Tropical Biomedicine 39(3): 328-331 (2022)
https://doi.org/10.47665/tb.39.3.004

RESEARCH ARTICLE

Serological review of *Bartonella henselae* and *Bartonella quintana* infection among Malaysian patients with unknown causes of febrile illnesses

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ARTICLE HISTORY

Received: 14 May 2022
Revised: 4 June 2022
Accepted: 7 June 2022
Published: 15 August 2022

ABSTRACT

Limited information is available on human exposure to *Bartonella* infection, i.e., *Bartonella henselae* (causative agent of cat scratch disease) and *Bartonella quintana* (causative agent of trench fever) in West Malaysia. This study reports a review of serological findings obtained from patients attending to a teaching hospital in Klang Valley, Malaysia. An indirect immunofluorescence assay (IFA) was used to determine IgG and IgM antibody titers against *B. henselae* and *B. quintana.* In a pilot study conducted between 2013-2015, IgG antibodies against *Bartonella* spp. (either *B. quintana* and *B. henselae*) were detected in 14 (36.8%) of 38 patients who were clinically suspected of rickettsial infections, while IgM antibody was detected in 4 (10.5%) patients. This has prompted us to investigate the serologic responses of patients who were clinically suspected of other febrile causes besides rickettsial infection. Of the 59 serum samples analysed in a follow-up investigation, *Bartonella* IgG antibodies were detected from 7 (11.9%) patients, of which 5 (27.8%) and 2 (18.2%) patients were clinically suspected of rickettsial infection (*n*=18) and dengue (*n*=11), respectively. None of the sera obtained from the leptospirosis (*n*=10), legionellosis (*n*=10) and mycoplasma infection (*n*=10) groups were seropositive to *Bartonella* spp. The review of *Bartonella* serological findings in this study highlights that *Bartonella* infection is not uncommon and should be considered as one of the causes for febrile illness in Malaysia.

Keywords: *Bartonella henselae*; *Bartonella quintana*; IFA; febrile illness.

INTRODUCTION

Bartonella spp. are small, fastidious, intra-erythrocytic Gram-negative bacteria that are responsible for a number of human diseases including cat scratch disease (causative agent: *Bartonella henselae*), trench fever (causative agent: *Bartonella quintana*) and Oroya fever (causative agent: *Bartonella bacilliformis*). Zoonotic transmission of *Bartonella* spp. can occur following animal scratches or bites or via exposure to hematophagous arthropod vectors, including fleas, lice, sand flies, and ticks (Regier *et al*., 2016).

Little is known about the prevalence and clinical presentation of *Bartonella* infection in Malaysia. Cat scratch disease (CSD) is mostly caused by *B. henselae* or *B. clarridgeae* following scratches or bites by cats infested with *Ctenocephalides* fleas (Kalinova, 2007). Various *Bartonella* species including *B. henselae*, have been reported in small mammals (such as rodents and cats) and arthropod vectors (mainly fleas) in previous Malaysian studies, suggesting potential human exposure to *Bartonella* infection (Tay *et al*., 2014; Hassan *et al*., 2017; Kho *et al*., 2017). So far, there have been no reports concerning infection caused by *B. quintana* and *B. bacilliformis* in Malaysia.

Research is needed to confirm *Bartonella* infection as a cause of acute undifferentiated febrile illness. The diagnosis of *Bartonella* infection has been hampered by the lack of appropriate laboratory tests, especially in resource-limited countries. Isolation of *Bartonella* spp. from blood and infected tissue is not routinely performed in the clinical microbiology laboratory, due to the fastidious nature of the organisms. Up to now, IFA is the only laboratory test that has been validated with clinical diagnosis of *Bartonella* infections (Allizond *et al*., 2019). A review of *Bartonella* IFA results obtained from febrile patients may reveal the extent of human exposure to *Bartonella* spp. Hence, this study was carried out to examine the presence of IgG and IgM antibodies against *B. henselae* and *B. quintana* in patients clinically suspected of rickettsial, dengue, leptospirosis, legionella, and mycoplasma infection.

MATERIALS AND METHODS

Serum samples

The collection and use of the serum samples have been approved by University Malaya Medical Center (UMMC) Medical Ethics Committee (MEC ID No.: 944.20b and 20159-1658). A pilot study was conducted to screen *Bartonella* antibodies in 38 febrile patients who were clinically suspected of rickettsial infections (Kho *et al*., 2015). There were 13 females and 25 males, with the age ranging from 16-76 years old.

Following detection of *Bartonella* antibodies from some febrile patients in the pilot study, a follow-up investigation was conducted to analyse the serologic responses of patients who were clinically suspected of other febrile causes, besides rickettsial infections. The

serum samples (*n*=59), kindly provided by the Serologic Diagnostic Unit, Microbiology Diagnostic Department, UMMC, were obtained from patients who were clinically suspected of rickettsial infection (*n*=18), dengue (*n*=11), leptospirosis (*n*=10), legionellosis (*n*=10) and mycoplasma infection (*n*=10) from 2015-2018. There were 29 female and 30 male patients, with the ages ranging from 14 to 59 years old. The serum samples had been previously diagnosed as negative using Rickettsia IFA (ARRL, Australia), SD BIOLINE Dengue Duo (Abbott, USA), in-house Leptospira microscopic agglutination test, *Legionella pneumophila* IFA [IgM (Vircell, Spain), IgG (Trinity Biotech, Ireland)] and Mycoplasma SERODIA®-MYCO II test (Fujirebio, Japan).

Bartonella indirect immunofluorescence assays

Determination of IgG and IgM antibodies against *B. quintana* and *B. henselae* was performed using an indirect immunofluorescence assay (IFA) (Focus Diagnostic, Cypress, California, USA) in accordance with the manufacturer's instructions. For *Bartonella* IgM assay, 5 µL of each serum sample was diluted to a ratio of 1: 20 with 95 µL IgM pretreatment diluent (provided by the manufacturer), mixed and left for five minutes. The diluted sample was centrifuged at 13 500 rpm at room temperature for one min prior to use. For *Bartonella* IgG assay, 5 µL of each serum sample was diluted to a ratio of 1:64 with 315 µL IgG sample diluent working solution (provided by the manufacturer) prior to use. A volume of 20 µL diluted serum sample was then added to each well on an antigen slide. After incubation at room temperature for 60 min (IgG) and 90 min (IgM) in a humidified incubator, the antigen slide was submerged in PBS for 10 min before dipping briefly in the distilled water and air dried. A volume of 20 µL IgM or IgG conjugate (supplied by the manufacturer) was then added to each well and incubated for 30 min at room temperature in dark. After several washing steps, the antigen slide was added with a few drops of mounting medium, covered with a glass slip, and viewed under 400x magnification using a fluorescence microscope (DM4000B LED, Leica, Germany). The test was interpreted in accordance with the manufacturer's recommendation; whereby IFA titers of ≥1:20 and ≥1:64 were interpreted as positive for IgM and IgG, respectively. Whenever possible, positive serum samples were further diluted to determine the endpoint titers.

RESULTS

Table 1 summarises the analysis of the *Bartonella* IFA results. For the pilot study, IgG/IgM antibodies against *B. quintana* and/or *B. henselae* were detected from 14 (36.8%) patients, of which 5 (13.2%) also had antibody against *R. typhi*. IgM antibody against *B. quintana* alone was detected in 3 (7.9%) patients, while only 1 (2.6%) patient had IgM antibody against *B. henselae.* Overall, all *Bartonella*-positive serum samples exhibited low titers of IgG/IgM antibody against *B. quintana* and/or *B. henselae*.

In the follow-up investigation of 59 patients, IgG antibodies against *B. quintana* and/or *B. henselae* were detected from 7 (11.9%) patients, including 5 (27.8%) out of 18 patients suspected with rickettsial infection and 2 (18.2%) out of 11 patients suspected with dengue. IgM antibodies against *B. henselae* and *B. quintana* were not detected from any of the patients. None of the sera obtained from patients clinically suspected of leptospirosis (*n*=10), legionellosis (*n*=10) and mycoplasma infection (*n*=10) were serologically positive for *Bartonella* infection (Table 1).

DISCUSSION

Different clinical manifestations, including fever, granulomatous inflammation of the heart, liver, lymph nodes, endocarditis, bacillary angiomatosis, peliosis hepatis, uveitis and vasoproliferative tumors have been reported in patients infected with *Bartonella* spp. (Álvarez-Fernández *et al*., 2018). As the clinical manifestation of *Bartonella* infection have been reported to mimic many other infectious diseases, the infection may be left under diagnosed or reported in resource-limited settings (Florin *et al*., 2008; Noden *et al*., 2014).

The clinical cases of *Bartonella* infection reported in Malaysia are mostly associated with ocular manifestation, the most common nonlymphatic presentation of the infection (Ghazi & Sams, 2012). Lina *et al*. (2010) reported the first suspected case of cat scratch disease in a 29-year-old Malaysian febrile patient with painful left neck mass and lymphadenopathy. Raihan *et al*. (2014) reported unilateral and bilateral neuroretinitis in four patients presented with fever 2 weeks before the onset of eye symptoms. Ocular bartonelloses have also been reported in 19 Malaysians from two ophthalmology centres based on clinical histories of contact with cats, eye examination findings and positive serology test for *Bartonella* spp. (Tan *et al*., 2017). A retrospective review of the medical records of 13 Malaysian immunocompetent patients reported fever prior to ocular presentation (Tey *et al*., 2020). Although these studies suggest that *Bartonella* infection might not be rare among patients with ocular manifestations, there is no report yet on the investigation of *Bartonella* infection among febrile patients in West Malaysia.

In this study, IgM (1:20) and IgG antibody titers (1:64 to 1:128) were noted among 21 (21.6%) of the 97 patients investigated in this study, suggesting exposure to either *B. henselae* or *B. quintana*.The overall review of the *Bartonella* IFA results showed higher IgG prevalence than IgM against the *Bartonella* spp. As most patients positive for *B. quintana* antibody were also tested positive for *B. henselae* by IFA, a high possibility of serological cross reactivity between the two organisms is suspected. According to previous studies, serological differentiation between *B. henselae* and *B. quintana* infections is impossible, due to the closely relatedness

Table 1. Analysis of IFA findings of *B. quintana* and *B. henselae* from 2 groups of febrile patients investigated in this study

Patient group	No. (%) serum with IgG/IgM antibody detected	No. (%) serum with IgG antibody detected		No. (%) serum with IgM antibody detected	
	B. quintana/B. henselae	*B. quintana*	*B. henselae*	*B. quintana*	*B. henselae*
Pilot study (*n*=38)					
R. typhi seropositive (*n*=10)	5 (50)	3 (30.0)	5 (50.0)	1 (10.0)	1 (10.0)
R. typhi seronegative (*n*=28)	9 (32.1)	7 (25.0)	5 (17.9)	2 (7.1)	0 (0.0)
Total	**14 (36.8)**	**10 (26.3)**	**10 (26.3)**	**3 (7.9)**	**1 (2.6)**
Follow-up investigation (*n*=59)					
Rickettsial infection (*n*=18)	5 (27.8)	5 (27.8)	5 (27.8)	0 (0.0)	0 (0.0)
Dengue (*n*=11)	2 (18.2)	0 (0.0)	2 (18.2)	0 (0.0)	0 (0.0)
Leptospira, Legionella, and Mycoplasma (*n*=30)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	**7 (11.9)**	**5 (8.5)**	**7 (11.9)**	**0 (0.0)**	**0 (0.0)**

of the organisms (Dalton *et al*., 1995; Sander *et al*., 1998, 2001). Re-emergence of *B. quintana* infection has been reported among human populations with lice infestation, poor living condition and chronic alcoholism in Europe and the United States (Brouqui *et al*., 1999; Foucault *et al*., 2006).

The co-detection of antibodies against *Bartonella* and rickettsial organisms (*R. typhi*) in the pilot study suggests serological cross-reactivity between the two bacterial genera (Table 1). In fact, serological cross-reactivity has been reported with other rickettsial species, including *Rickettsia prowazekii*, and *Orientia tsutsugamushi* (previously known as *Rickettsia tsutsugamushi*) (Hollingdale *et al*., 1978; da Costa *et al*., 2005). Additionally, as animal ectoparasites (mites, fleas and ticks) are known to play an important role as vectors for both *Bartonella* and rickettsial infection, co-transmission of bacterial pathogens during a blood meal of the infected ectoparasites is highly possible and this may be an explanation for the co-detection of *Bartonella* and rickettial antibodies in our patients.

Two patients who were clinically suspected of dengue in this study were tested positive for *B. henselae* using IFA (Table 1). Serological cross-reactivity has not been described between dengue and *Bartonella* infection. In a study conducted by Chamberlin *et al*. (2000), the possibility of cross-reactivity between dengue and *B. bacilliformis* has been ruled out. Hence, there is a high likelihood that *Bartonella* infection may be under diagnosed in the febrile patients.

The serodiagnosis of *B. henselae* infection based on IgM antibody detection is challenging due to low antibody titers (≥1:20) observed in most studies. An Indian study reported that negative IgM results did not necessarily exclude disease as poor antibody response might be caused by antigenic variability between infecting *B. henselae* strains (Chaudhry *et al*., 2018). It has also been reported that IgM level decreases eighth week after infection and the antibody disappears around 100 days after the onset of the symptoms (Bergmans *et al*., 1996; Metzkor-Cotter *et al*., 2003; Vermeulen *et al*., 2007). IgM antibody may not be detected if CSD is diagnosed at a later stage when the IgM titer has decreased (Metzkor-Cotter *et al*., 2003).

This study provides serological evidence of *Bartonella* infection among febrile patients attending to our hospital. Ideally, the serological findings of the patients should be confirmed by testing their convalescent samples, two to four weeks after recovery, however; this was not possible due to the retrospective nature of the study. This review highlights that previous exposure of *B. henselae* may not be uncommon amongst febrile patients in our setting, and the infection should be considered for differential diagnosis of febrile illnesses. The same observation has been reported in Thailand, a neighbouring country, whereby *B. henselae* infection has been associated with acute febrile illness (Kosoy *et al*., 2010). Tsukahara *et al*. (2000) reported that 34% of patients serologically positive for *B. henselae* were found to have prolonged fever or fever of unknown origin, suggesting that generalized systemic *B. henselae* infection was not rare in febrile patients in Japan. More extensive studies are thus required to improve clinical and laboratory diagnosis, and understanding on the epidemiological and zoonotic aspects of *Bartonella* infection in Malaysia.

ACKNOWLEDGEMENTS

This research was funded by the Ministry of Science, Technology and Innovation E-Science Fund (SF014-2015) and the PPP fund (PG255-2016A). We thank Ms. Hooi Poh Sim, Harvinder Kaur and Lu Mei for their technical assistance provided in this study.

Conflict of interest statement

The author declares that they have no conflict of interests.

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