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Seroprevalence of Antibodies against *Bartonella hensalae* Infection in Cats and Dogs along the Northern Borders of Thailand

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Abstract

Bartonella hensalae is a causative agent of cat scratch disease that has serious zoonotic potential for people. The aim of the present study is to examine seroprevalence of antibodies against *B. hensalae* in cats and dogs along the northern borders of Thailand in Chiangrai, Maehongson and Nan provinces using the indirect fluorescent antibody test (IFA). A total of 169 serum samples derived from 56 cats and 113 dogs were collected from 3 provinces along the northern borders of Thailand. It was found that the average percentage of positive rate to *B. hensalae* in Chiangrai, Maehongson and Nan provinces was 73.0%, 52.0% and 0.0%, respectively with the cut-off value of 1:32. The seroprevalence of cats from the Thailand-Myanmar border (Chiangrai and Maehongson provinces) was significantly higher than that of cats from the Thailand-Laos PDR border (Nan province) (61.8% vs 0.0%, $p < 0.001$). There was no prevalence of antibodies against *B. hensalae* from dogs in the 3 provinces.

Keywords: *Bartonella hensalae*, cats, dogs, the northern borders of Thailand

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บทคัดย่อ

ความชุกของแอนติบอดีต่อการติดเชื้อ *Bartonella hensalae* ในแมวและสุนัขในพื้นที่ชายแดนไทยภาคเหนือ

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Bartonella hensalae เป็นสาเหตุของโรค Bartonellosis ซึ่งเป็นโรคติดต่อจากสัตว์สู่คน คณะผู้วิจัยจึงได้ศึกษาหาความชุกของแอนติบอดีต่อการติดเชื้อในสัตว์ที่ใกล้ชิดคน ได้แก่ สุนัขและแมว ในพื้นที่ชายแดนภาคเหนือ 3 จังหวัด ได้แก่ จังหวัดเชียงราย แม่ฮ่องสอน และน่าน โดยวิธีทดสอบแบบ indirect fluorescent antibody test (IFA) ในตัวอย่างทั้งสิ้น 169 ตัวอย่าง ซึ่งเป็นตัวอย่างซีรัมแมว 56 ตัวอย่างและซีรัมสุนัขจำนวน 113 ตัวอย่าง ผลของการศึกษาพบว่า ที่เกณฑ์ตัดสินโรค 1:32 ตัวอย่างซีรัมแมวของจังหวัดเชียงรายพบผลบวกร้อยละ 73 (11/15) จังหวัดแม่ฮ่องสอน พบผลบวกร้อยละ 52 (10/19) ส่วนของจังหวัดน่านไม่พบผลบวก ความชุกของแอนติบอดีต่อการติดเชื้อในแมวในพื้นที่ชายแดนไทย-พม่า มีอัตราการสัมผัสเชื้อสูงกว่าในแมวในเขตพื้นที่ชายแดนไทย-ลาวอย่างมีนัยสำคัญทางสถิติ (61.8% ต่อ 0.0%, $p < 0.001$) แต่ไม่พบอัตราการติดเชื้อ *B. hensalae* ในสุนัข

คำสำคัญ: *Bartonella hensalae* แมว สุนัข พื้นที่ชายแดนไทยภาคเหนือ

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Introduction

Genus *Bartonella* is small, gram-negative aerobic bacilli that is difficult to grow in culture. They are found in many different animals. *Bartonella* species are the cause of several different diseases associated with human disease including *B. bacilliformis*, *B. quintana*, *B. vinsonii berkhoffii*, *B. henselae*, *B. elizabethae*, *B. grahamii*, *B. washoensis*, *B. koehlerae*, *B. rocha-limae* and *B. tamiiae* (Lamas et al., 2008).

Bartonella henselae is a proteobacterium that can cause bacteremia, endocarditis, bacillary angiomatosis and peliosis hepatitis in human (Anderson and Neuman, 1997). It is also the causative agent of cat scratch disease, occurring after a cat bite or scratch. The disease is characterized by lymphadenopathy and fever (Chomel et al., 2004; Inoue et al., 2009).

The study was conducted to understand the serological level of the infection of *Bartonella henselae* in cats and dogs in the areas along the Thailand-Myanmar-Laos PDR borders. The relationship of dogs, cats and prevalence of *Bartonella* spp. was also

examined in the present study. The data will benefit public health workers and patients who have a history of contact with commensal vectors such as flea and tick. It will be useful for the practice of public health in controlling vector-borne disease. It can also benefit for the management of the regional offices of Disease Prevention and Control in the province along Thailand-Myanmar-Laos PDR borders.

Materials and Methods

Sera: A total of 56 pet cats and 113 pet dogs sera were collected from 3 provinces along the Thailand-Myanmar border (Maesai district: Chiangrai province, Muang district: Mae Hong Son province) and Thailand-Laos PDR border (Chalermprakit district: Nan province), from February to March in 2008. Before the collection of blood samples, gender, type and age of each cat and dog were recorded. The dogs and cats were restrained and 3 ml of fresh blood from the cephalic, saphenous or femoral veins was aseptically collected and placed in vials. Sera were separated and stored at -20°C until tested.

Table 1 The gender and age of cats and dogs samples along the northern borders of Thailand

Species	Thailand-Myanmar border				Thailand-Lao PDR border		Total
	Chiangrai province		Maehongson province		Nan province		
	Male/Female	Young*/Adult**	Male/Female	Young/Adult	Male/Female	Young/Adult	
Cat	3/12	6/9	6/13	7/12	10/12	2/20	56
Dog	9/6	1/14	15/23	15/23	27/23	25/35	113

*Young: Cat/Dog age < 1 year **Adult: Cat/Dog age ≥ 1 year

Serological test: Anti-*B. henselae* detection was performed in the Laboratory of Veterinary Public Health, College of Bioresource Sciences, Nihon University, Kanagawa, Japan. The antibody titers to *B. henselae* were determined by the indirect fluorescent antibody test (IFA), using *B. henselae* (ATCC 49882) as an antigen. The procedure followed a previous study by Maruyama et al. (2000). In brief, a volume of 10 µl of diluted serum (diluted in PBS containing 5% skim milk) was put onto test holes and slides were incubated at 37°C for 1 hour in a humid chamber. Then, the slides were washed twice with PBS for 15 min. Fluorescein-conjugated goat anti-cat immunoglobulin G (Cappel Products, Organon Teknika Corp, USA) was diluted 1:800 in PBS with 0.001% Evan's blue and 10 µl of the mixture was applied into each well. Fluorescein-conjugated anti-dog immunoglobulin G was also employed in the IFA. The slides were incubated at 37°C for 1 hour, washed twice with PBS for 15 min, and then washed again with double distilled water for 10 min. The intensity of the bacillus-specific fluorescence was scored subjectively from +1 to +4, and the fluorescence score of ≥ +2 at a dilution of 1:32 was considered to be positive. Serum samples were screened at 1:32 and any positive sample at 1:32 dilution was titrated in a series of two fold dilutions up to 1:1,024.

Statistical analysis: Pearson Chi-square tests were used to examine statistical significance; $p < 0.001$ was considered significant.

Results and Discussion

The cat samples collected from the 3 provinces range from 15-22 samples/province (an average of 18.7) (Table 1). All cat samples were mixed type. The number of antibody titers against *B. henselae* in cats at the cut-off value 1:32, 1:64 and 1:128 were 5, 11, 5 samples, respectively (Table 2). The prevalence of antibodies to *B. henselae* varied by provinces, ranging from 0.0 to 73.0%. The average percentage of positive rate to *B. henselae* in Nan, Maehongson and Chiangrai provinces was 0.0, 52.0 and 73.0%, respectively with the cut-off value of 1:32 (Table 3). We found that the prevalence of antibodies to *B. henselae* in the 3 provinces had statistical differential ($p < 0.001$). The prevalence of *B. henselae* in Chiangrai and Maehongson provinces was significantly higher

than that of Nan province ($p < 0.001$) but the prevalence of *B. henselae* in Chiangrai and Maehongson provinces had no statistical differences. In this study, the gender and age of the cat had no statistical differences associated with seroprevalence. Examining the prevalence of antibodies to *B. henselae* in the cats in the two different border parts of Thailand, we found that the prevalence of antibodies to *B. henselae* from Thailand-Myanmar border (Chiangrai and Maehongson provinces, 61.8%) was significantly higher than that of Thailand-Lao PDR border (Nan province, 0.0%) ($p < 0.001$). The dog samples collected from the 3 provinces range from 15-60 samples/province (an average of 38) (Table 1). All dogs sample were mixed type. This study found that the dogs in the 3 provinces along Thai-Myanmar-Lao PDR had no prevalence of antibodies against *B. henselae*.

Table 2 Antibody titers against *B. henselae* in cats along the northern borders of Thailand

Titer	Province			Total (56)
	Chiangrai	Maehongson	Nan	
<1:32	4	9	22	35
1:32	3	2	0	5
1:64	5	6	0	11
1:128	3	2	0	5

Table 3 The seroprevalence of *B. henselae* IgG in cats along the northern borders of Thailand (cut off 1:32)

Border	Thailand Myanmar		Thailand Lao PDR	
	Chiangrai	Maehongson	Nan	Total
Positive/Samples	11/15	10/19	0/22	21/56
% Positive	73	52	0	37

The seroprevalence of *B. henselae* in cats was found in Thailand-Myanmar Border (Chiangrai and Maehongson provinces), but not found in Thailand-Lao PDR border (Nan province). The result correlated with report of isolate *B. henselae* from cats and cat fleas in Thailand-Myanmar Border (Parola et al., 2003). The seroprevalence in cats was higher in Chiangrai and Maehongson provinces, while no positive cats were found in Nan province. Chiangrai and Maehongson provinces are located in city site, but Nan province is located in rural and mountain areas. The seroprevalence of *B. henselae* in cats may depend upon the areas that the samples of sera were collected. The result also correlated with a report

stating that warm and humid climates are strongly associated with the presence of antibodies against *B. henselae* and ectoparasite infestation in cats (Maruyama et al., 2001; Chomel et al., 2004). The antibody to *B. henselae* was found only in the cats and not found in the dogs captured from the 3 areas suggesting that cats are important reservoirs of *B. henselae* in these areas and host specificity may be responsible for the association of *B. henselae* with the feline reservoir (Breitschwerdt and Kordick, 2000). It is known that dogs serve as the reservoir of *B. vinsonii* subsp. *berkhoffii*. Recently, there was a report that dogs could be accidentally infected with *B. henselae* and *B. clarridgeiae* (Diniz et al., 2007; Inoue et al., 2009). Therefore, the other panels, *B. vinsonii* subsp. *berkhoffii* and *B. clarridgeiae*, should also be examined in the dog sera.

In this study, we didn't collect other kinds of samples e.g. mite, chigger, tick, flea, rat and humans to examine for *B. henselae* by using isolation, purification, PCR, sequencing and phylogenetic classification techniques. Therefore, further studies should be conducted to clarify the epidemiology of the other reservoirs as the sources of *Bartonella* spp. infection along Thailand borders.

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