

BARTONELLA SEROPREVALENCE IN RURAL THAILAND

Saithip Bhengsr¹, Henry C Baggett¹, Leonard F Peruski¹, Christina Morway², Ying Bai², Tamara L Fisk¹, Anussorn Sitdhirasdr³, Susan A Maloney¹, Scott F Dowell⁴ and Michael Kosoy²

¹International Emerging Infections Program, Thailand MOPH – US CDC Collaboration, Nonthaburi, Thailand; ²Centers for Disease Control and Prevention, Fort Collins, Colorado, The United States of America; ³Ministry of Public Health, Nonthaburi, Thailand; ⁴Centers for Disease Control and Prevention, Atlanta, Georgia, The United States of America

Abstract. We estimated the prevalence of anti-*Bartonella* antibodies among febrile and non-febrile patients presenting to community hospitals in rural Thailand from February 2002 through March 2003. Single serum specimens were tested for IgG titers to four *Bartonella* species, *B. henselae*, *B. quintana*, *B. elizabethae* and *B. vinsonii* subsp *vinsonii* using an indirect immunofluorescent assay. A titer $\geq 1:256$ was considered positive. Forty-two febrile patients (9.9%) and 19 non-febrile patients (19%) had positive serology titers to at least one *Bartonella* species. Age-standardized *Bartonella* seroprevalence differed significantly between febrile (10%) and non-febrile patients (18%, $p = 0.047$), but did not differ by gender. Among all 521 patients, IgG titers $\geq 1:256$ to *B. henselae* were found in 20 participants (3.8%), while 17 (3.3%) had seropositivity to *B. quintana*, 51 (9.8%) to *B. elizabethae*, and 19 (3.6%) to *B. vinsonii* subsp *vinsonii*. These results suggest exposure to *Bartonella* species is more common in rural Thailand than previously suspected.

Keywords: *Bartonella*, seroprevalence, Thailand

INTRODUCTION

Bartonella are fastidious gram-negative bacteria that infect erythrocytes of mammalian hosts and have a worldwide distribution. At least 10 *Bartonella* spe-

cies are known to cause human disease (Saisongkorh *et al*, 2009). In Thailand, *Bartonella* species have been reported from mammalian hosts, including humans, and potential arthropod vectors, (Maruyama *et al*, 2001; Parola *et al*, 2003; Castle *et al*, 2004) but data on human infections are limited (Maruyama *et al*, 2000; Kosoy *et al*, 2008; Paitoonpong *et al*, 2008; Bhengsr¹ *et al*, 2010). In a recent study in northern and northeastern Thailand, 27% of patients with undifferentiated febrile illness had serological evidence (*ie*, 4-fold antibody rise) of *Bartonella* infection (Bhengsr¹ *et al*, 2010). The one published *Bartonella* seroprevalence study in Thailand reported

Correspondence: Saithip Bhengsr¹, International Emerging Infections Program (IEIP), Thailand Ministry of Public Health (MOPH) – US Centers for Disease Control and Prevention Collaboration, 3rd Floor, Building 7, Department of Disease Control, Ministry of Public Health, Nonthaburi, 11000, Thailand.

Tel: +66 (0) 2580 0669 ext 331; Fax: +66 (0) 2580 0911

E-mail: saithipb@th.cdc.gov

5.5% of apparently healthy individuals, mainly blood donors, had antibodies to *B. henselae* (Maruyama *et al*, 2000). No studies have assessed *Bartonella* seroprevalence using species other than *B. henselae*. We estimated *Bartonella* seroprevalence among patients presenting to hospitals in north and northeastern Thailand based on antibody titers to four *Bartonella* species.

MATERIALS AND METHODS

Setting

This analysis utilized data from a prospective study that was designed to determine causes of acute febrile illness in northern and northeastern Thailand. Chiang Rai Province is in northern Thailand, borders Lao PDR and Myanmar, and has a population of 1,167,780. Khon Kaen is in northeastern Thailand and has a population of 1,801,016. Both provinces have primarily agrarian economies. In the current study, we present the seroprevalence of four *Bartonella* species based on testing of single sera specimens among febrile patients and non-febrile control patients.

Patients

Febrile and non-febrile patients presenting to the outpatient and inpatient departments of four community hospitals between 8 AM and 5 PM from February 4, 2002 through March 25, 2003 were enrolled in the study. Eligible febrile patients were those ≥ 7 years old with a documented fever $> 38^{\circ}\text{C}$ for < 2 weeks. Patients with infection that could be initially diagnosed by physicians were excluded. Eligible non-febrile control patients were persons ≥ 14 years old who presented to the inpatient or outpatient departments of participating hospitals for routine health maintenance or noninfectious conditions. Interviews were conducted with all parti-

cipants using a standardized questionnaire to collect demographic information and exposure history (*eg*, animal exposures and outdoor activities). A single serum specimen was collected from each febrile and non-febrile patient for IgG antibodies against *B. henselae*, *B. quintana*, *B. elizabethae* and *B. vinsonii* subsp *vinsonii*, for a total of 521 patients: 423 febrile patients and 98 non-febrile patients.

Ethical considerations

Written informed consent was obtained from each adult participant and from a guardian of each participant under 18 years old. Verbal assent was obtained from participants aged 7 to 17 years. The protocol was approved by the Institutional Review Board of the US Centers for Disease Control and Prevention and the Ethical Review Committee, Ministry of Public Health, Thailand.

Laboratory testing

Sera were shipped on dry ice for serologic testing to the *Bartonella* laboratory at the US Centers for Disease Control and Prevention (CDC) in Fort Collins, Colorado, USA where the immunofluorescent assay (IFA) testing was carried out. Strains used for antigen production were: *B. elizabethae* (F9251), *B. henselae* (Houston-1), *B. quintana* (Fuller), *B. vinsonii* subsp *vinsonii* (Baker). Hyperimmune sera against *Bartonella* species were produced in BALB/c mice as previously described (Kosoy *et al*, 1997). The sera were screened for IgG antibodies against each *Bartonella* species starting at a dilution of 1:32. Positive sera were titrated in 2-fold dilutions with phosphate-buffered saline to an endpoint of 1:2048. The IFA slides were evaluated independently by two researchers. A titer $\geq 1:256$ was considered positive.

Statistical analysis

Data were double entered and vali-

dated to exclude data entry errors using Epi Info version 6.04 (CDC, Atlanta, GA) and analyzed using SPSS version 12.0 (SPSS, Chicago, IL). For seroprevalence estimates, we controlled for age in the febrile and non-febrile patients by calculating age standardized seroprevalence using the combined age group as the standard population (PAHO/WHO, 2002). A *z*-test was calculated based on the standardized prevalence difference to compare the two groups (Flanders, 1984). Dichotomous variables were compared with the chi-square or Fisher's exact test, and continuous variables with the Student's *t*-test. Exposures were compared between *Bartonella* seropositive and seronegative patients using odds ratios adjusted for age and sex and presented as adjusted odds ratios (AOR) with 95% confidence intervals (CI).

RESULTS

Forty-two febrile patients (9.9%) and 19 non-febrile patients (19%) had IgG titers $\geq 1:256$ to at least one *Bartonella* species, which was considered evidence of past infection for this analysis. Age-standardized *Bartonella* seroprevalence differed significantly between febrile (10%) and non-febrile patients (18%, $p = 0.047$), but did not differ by gender. Except for the oldest age-group, seropositivity increased slightly with age [age 7-13 years (15/155, 10%); 14-29 years (16/142, 11%); 30-44 years (16/131, 12%); 45-59 years (12/70, 17%) and ≥ 60 years (2/23, 9%)]. Of the 521 total patients, IgG titers $\geq 1:256$ to *B. henselae* were found in 20 patients (3.8%), seropositivity to *B. quintana* was found in 17 patients (3.3%), seropositivity to *B. elizabethae* was found in 51 patients (9.8%), and seropositivity to *B. vinsonii*, was found in 19 patients (3.6%) (Table 1).

Of the 61 patients seropositive for at least one *Bartonella* species, 32 (52%) were positive for only one species; 18 patients (29.5%) were positive for two species (5 positive for *B. elizabethae* and *B. vinsonii*, 6 positive for *B. quintana* and *B. elizabethae*, 2 positive for *B. henselae* and *B. vinsonii*, and 5 positive for *B. henselae* and *B. elizabethae*), 5 patients (8.2%) were positive for three species (1 positive for *B. henselae*, *B. quintana* and *B. elizabethae*, 4 positive for *B. quintana*, *B. elizabethae* and *B. vinsonii*); and 6 patients (9.8%) were positive for all four species. Patients with positive serology to ≥ 1 *Bartonella* species were no more likely than seronegative patients (IgG titers $< 1:256$) to report owning dogs (57% vs 48%; AOR 1.5; 95% CI 0.9-2.5) or cats (26% vs 29%; AOR 0.9; 95% CI 0.5-1.6) or to have had rodent exposure during the 2 weeks prior to the onset of illness (70% vs 59%; AOR 1.6; 95% CI 0.9-2.9). Thirty-five percent of patients with positive serology against *B. henselae* reported cat ownership similar to the seronegative patients (28%; AOR 1.3; 95% CI 0.5-3.4).

DISCUSSION

Our study is the first to report the prevalence of antibodies to *Bartonella* species other than *B. henselae* among humans in Thailand. We found a high prevalence of IgG antibodies against *Bartonella* species: 10% of patients with undifferentiated febrile illness and 19% of non-febrile patients. Our findings build on recent work confirming *Bartonella* infections in Thailand based on a four-fold rise in antibody titers (Bhengsi *et al*, 2010) and by culture and sequence identification (Kosoy *et al*, 2010); the seroprevalence data reported here indicate *Bartonella* infections may be more common in Thailand than previously suspected.

Table 1
IgG antibody titers against *Bartonella* species from single serum samples among febrile ($n = 423$) and non-febrile patients ($n = 98$).

Acute IgG titer	<i>B. henselae</i>			<i>B. quintana</i>			<i>B. elizabethae</i>			<i>B. vinsonii</i>		
	Febrile no. (%)	Non-febrile no. (%)	Total no. (%)	Febrile no. (%)	Non-febrile no. (%)	Total no. (%)	Febrile no. (%)	Non-febrile no. (%)	Total no. (%)	Febrile no. (%)	Non-febrile no. (%)	Total no. (%)
	<1:32	354 (84)	77 (79)	431 (83)	306 (72)	62 (63)	368 (71)	226 (53)	42 (43)	268 (51)	312 (74)	69 (70)
1:32	29 (6.9)	4 (4.1)	33 (6.3)	49 (12)	15 (15)	64 (12)	81 (19)	21 (21)	102 (20)	54 (13)	9 (9.2)	63 (12)
1:64	20 (4.7)	6 (6.1)	26 (5.0)	33 (7.8)	10 (10)	43 (8.3)	41 (9.7)	15 (15)	56 (11)	33 (7.8)	9 (9.2)	42 (8.1)
1:128	7 (1.7)	4 (4.1)	11 (2.1)	21 (5.0)	8 (8.2)	29 (5.6)	38 (9.0)	6 (6.1)	44 (8.4)	10 (2.4)	6 (6.1)	16 (3.1)
1:256 ^a	4 (0.9)	4 (4.1)	8 (1.5)	8 (1.9)	1 (1.0)	9 (1.7)	19 (4.5)	6 (6.1)	25 (4.8)	3 (0.7)	0 (0.0)	3 (0.6)
1:512	5 (1.2)	2 (2.0)	7 (1.3)	3 (0.7)	2 (2.0)	5 (1.0)	12 (2.8)	4 (4.1)	16 (3.1)	6 (1.4)	4 (4.1)	10 (1.9)
1:1024	4 (0.9)	1 (1.0)	5 (1.0)	3 (0.7)	0 (0.0)	3 (0.6)	4 (0.9)	4 (4.1)	8 (1.5)	5 (1.2)	1 (1.0)	6 (1.2)
1:2048	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.5)	0 (0.0)	2 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)
Total \geq 1:256 ^b	13 (3.1)	7 (7.1)	20 (3.8)	14 (3.3)	3 (3.0)	17 (3.3)	37 (8.7)	14 (14)	51 (9.8)	14 (3.3)	5 (5.1)	19 (3.6)

^a Cut-off level for a positive titer.

^b Forty-two febrile and 19 non-febrile patients had a positive titer to at least one *Bartonella* species. Thirty-two patients (52%) were positive for > 1 species; 18 (29.5%) were positive for 2 species (5 positive for *B. elizabethae* and *B. vinsonii*, 6 positive for *B. quintana* and *B. elizabethae*, 2 positive for *B. henselae* and *B. vinsonii* and 5 positive for *B. henselae* and *B. elizabethae*), 5 patients (8.2%) were positive for 3 species (1 positive for *B. henselae*, *B. quintana* and *B. elizabethae* and 4 positive for *B. quintana*, *B. elizabethae* and *B. vinsonii*) and 6 patients (9.8%) were positive for all four species.

Bartonella serology testing is known to be cross-reactive among *Bartonella* species. These data provide evidence of the relative seroprevalence of the four species examined, but species-specific seroprevalence should be interpreted cautiously. Serology testing in general can be non-specific, raising concerns of cross-reactivity with other pathogens (La Scola and Raoult, 1996; Maurin *et al*, 1997). However, IFA against *B. henselae* has been shown to have high specificity (94-96%) (Regnery *et al*, 1992; Zangwill *et al*, 1993; Dalton *et al*, 1995). We intentionally used conservative cut-offs ($\geq 1:256$) for antibody titers to define seropositivity and believe our estimates represent a minimum seroprevalence. A previous study among blood donors in Thailand found IgG seropositivity to *B. henselae* of 5.5% among those tested using a cut-off of $\geq 1:64$ (Maruyama *et al*, 2000). Seropositivity against *B. henselae* in our study would be more than double that of the above study if we used this lower cut-off value (Table 1). We found no associations between seropositivity and pet or rat exposure, but we only assessed rat exposure during the previous 2 weeks, which might not have adequately determined longer-term exposure and led to misclassification.

We do not know why *Bartonella* seroprevalence was more common among non-febrile patients than febrile patients. Previous infection with *Bartonella* species may be relatively protective against current febrile disease, and febrile patients with acute *Bartonella* infections may not have yet seroconverted at the time the specimen was collected. Some non-febrile patients had symptoms associated with *Bartonella* infection [eg, muscle pain (46%), rash (25%), anemia (25%), eye pain or conjunctivitis (15%)]. There may also have been unmeasured confounders for which

we did not control.

Although these data do not provide information about active *Bartonella* infections, they do show human *Bartonella* infections are common in rural Thailand and multiple species are may be involved. The hospital-based enrollment may limit generalizability, but we believe that at least the non-febrile patients were fairly representative of the general population in these provinces. Studies using culture-based diagnostics or validated molecular assays are needed to determine the frequency of specific *Bartonella* species causing acute infection. Epidemiologic and animal transmission studies are also needed to improve understanding of the risk factors for *Bartonella* infection and to determine key animal reservoirs and vectors.

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