Surveillance of *Bartonella* Species from Pet Cats in Bangkok by Polymerase Chain Reaction Amplification of the 16S-23S rRNA Intergenic Region

C. Rodkhum*, P. Satranarakun, R. Pusoonthornthum

1Department of Veterinary Microbiology, 2Department of Veterinary Medicine, Faculty of Veterinary Sciences, Chulalongkorn University, Bangkok 10330, Thailand,

*Corresponding author: channarong_r@yahoo.com

Keywords: *Bartonella* species, pet cats, polymerase chain reaction, surveillance,

**Introduction**

*Bartonella* species are fastidious, Gram-negative bacilli or cocccobacilli and intra-erythrocytic bacterium. Several *Bartonella* species have been isolated from feline species including *B. henselae*, *B. charridegiae*, *B. koehlerae*, *B. weissii*, *B. quintana*, and *B. bovis*. They are considered as zoonotic human pathogens (1, 2). Despite many research and information of *Bartonella* sp. worldwide during the past decade, *Bartonella* sp. still is a mystery for veterinary and medical science in Thailand. In the year 1997-1998, *Bartonella* sp. have been isolated from difference geographical regions of Thailand including Chiang Mai (23.3%), Kanchanaburi (21.4%), Ratchaburi (34.8%), Bangkok (16.7%), Khon Kaen (50.1%), Roi Et (36.8%), Ubon Ratcharthani (21.6%), Nakhonratchasima (20.0%) and Songkhla (12.8%) (4). Objective of this study is to surveillance the present rate of *Bartonella* sp. infected pet cats in Bangkok.

**Materials and Methods**

A total of 75 pet cat blood samples were collected from pet cats in Bangkok province of Thailand in the year 2008-2009. The pet cats including in this experiment have not been treated with any antimicrobials before collection of samples. Approximately 1.5 ml of blood containing EDTA. In order to isolation of *Bartonella* sp., 100 µl of each blood sample was spread on the surface of tryptic soy agar (TSA) supplemented with 5% sheep blood. Then, incubated at 35°C under 5% CO<sub>2</sub> atmosphere. The cultured plates were observed for present of bacterial colony everyday and kept in CO<sub>2</sub> incubator for 1 month. The remained samples were stored at -20°C until used.

DNAs from blood samples were prepared using the QIAamp DNA blood mini kit (QIAGEN Inc., USA). PCR for amplification of the 16S-23S rRNA intergenic region was performed using method of Maggi and Breitschwerdt (3) with some modification. *Bartonella henselae* field strain (personal contact) was used as a positive control. The oligonucleotide primers used are listed in table 1

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Primer name</th>
<th>Oligonucleotide sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S-23S rRNA Intergenic Region</td>
<td>Bar321s-sense</td>
<td>5’-AGATGATGATCCCAAGCCTTCTGG-3’</td>
</tr>
<tr>
<td></td>
<td>Bar983as-antisense</td>
<td>5’TGTCTTCTAACAACATGATGATG-3’</td>
</tr>
</tbody>
</table>

**Results and Discussion**

The conventional isolation and culture of *Bartonella* sp. on TSA blood agar plate under 5% CO<sub>2</sub> atmosphere was not success due to the cultured plates were contaminated with many kinds of other bacteria. The further study should be modified for improve the efficacy of *Bartonella* sp. culturing method. The percentage of *Bartonella* sp. identified from 75 blood samples (n=75) was 1.33% (1/75). The only one PCR positive sample was *Bartonella henselae*. However, these results can not be absolutely interpreted as prevalence of *Bartonella* sp. in Bangkok due to low number of bacteria in blood or low number of bacteremia may caused of PCR negative result. Furthermore, the blood samples collected may or may not in the period of *Bartonella* septicemia. The PCR positive rate was difference from previous report (4) that found 16.7% of *Bartonella* sp. in Bangkok. Therefore, an effective *Bartonella* culturing method and serological studies should be implemented for confirmation of an epidemiological status of *Bartonella* sp. infection among Thai cat populations.

**Acknowledgements:** This research was supported by Chulalongkorn University-Veterinary Science Research Fund RG 8/2550

**References**