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Detection of *Babesia vogeli* in stray cats of metropolitan Bangkok, Thailand

Patcharathorn Simking[a], Sirichai Wongnakphet[b], Roger W. Stich[c], Sathaporn Jittapalapong[a,*]

[a] Department of Parasitology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand
[b] Department of Veterinary Diagnostic Services, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand
[c] Department of Veterinary Pathobiology, University of Missouri, Columbia, MO 65211, USA

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ABSTRACT

The combination of a rapidly growing stray animal population and the lack of animal control in Bangkok has resulted in a unique opportunity to evaluate the potential role of companion animals as sentinels and reservoirs of infectious diseases, including several of those caused by vector-borne parasites. The purpose of this study was to determine the prevalence and factors associated with the distribution of *Babesia* species infections among stray cats in Bangkok. Blood samples were collected from 1490 stray cats residing in 140 monasteries of 50 metropolitan districts of Bangkok, and assayed with light microscopy and PCR for evidence of *Babesia* spp. Pear-shaped merozoites were observed microscopically from two (0.13%) of these cats, while a nested 18S rDNA-based PCR assay detected babesial infections in 21 (1.4%) of the cats tested. The prevalence of infection was significantly different between sexes ($p < 0.05$), and PCR-positive cats were found in 30% (15/50) of the districts surveyed. All 21 amplicon sequences were identical, and were determined to be closest to that reported for *B. vogeli* (98% identity). These results represent the first molecular confirmation that a *Babesia* sp. is enzootic among stray cat populations in Thailand, and suggest that the presence of pet companion animals could be a risk factor for exposure of stray cats to vector-borne parasites.

1. Introduction

Babesia species are tick-borne intraerythrocytic apicomplexan parasites (Uilenberg et al., 1989). Members of this genus naturally infect a broad range of mammals, including rodents, canids and bovids, and some *Babesia* spp. can be zoonotic. Babesial infections of domestic dogs include large piroplasms originally described as *B. canis* and smaller piroplasms usually grouped under the species *B. gibsoni*. The larger piroplasms, once collectively recognized as *B. canis*, are now recognized as no fewer than least three species, *B. canis*, *B. vogeli* and *B. rossi*, based on pathology, antigenic properties, tick vectors and genetic characterization (Carret et al., 1999; Depoix et al., 2002; Hauschild and Schein, 1996; Passos et al., 2005; Schetters et al., 1997; Zahler et al., 1998).

The first report of a *Babesia* infection in a cat (*Felis catus*) was from India (Mudaliar et al., 1950) and sporadic cases of infection among domestic cats by unidentified *Babesia* parasites were since reported in France, Germany, Thailand and Zimbabwe (Bourdeau, 1996; Moik and Gothe, 1997; Jittapalapong and Jansawan, 1993; Stewart et al., 1980). However, babesiosis in cats has primarily been reported from South Africa, where infection is mainly due to *B. felis*, a small piroplasm that causes anemia and icterus (Schoeman

* Corresponding author. Tel.: +66 2 942 8438; fax: +66 2 942 8438.
E-mail addresses: fvetspj@hotmail.com, fvetspj@yahoo.com (S. Jittapalapong).

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et al., 2001). Initial molecular evidence for *Babesia* infections in cats was reported after a partial small subunit ribosomal RNA gene was amplified from blood of cats in Spain and Portugal, which was identified as *B. canis* through amplicon sequence analysis (Criado-Fornelio et al., 2003).

It is important to determine the species and genotypes that cause babesiosis in order to prescribe the most suitable treatment, because these different infections result in different degrees of disease severity, prognoses and responses to antibiotic treatments (Brikenheuer et al., 2003). Diagnosis of canine babesiosis often includes visualization of piroplasms in Giemsa-stained thin blood smears with light microscopy. However, *Babesia* parasites can be difficult to detect microscopically, especially during the chronic stage of babesiosis when parasitemias are low. Indirect immunofluorescent antibody and enzyme-linked immunosorbent assays were developed to detect antibodies to *B. gibsoni* (Fukumoto et al., 2001). More recently, an 18S rRNA gene (rDNA)-based PCR method was developed to provide greater sensitivity and specificity in terms of detection and differentiation of *Babesia* species (Brikenheuer et al., 2003). Furthermore, in addition to surveys for specific pathogens, the geographic information system (GIS) is widely utilized as a public health tool for investigations of chronic diseases, zoonoses, injuries and accident patterns, allocation of health resources, occupational morbidity and environmental exposure (Zenilman et al., 2005). Thus, the combined use of molecular diagnostic and GIS technologies makes it possible to implement strategies to identify the relationship of the environment for prediction of vector-borne pathogen transmission (Melone et al., 2001).

The combination of a rapidly growing stray animal population and the lack of animal control in Bangkok provides a unique opportunity to evaluate risk factors associated with feline exposure to these parasites. However, to the best of our knowledge, molecular evidence of *Babesia* infections among cats in Thailand has not been reported to date. Thus, the objectives of this study were to utilize an 18S rDNA-based PCR assay to survey stray cats located in monasteries of 50 districts from the Bangkok metropolitan areas, and to utilize GIS to integrate spatial data to determine the influence of climate and other environment factors on the distribution of these infections.

2. Materials and methods

2.1. Study area

Stray cat samples were selected by simple randomization. Blood was sampled from 10 stray cats from each of three monasteries per district, or 30 or 15 cats were sampled as representative of districts that only had one or two monasteries, respectively. Fifty districts were sampled in Bangkok.

2.2. Samples

A total of 1490 blood samples were collected from March to May, 2004. The animals were gently restrained, and 3–5 ml of blood was drawn from the jugular vein, preserved in sodium citrate vacuum tubes and stored at −40 °C until use. Each cat was thoroughly examined and searched for ectoparasites, and age, sex, health status and environmental conditions were recorded. Cat ages were estimated with information provided by monastery caretakers and through the results of dental examinations that were evaluated based on guidelines provided by the Humane Society of the United States. For example, cats with clean white teeth were considered less than 1-year old; yellowing on back teeth indicated 1–2 years of age; tartar build up on all teeth and some tooth wear indicated 3–5 years. Health condition criteria were as follows: (1) healthy: good body score, no dehydration, no clinical signs of disease (e.g. normal mucous membranes); (2) unhealthy: lower body score (presenting crest of Ilium), but no dehydration nor clinical signs; and (3) sick: weak, dehydrated, purulent ocular or nasal discharge, or some clinical signs observed. Environmental conditions were also scored as (1) good: monastery grounds were clean with good administration of animal habitation; (2) fair: monastery grounds were clean with some filth or construction present, but no left over food in animal habitation; and (3) poor: dirty, disordered monastery grounds and poor administration of animal habitation.

2.3. Microscopic examination

Thin blood smears were prepared in the field and fixed with methanol for 2 min. Fixed slides were transported back to the laboratory and stained with Modified Giemsa solution for 5 min, and washed for 10 min in distilled water prior to examination under a light microscope.

2.4. PCR assays

Duplicate blood samples (100 μl each) were lysed with 500 μl of denaturing solution (4 M guanidinium thiocyanate, 25 mM sodium citrate, pH 7, 0.1 M 2-mercaptoethanol, 0.5% N-lauroylsarcosine), and shaken for 5–10 min. Proteins were extracted with phenol–chloroform extraction and DNA precipitated in ethanol as previously described elsewhere (Sambrook and Russell, 2001), resuspended in TE buffer (50 mM Tris, pH 8.0, 1 mM EDTA) and stored at −20 °C until use.

DNA was assayed with the PCR method described by Ano et al. (2001). The 25 μl reactions contained 1× buffer (10 mM Tris–HCl pH 8.8, 50 mM KCl and 0.1% Triton X-100), 1.6 mM $MgCl_2$, 12.5 pmol of each primer, 0.2 mM of each dNTP, 0.75 Units of Taq DNA polymerase (DyNAzyme, FINNZYMES) and 1 μg of DNA template. The primers PIRO-F (5′-AGTCATATGCTTGTCTTA-3′) and PIRO-R (5′-CCATCATTCCAATTACAA-3′), which amplified approximately 500 nucleotides of the small subunit ribosomal RNA gene, were designed according to the nucleotide sequence in Genbank (accession number LI3729). A nested PCR was performed to generate a 327 bp amplicon with the re-designed internal primers PIRO2-F (5′-ATAACCGTGCTAATTGTAGG-3′) and PIRO2-R (5′-TGTTATTTCTTGTCACTACC-3′). Reactions were incubated at 90 °C for 2 min followed by 30 cycles of denaturation at 90 °C for 30 s, annealing at 55 °C for 2 min and