

## Article

# *Tropheryma whipplei* in immunocompromised children in Iran: a preliminary study

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## Abstract:

**Background:** *Tropheryma whipplei* is the causative pathogen of Whipple's disease and other acute and chronic manifestations. Children have been identified as reservoirs of this bacterium especially in low-middle income countries. No information is currently available on the dissemination of *T. whipplei* in Iran. Therefore, the aim of this study was to investigate the presence of *T. whipplei* in children with immunodeficiency. **Methods:** This cross-sectional study was performed from July 2018 to February 2019 in Qom province (central Iran). Stool samples were collected from immunocompromised children. *T. whipplei* was tested by SYBR Green and Taq-Man Real-time PCR assays. For confirmation, sequencing of the isolated bacteria was done. **Results:** One hundred and 30 children with a mean age of 56.7 months were enrolled. Acute lymphocytic leukemia was the most reported immunodeficient disease (77%), followed by non-Hodgkin lymphoma and retinoblastoma. The majority of the children were undergoing chemotherapy during the study. Thirteen (10%) children had *T. whipplei* DNA in the collected stools. Sequencing results confirmed *T. whipplei* identification in all the cases. Eight out of 70 (11.4%) children under 5 years old resulted positive. **Conclusion:** This is the first study showing the circulation of *T. whipplei* among immunocompromised children in Iran. More epidemiological studies are needed to evaluate the prevalence of this pathogen in different risk groups in Iran and to increase the knowledge of its rare clinical manifestations.

**Keywords:** *Tropheryma whipplei*; epidemiology; children; immunocompromised; Iran.

## 1. Introduction

*Tropheryma whipplei*, a rod-shaped bacterium of the group of actinomycetes, is the causative agent of Whipple's disease and other acute and chronic clinical manifestations [1]. According to the literature, Whipple's disease is a rare infective condition that affects predominantly male (73-87%), in the 48 - 54 years age-range, with a genetic predisposition for a lifetime susceptibility to the bacteria [2,3]. The classical symptoms reported by this patients starts with arthritis (73-80%) followed by persistent diarrhea, other chronic gastrointestinal symptoms (72-81%), and weight loss (79-93%) [2,3]. Other acute and

chronic *T. whipplei* localized manifestations are also been reported in the absence of the classic gastrointestinal involvement [4-9].

*T. whipplei* is transmitted through oral-oral and oral-fecal routes from human reservoirs [2,3]. After developing an immune response to the first exposure, these can carry the microorganism for a long time, causing its spread in community. Two studies have shown that 48% [10] and 72% [11] of the general population in Europe and Senegal, respectively, have antibodies against *T. whipplei*. Nevertheless, clinical manifestations of this infection are rarely reported worldwide.

One cause of this lack of information is the necessity to perform histological analysis of small-bowel or other tissues (positive periodic acid-Schiff staining and/or *T. whipplei* immunohistochemistry), to obtain the definitive diagnosis of Whipple's disease. Another reason is the inaccessibility of other diagnostic tools. Culture of *T. whipplei* is implemented only in few laboratories and availability of molecular techniques has been introduced only recently in clinical practice. Currently, real-time polymerase chain reaction (rt-PCR) to identify *T. whipplei* DNA in biological specimens is the preferred method, since it is highly sensitive, specific, and cost-effective [12]. This diagnostic tool allows a non-invasive detection of the pathogen in various biological specimens, including stool, saliva, urine, blood, bronchoalveolar, cerebrospinal and synovial fluids. However, a conclusive diagnosis requires more invasive procedures for the identification of the pathogen in biopsies, such as stomach, small intestine, lymph node, heart valve, myocardium, skeletal muscle, lung, spleen, or liver [13]. Rt-PCR positivity in these biological samples has a high positive predictive value if classical symptoms are been reported, allowing the selection of patients to be further investigated with the histological analysis [14].

The prevalence of *T. whipplei* DNA in stool samples of asymptomatic European people (intestinal colonization) ranges from 1.5 to 7% [14-18]. The rate of intestinal colonization in sewage workers, HIV-positive people, and homeless is higher (12-25%)[16,17,19]. A molecular-epidemiological survey conducted in Italy, for evaluating *T. whipplei* intestinal colonization in Italian and migrant population, showed an overall prevalence of 6.9%[20]. The study revealed a higher prevalence in patients coming from Africa (11.2%) and Latin America (10.8%), particularly in <10 years old children (19.3 and 25%, respectively). In a study conducted in Senegal, intestinal colonization rate in healthy children younger than 5 years old reached 75%[11]. Differently, in children 5–10 years old and older the prevalence was 30% and 17.4%, respectively[11]. No study has been conducted on children with any form of immunodeficiency.

This preliminary study was performed in order to identify *T. whipplei* DNA in stool samples of a cohort of children with immunodeficiency in Iran.

## 2. Materials and Methods

### Sample collection

This cross-sectional study was conducted from August 2018 to March 2019 in Qom (in central Iran). Children suffering of acute lymphocytic leukemia (ALL), non-Hodgkin lymphoma (NHL) and retinoblastoma referred to Hazrat Masoumeh Children's Hospital in Qom, Iran, were included in the study. About 10 gr of stool were collected from each child in sterile containers and immediately transferred to the bacteriological laboratory. If the patients were receiving chemotherapy, the samples were collected during or after it. A questionnaire including demographic characteristics, clinical symptoms and treatment history was completed for each patient.

## Ethics approval

The present study was reviewed and approved by the Ethics Committee of Qom University of Medical Sciences (Code: IR.MUQ.REC.1396.89). A written consent form was obtained from the children's parents.

## DNA extraction and molecular detection

DNA was extracted from stool samples using the method described by Yang et al[21]. Briefly, 20 mg of each sample was dissolved in 1 ml PBS and then centrifuged at 100 xg for 15 min. The supernatant was then removed and the pellet was discarded. The supernatant was transferred to new sterile microtube and centrifuged at 13 000 xg for 10 min. The supernatant was discarded and the pellet was washed three times with 300 µl of acetone to remove PCR inhibitors (each washing step was centrifuged at 13 000 xg for 10 minutes). At the final stage, the acetone was discarded and pellet was dissolved in 200 µl of TE buffer. In order to extract the genome, the samples were boiled in a hot water bath at 100 °C for 15 min. The samples were then centrifuged at 10 000 xg for 10 min. Finally, the supernatant was transferred to a new sterile microtube and stored at -20 °C until the analysis.

All extracted DNA samples were tested using both SYBR Green and Taq-Man probe rt-PCR for the diagnosis of *T. whipplei*. For the SYBR Green method, TWS1F primer (5'-AGAGAGATGGGGTGCAGGAC-3') and TWS1R primer (5'-AGCCTTTGCCAG-ACAGACAC-3') were used[22]. The final volume of each reaction was 20 µl, containing 10 µl of RealQ Plus 2x Master Mix Green Low ROX™ (Ampliqon, Denmark), 900 nmol of each primer, and 4 µl of extracted DNA. The temperature program was performed with initial denaturation step at 95 °C for 15 min, followed by 40 cycles in three stages (95 °C for 15 sec, 60 °C for 30 sec, and 72 °C for 30 sec) associated to the melting step[22]. This test was done using the Corbett 6000 Rotor-Gene system (Corbett, Victoria, Australia). Samples with amplification curves and melting temperatures of ~ 81° C were considered as positive. For confirmation, the positive samples were sequenced (Genomine Company, Tehran, Iran).

All samples were re-tested using Taq-Man RT-PCR. The assay was performed with a total volume of 20 µl, containing 10 of µl RealQ Plus 2x Master Mix (Ampliqon, Denmark), 900 nmol of Twhi3F primer (5'-TTGTGTATTTGGTATTAGATGAAACAG-3'), 900 nmol of Twhi3R (5'-CCCTACAATATGAAACAGCCTTTG-3'), 200 nmol of probe (FAM-5'-GGGATAGAGCAGGAGGTGTCTGTCTGG-3'BHQ-1)[23], and 4 µl of template DNA. The test was performed using a Corbett 6000 Rotor-Gene system (Corbett, Victoria, Australia) with a temperature of 95 °C for 10 min followed by 45 cycles at 94 °C for 15 sec and 60 °C for 60 sec. [24].

In all the runs, control DNA from *T. whipplei* positive samples (prepared in a lyophilized form from IRCCS Sacro Cuore Don Calabria Hospital, Italy) and distilled water were used as positive and negative controls, respectively.

## Statistical analysis

Descriptive statistics were used to analyze the characteristics of the entire cohort of children and then separately for individuals infected and non-infected with *T. whipplei*.

## 3. Results

### Description of the study population

One hundred and 30 children were enrolled in the study. Demographic data and clinical characteristics of this population study are reported in the Table 1.

**Table 1:** demographic data and clinical characteristics of 130 children referred to Hazrat Masoumeh Children's Hospital in Qom, Iran.

	N (%)
Male, n (%)	79 (60.8)
Cancer diseases	
ALL, n (%)	101 (77.7)
NHL, n (%)	19 (14.6)
Retinoblastoma, n (%)	10 (7.7)
Symptoms	
Diarrhea, n (%)	128 (98.5 )
Fever, n (%)	120 (93.0)
Anorexia, n (%)	115 (89.1)
Vomiting, n (%)	98 (75.4)
Weight loss, n (%)	80 (61.5 )
Cramp, n (%)	55 (42.3)

Acute lymphocytic leukemia, ALL; non-Hodgkin lymphoma, NHL.

Seventy (53.8%) children had less than 5 years. The mean age of the children was 56.72±40.49 months (range, 2 - 192 months); 60% were male. Most patients (n= 101, 77.7%) had ALL, 19 (14.6%) had NHL, and 10 (7.7%) children had retinoblastoma cancer. One hundred and 21 (93%) children were undergoing chemotherapy during the study for a duration ranging between 1-72 months (mean ±SD of 15.23±11.9 months). Seventy-five (57.7%) children were treated with antibiotics.

### PCR analysis for *T. whipplei*

A total of 13 (10%) children resulted positive to both rt-PCRs for *T. whipplei*. Sequencing also confirmed the results of the molecular evaluation (Recorded accession number in gene bank: MT380911). Eight out of 70 (11.4%) children younger than 5 years old resulted positive. The mean age of the *T. whipplei* positive children was lower respect negative children ( $48.5 \pm 32.2$  vs  $57.6 \pm 41.3$  months). Seven (53.8%) were male. Twelve cases (92.3%) had ALL and one (7.7%) had NHL. All 13 children had bloody diarrhea and anorexia, 12 cases had vomiting, and 11 of them had fever and weight loss.

### 4. Discussion

There are few epidemiological data available worldwide on rate of *T. whipplei* intestinal infection in children but none in children with immunodeficiency [11,14-18,20,25-27]. Moreover, the epidemiology of *T. whipplei* in Iran is unknown. Only a case of Whipple's disease confined to the central nervous system has been reported [28]. The aim of the study was to explore the presence of *T. whipplei* in children hospitalized for ALL, NHL and retinoblastoma, in our center in Iran.

Our investigation demonstrates for the first time *T. whipplei* intestinal colonization in hospitalized children in Iran. Our data highlighted that the 10% of children younger than 16 years old, mostly affected by a haematological malignancy, resulted colonized by *T. whipplei*. In particular, in the subgroup of children less than 5 years old the rate of infection was 11.4%. This percentage is higher compared to prevalence rate found in asymptomatic children in European studies (range from 1.5% to 7%) [14-18,20]. In a recent study, the rate of intestinal colonization found in Italian children younger than 10 years was 5.6%, but it was higher in African and American children of the same age (19.3% and 25%, respectively) [20]. Studies conducted in low-middle income countries showed higher rate of *T. whipplei* intestinal colonization in asymptomatic children. The prevalence rates were 40% and 36.4% among 4-years and 5–10 years old in Gabon [25], 44% in children 2–10 years of age in Senegal [26], 48% in children aged 1–7 years in Laos [27], and 75% in healthy children younger than 5 years old in Senegal [11]. However, all these studies are no comparable to our study, in which immunosuppressed and symptomatic children characterized the population analyzed.

*T. whipplei* can cause acute clinical manifestations during primary infection as gastroenteritis, bacteremia, or pneumonia [4]. There are some studies conducted in European and African children showing a significantly association between *T. whipplei* and acute diarrhea [15]. Our entire positive study population reported diarrhea. However, most of these children were having the chemotherapy causing neutropenic enterocolitis that affects the developmental integrity of the intestinal mucosa [29]. The aim of our study was not to demonstrate that *T. whipplei* is a causative agent of diarrhea, as other pathogens causing diarrhea have not been investigated. In fact, bacteria and protozoa often cause diarrhea in immunodeficiency population. Moreover, intestinal microbiome in patients undergoing chemotherapy can be altered and replaced by pathogenic bacteria.

However, identification of *T. whipplei* intestinal colonization in children with cancer is an important epidemiological result to highlight the circulation of this unknown pathogen in Iran. Particularly if considering that undiagnosed and untreated Whipple disease can be lethal in adulthood.

The most important limitation of the study was the lack of a control group, which make results interpretation more difficult. Finally, the small size of the analyzed sample,

and the narrow geographical area in which the study was conducted, were other important bias.

## 5. Conclusions

This preliminary study showed the circulation of *T. whipplei* among children with immunodeficiency in Qom-Iran area. A new study with a larger population sample, conducted in larger areas of the country, including immunocompetent and immunosuppressed children, symptomatic and asymptomatic, should be designed to obtain more accurate epidemiological information.

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**Institutional Review Board Statement:** The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of Qom University of Medical Sciences (Code: IR.MUQ.REC.1396.89).

**Informed Consent Statement:**

Written informed consent has been obtained from the children's parents.

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**Conflicts of Interest:**

The authors declare no conflict of interest.



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