18)	2/117 (1.71)	5/637 (0.78)	5/526 (0.95)
2003	2/23 (8.70)	3/32 (9.37)	0/1 (0)	4/135 (2.96)	4/77 (5.19)	1/170 (15.7)	0/2 (0)	0/163 (0)	1/225 (0.44)	0/2 (0)	0/0 (0)	2/4 (50.00)	12/98 (12.24)	39/832 (4.69)	23/597 (3.85)
2004	5/86 (5.81)	4/174 (2.30)	0/4 (0)	3/330 (0.90)	11/135 (8.15)	11/229 (4.80)	1/3 (33.3)	2/195 (1.02)	3/235 (1.28)	0/2 (0)	0/1 (0)	4/11 (36.36)	17/170 (10.00)	61/1,575 (3.87)	37/777 (4.76)
2005	45/168 (26.79)	8/204 (3.92)	0/3 (0)	10/407 (2.46)	16/175 (9.14)	9/384 (2.34)	0/3 (0)	14/168 (8.33)	0/158 (0)	3/10 (30.00)	1/2 (50.00)	2/8 (25.00)	13/139 (9.35)	121/1,829 (6.62)	47/818 (5.75)
2006	47/250 (18.80)	12/290 (4.14)	0/2 (0)	9/455 (1.98)	17/204 (8.33)	13/245 (5.31)	0/4 (0)	1/196 (0.51)	2/255 (0.78)	0/7 (0)	0/34 (0)	7/12 (58.33)	3/86 (3.49)	111/2,040 (5.44)	63/1,130 (5.58)
2007	45/267 (16.85)	24/335 (7.16)	1/6 (16.67)	6/472 (1.27)	15/178 (8.43)	6/228 (2.63)	0/2 (0)	1/240 (0.42)	0/265 (0)	3/16 (18.75)	2/42 (0.83)	4/20 (20.00)	9/145 (6.21)	116/2,216 (5.23)	50/1,242 (4.03)
2008	65/1,207 (5.39)	20/368 (5.43)	4/15 (26.67)	10/581 (1.72)	13/186 (6.98)	5/203 (2.46)	0/8 (0)	0/253 (0)	1/145 (0.69)	1/6 (16.67)	0/69 (0)	3/57 (5.26)	6/144 (4.17)	128/3,242 (3.95)	79/1,806 (4.37)
2009	128/1,125 (11.37)	31/691 (4.49)	6/21 (28.57)	5/807 (0.62)	27/273 (9.89)	8/249 (3.21)	0/1 (0)	0/246 (0)	1/82 (1.22)	2/23 (8.70)	1/125 (0.8)	1/64 (1.56)	33/283 (11.31)	242/3,990 (6.07)	121/2,197 (5.51)
2010	74/917 (8.07)	30/915 (3.38)	2/8 (25.00)	4/1,100 (0.36)	21/312 (6.73)	5/278 (1.80)	0/2 (0)	2/241 (0.83)	0/72 (0)	1/48 (2.08)	1/261 (0.38)	6/78 (7.69)	22/372 (5.91)	168/4,604 (3.65)	104/2,413 (4.31)
2011	121/1,237 (9.78)	50/1,165 (4.29)	1/28 (3.57)	11/1,583 (0.69)	22/309 (7.12)	4/261 (1.53)	1/4 (25.00)	9/358 (2.51)	0/269 (0)	4/114 (3.51)	1/356 (0.28)	3/193 (1.55)	10/412 (2.43)	237/6,289 (3.77)	175/3,391 (5.16)
Total	532/5,285 (10.07)	182/4,204 (4.3)	14/91 (15.4)	63/5,946 (1.06)	155/2,140 (7.24)	72/2,185 (3.3)	2/34 (5.9)	30/2,447 (1.2)	8/1,965 (0.4)	14/234 (5.9)	6/890 (0.7)	34/467 (7.28)	129/2,035 (6.3)	1,241/27,923 (4.44)	717/15,473 (4.63)

<sup>a</sup> Number of positive samples/total number of samples received (percentage).  
<sup>b</sup> Total number of positive samples/total number of samples received (percentage).  
<sup>c</sup> Number of patients with a positive result/total number of patients (percentage).

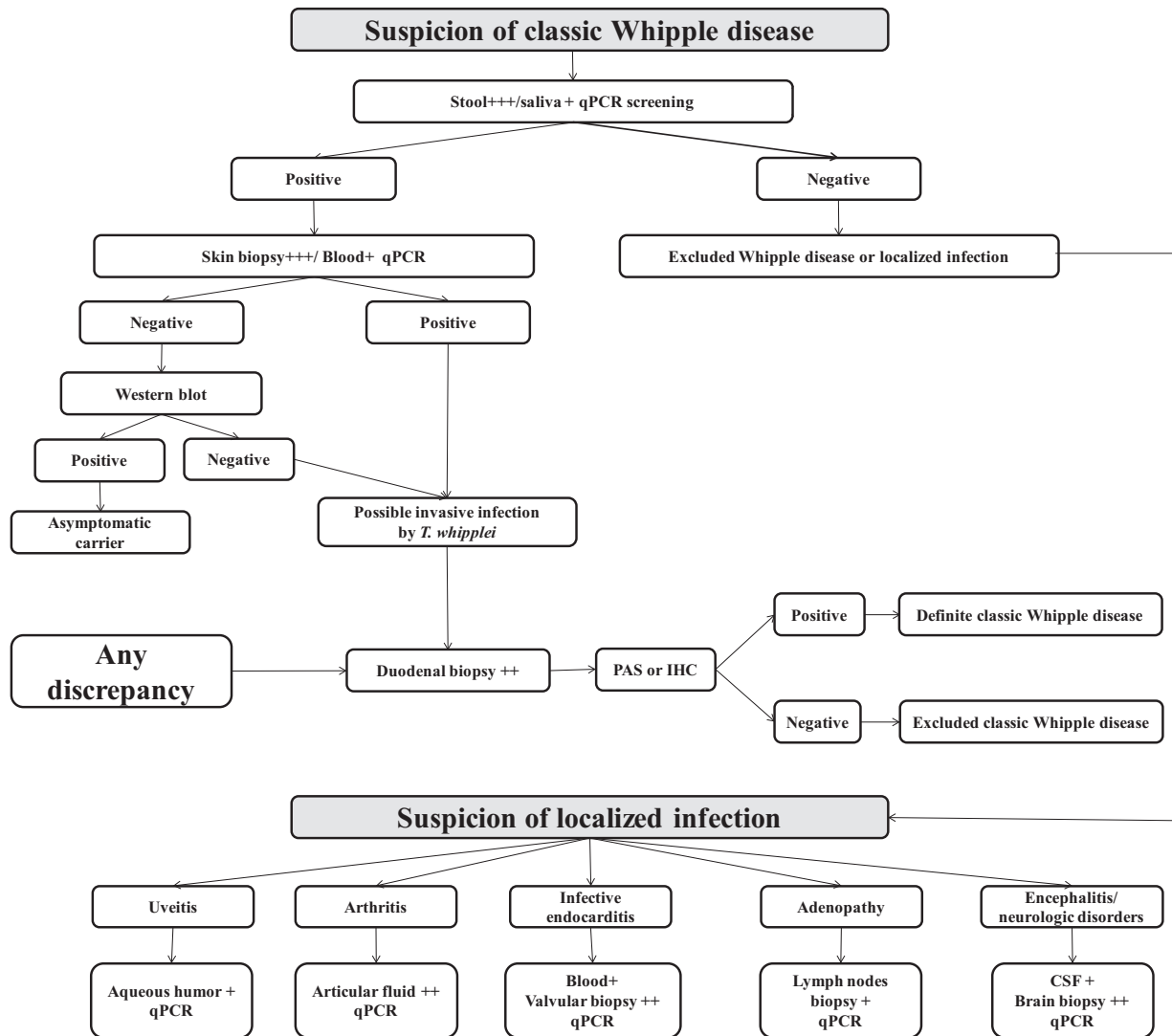


FIG 1 Strategy for the diagnosis of classic, localized carriage of Whipple's disease based on qPCR results. We identified samples with a high prevalence of positivity (>10%) (+++), with a moderate prevalence of positivity (5 to 10%) (++), and with a low percentage of positivity (<5%) (+). PAS, periodic acid-Schiff stain; IHC, immunohistochemistry.

Here, we propose an update of the diagnostic strategy for WD when using PCR (Fig. 1). When qPCR results indicate positivity in stool and/or saliva samples, the diagnosis must be completed by qPCR on more invasive samples, such as blood, a skin biopsy specimen, or others, in accordance with clinical findings. Digestive biopsies were performed to confirm classic WD and for patient follow-up. Digestive biopsy specimens were also used to complete the diagnosis using immunohistochemical analysis or periodic acid-Schiff staining to define classic WD or other clinical entities (11, 13). Skin biopsies were performed in an area under the arm, thus avoiding the possibility of fecal or salivary contamination. The skin may be a reservoir for *T. whipplei* in patients with classical WD without skin manifestations; therefore, skin biopsy specimens are non-invasive samples that highly contribute to the diagnosis of WD (1). To confirm a localized infection, we recommended performing qPCR and histological investigation on the appropriate samples (for example, valvular biopsy specimens,

intra-articular fluid, aqueous humor, CSF, and lymph nodes). For the patients with exclusive positive qPCR results from fecal and/or saliva samples, only serological testing with Western blotting is able to differentiate asymptomatic carriers from patients with possible infection by *T. whipplei* (4). Paradoxically, the patients with classic WD presented a lack of or a lower reactivity in serological tests than the asymptomatic carriers (4).

The number of positive cases of WD has significantly increased over the last 12 years in our reference center, although the positive ratio has not changed. Real-time qPCR is a useful, sensitive, specific, and rapid tool for the screening and diagnosis of WD (7, 11). Between 2000 and 2011, the activity of our laboratory increased 30-fold for specific qPCR for *T. whipplei*. The development of better diagnostic tools, such as qPCR, has greatly contributed to the emergence and better knowledge of the fastidious bacterium *T. whipplei* and, consequently, has intensified physician interest in WD.

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