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Research Article

First Case of *Babesia Vogeli* in a Cat in Vietnam

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Abstract

A 15 years-old female British long hair cat was presented to a private veterinary clinic in Hanoi, Vietnam showing anemia, weakness, lethargy, weight loss and nose bleeding. Clinical examination of the cat showed pale mucous membrane, high fever, and shortness of breath.

Amplification and sequence of the 18S *rRNA* gene follow by phylogenetic analysis, *Babesia vogeli* was confirmed with 100% and close to reported sequences from China, Thailand, and Japan. This is the first report of *Babesia vogeli* in cat in Vietnam.

Keywords: *Babesia vogeli*; Cat; Vietnam; PCR

Introduction

Babesia species are intracellular apicomplexa parasites transmitted by ticks [1]. Members of this genus are naturally infectious to a wide variety of mammals, inclusive of cats. Babesia in domestic cats is a rarer infection in comparison with its canine infection. Subsequent cases of infection were also published in some countries such as: Brazil, France, Germany, Thailand and Zimbabwe [2-4]. The first molecular evidence of *Brucella canis* infection in cats was reported after a partial amplification of the *rRNA* gene from feline blood in Spain and Portugal [5].

In 2003, an 18S *rRNA* gene-based PCR method was developed to the detection and distinction expressly of Babesia species [6]. Currently, in Vietnam to diagnosis of Babesiosis, it is common to use Giemsa-stained method and observe the morphology and developmental stages of *Babesia* under the light microscope. However, Babesia parasites are hard to detect by light microscope, specifically during the chronic phase of Babesiosis when parasitemias are low. Besides, the occurrence of molecular evidence of *Babesia* infection among normal cats in Vietnam has not previously been reported to date. Therefore, the purpose of this research was to use PCR assay based on 18S *rRNA* gene to accurately determine the prevalence of Babesia species in Vietnam.

Case Presentation

A 15 years-old female British long hair cat was presented to a private veterinary clinic showing anemia, weakness, lethargy, and epistaxis. Clinical examination of the cat showed pale mucous membrane, high fever (40°C) and weigh lost with histological of tick infection.

Approximately 2 ml of blood was obtained aseptically from the jugular vein for hematological and biochemical analysis. Complete blood count revealed the strongly regenerative anemia (Hematocrit = 7.3%), accompanying a dramatic decrease of erythrocyte (3.8×10^{12}/l), hemoglobin (8.7 g/dl) and platelets (170×10^{9}/l). Serum biochemistry showed a moderate increase in alanine transaminase (ALT; 240 IU/l) and AST (370 IU/I) with increasing of creatinine level (434 mg/dl) (Table 1).

Thin blood smears were prepared and used Giemsa-stained method, after that examination under a light microscope. Molecular identification of *B. vogeli* was performed by amplifying the 18S *rRNA* gene-based PCR method. The DNA was extracted using the DNeasy Blood & Tissue Kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions. Fragment of the 18S rRNA of *Babesia* spp. amplified a 422 bp to 440 bp using conventional PCR with a primer set, 18SR-reverse: CAAGACAAAAGTCTGCTTGAAAC and 18SF-forward: GTTTCTGMCCCATCAGCTTGA [7], following the conditions: denaturation at 94°C for 10 min, followed by 40 amplification cycles (94°C for 30 sec, 60°C for 30 sec and 72°C for 30 sec) and a final extension at 72°C for 5 min. Phylogenetic analysis was, realize based on the variable region of the Babesia 18S *rRNA* gene, the sequences identified in this study were compared with the homologous sequences on GenBank, the phylogenetic tree were constructed by the neighbor-joining method using the Kimura 2-parameter model, Bootstrap value were 1000 replicates with MEGA X software. *B. vogeli* in this study was 100% to the sequences reported from China, Thailand, and Japan. (KY073363.1, KF621074.1, AY077719.1) (Figure 1).

Discussion

The results of this study are the first molecular evidence of *B. vogeli* infection in cats in Vietnam. The first report of *B. vogeli* infection in cats was from Bangkok, Thailand with infection rate of 1.4% out of a total of 1,490 stray cat's positive [8]. *B. vogeli* is commonly reported in dogs, however, seems to be increasingly widespread in domestic cats, with reports of high prevalence of this species in cats, for example, 16% on Brazil [9,10], 13% on St Kitts [11] in the Americas, 8.1% on Portugal [12] in Europe, 2.9% on Qatar in the Middle East [13] and Thailand [8] in Asia.

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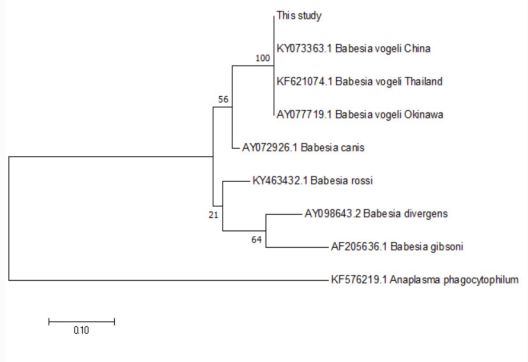

Figure 1: The phylogenetic relationship of *Babesia vogeli* isolated in this study with other studies is based on partial sequencing of the 18S *rRNA* gene.

Cats infected with *Babesia* spp. are related to anorexia, lethargy, anemia, and icterus. In a research on *B. felis*, most cats showed symptoms of anorectic, lethargic and hypochromic microcytic anemia; In addition, hyperbilirubinemia and Alanine Aminotransferase Activity (ALT) at a high rate (86% to 89%) [14].

Main clinical signs commonly found with *B. vogeli* infection: fever, lethargy, anorexia, jaundice, Immune mediated Haemolytic anemia, non-regenerative anemia, leukocytosis, leukopenia, and thrombocytopenia [15]. Although *B. vogeli* in cats has been detected in some countries around the world by molecular methods, but, the clinical features of *B. vogeli* infection in cats have not been well described. In this case, the cat showed anemia, weakness, and epistaxis. The clinical sign of weakness and anemia may cause by rapid increasing of Babesia organisms in peripheral blood vessels leading to decrease in level of hemoglobin, erythrocyte and hematocrit (Table 1). Epistaxis evident was well reported in various infections including babesiosis [16] and nose bleeding in this study case may cause by platelet destruction resulting by the infection. On the other hand, the dysfunction of liver and kidney caused by *Babesia* infection well was reported [17,18]. In this case, *Babesia* infection may affected to liver and kidney function presenting by the rise of ALT, AST, and creatinine level (Table 1).

Table 1: Hematological and biochemical analysis of the *Babesia vogeli*-infected cat.

Parameter	Value	Reference range
WBC	4.9	3.5-16
RBC	3.8	5.9-9.9
Hemoglobin	8.7	9.3-15.9
Lymphocytes	3.2	1.5-7
Neutrophils	3.5	2.5-12.8
Hematocrit	7.3	37
MCV	52	39-55
MCH	16.3	12.5-17.5
Platelets	170	200-500
AST	370	10-100
ALT	240	10-100
GGT	4	0-7
ALP	32.03	6-102
Creatine	434	60-163
Urea	6.81	4.8-11.6

WBC: White Blood Cell; RBC: Red Blood Cell; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; GGT: Gamma-Glutamyl Transpeptidase; ALP: Alkaline Phosphate

Last decade, detection of Babesia species base on morphology identification by the form or dimensions of the organism. In advent of molecular technique, distingue of *Babesia* species base on the18S *rRNA* gene sequences have been widely used as a tool for taxonomy and phylogenetic analysis for the purpose of identifying *Babesia* species. In 2010, the first report of *B. vogeli* infection in cats based on 18S *rRNA* gene sequences was from Bangkok, Thailand [8] with 98% homologous of a *B. vogeli* strain from dogs in Brazil [19]. In this study case, The 18S rRNA and phylogenetic analysis indicated that Babesia species found related to *B. vogeli* (100%) closely to reported sequence from China, Thailand and Japan. (GenBank number KY073363.1, KF621074.1, AY077719.1) (Figure 1).

Evidence of this cat with tick infected histologically was confirmed by the owner but the tick species was not clarified. It is well known that *Rhipicephalus sanguineusis* commonly found in Vietnam [20,21]. On the other hand, evidence of *B. vogeli* in *Rhipicephalus sanguineus* tick in Vietnam was reported recently [22]. Present of tick may prove as an important risk factor for transition *B. vogeli* in this study.

This report raises awareness for cat owners in controlling of blood sucking agent in the animal and environment surrounding, especially in the endemic area to prevent this infection. Further research is required to better understand this pathogen, including host susceptibility factors. Moreover, if any abnormal clinical signs have been observed on the animal such as weakness, anemia, weigh loss it should be delivered to the animal clinic to promptly receive the appropriate treatment.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethics Approval and Consent to Participate

Informed consent and agreement were obtained from cat's owner. The examination of animal was conducted with regards to their welfare.

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