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Incidence of *Babesia gibsoni* in dogs in and around Bengaluru

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Abstract

Canine babesiosis is a serious disease that affects domestic and wild canids worldwide. It is caused by intraerythrocytic protozoa of the genus *Babesia*, family Babesiidae, Order Piroplasmida, within the phylum Apicomplexa. The most common species that cause canine babesiosis include *Babesia canis* and *Babesia gibsoni*, which could be differentiated based on their size within the parasitized erythrocytes. *Babesia canis* is the large form (2.5-5.0 µm), while *Babesia gibsoni* is a small pleomorphic organism (1-2.5 µm) and appears most commonly as ring form (Obeta, 2020). Incidence of *Babesia gibsoni* was estimated by history, clinical signs and presence of *B. gibsoni* organisms in the geimsa stained blood smear and by PCR. A total of 136 suspected cases were examined out of which 64 cases were positive for *B. gibsoni* accounting to an incidence of 47.05 percent. Out of 136 samples screened 64 (47.05%) cases and 19 (13.97%) cases were positive by PCR and blood microscopy respectively. Higher incidence was observed in the age group of 1-3 years, breed wise in Labrador Retrievers and gender wise in male dogs and season wise in summer season.

Keywords: Haemophysalis, incidence, dogs, *Babesia gibsoni*

Introduction

Canine babesiosis is a serious disease that affects domestic and wild canids worldwide. It is caused by intraerythrocytic protozoa of the genus *Babesia*, family Babesiidae, order Piroplasmida, within the phylum Apicomplexa. The most common species that cause canine babesiosis include *Babesia canis* and *Babesia gibsoni*, which could be differentiated based on their size within the parasitized erythrocytes. *Babesia canis* is the large form (2.5-5.0 µm), while *Babesia gibsoni* is a small pleomorphic organism (1-2.5 µm) and appears most commonly as ring form (Obeta, 2020) [21]. *Babesia Canis* is transmitted by tick *Dermacentor reticulatus*, whereas the ticks *Rhipicephalus sanguineus* and *Haemophysalis longicornis* serves as a potential vector for *Babesia gibsoni*. *B. gibsoni* is distributed throughout the world including Middle East, Northern Africa, and South Asia (Salem and Farag., 2014) [22].

Clinical signs and physical examination of dogs infected with *Babesia gibsoni* include pyrexia, lymph node enlargement, dullness, depression, inappetance, pale mucous membrane, tachycardia, tachypnoea, epistaxis, ascites, loss of body weight, hepatomegaly, splenomegaly, nervous deficits, constipation, diarrhoea, icterus, and nephropathy (Varshney *et al.* 2008, Teodorowski *et al.* 2022) [32, 28].

Direct microscopic analysis of the stained blood smear is the most widely used approach for diagnosing canine babesiosis since it is a conclusive, practical, and economical procedure. However, the direct microscopic examination of stained blood smears do not detect parasites in inapparent or chronic infections (Caccio *et al.*, 2002) [6]. The serological methods such as indirect fluorescent antibody test (IFAT) and enzyme-linked immune sorbent assay (ELISA) for *B. gibsoni* parasites are highly sensitive but moderately specific due to antigenic cross reaction to *B. canis* (Yamne *et al.*, 1993) [34]. The advances in molecular biology techniques such as PCR have improved the sensitivity and specificity to identify piroplasm to a greater extent as compared to conventional approaches (Ionita *et al.* 2012) [11].

Materials and Methods

A total of 136 samples were collected from dogs with a history of tick infestation and exhibiting clinical signs of high fever, pale / icteric mucous membrane, inappetence, anaemia, lethargy, vomiting, diarrhoea, hemoglobinuria from different breeds, gender and age group of dogs during the period from July 2022 to August 2023, which were presented to Veterinary

College Hospital, Hebbal, Bengaluru. Blood samples were collected in vacutainer containing EDTA as anticoagulant and stored at -20 °C for further processing. Blood samples were screened by PCR.

Polymerase Chain Reaction (PCR)

DNA was extracted from the whole blood sample using QIA amp DNA blood mini kit (M/s QIAGEN Germany) according

to manufacturer’s instructions. DNA samples were amplified using *B. gibsoni* species specific primers (published by Inokuma *et al.* 2004)^[12]. The specific amplicon size of 662 bp was analysed by 1.2% agarose ethidium bromide gel electrophoresis.

Primers: The primers for *Babesia gibsoni* were procured from M/s Barcode Biosciences, Bangalore.

Table 1: Species specific primer for *Babesia gibsoni* (Inokuma *et al.* 2004)^[12]

Haemoparasite (gene targeted)	Primers	Product Size
<i>B. gibsoni</i> (18S rRNA)	Forward primer Gib599: 5'- CTCGGCTACTTGCCTTGTC-3' Reverse primer Gib1270: 5'- CCGAAACTGAAATAACGGC-5'	662 bp

Table 2: Composition of reaction mixture employed to amplify 18S rRNA gene of *B. gibsoni*

Sl. No.	Reaction mix	Quantity
1	Forward Primer	1.5 µl
2	Reverse Primer	1.5 µl
3	Template DNA	1.5 µl
4	Master Mix	12 µl
5	Nuclease Free Water	5 µl
	Total volume	21.5 µl

Table 3: The following PCR cycle condition was used in the thermal cycler (Eppendorf, Germany)

PCR Stage	Step No.	Name of the step	Temperature	Time	No. of cycles
1	1	Initial denaturation	95 °C	5 Min.	1
2	2	Denaturation	95 °C	1 Min.	28
	3	Annealing	58 °C	1 Min.	
	4	Extension	72 °C	1 Min.	
3	5	Final extension	72 °C	5 Min.	1

Results

Out of 136 blood samples suspected for *B. gibsoni* infection, 64 dogs (47.05%) were positive for *B. gibsoni*.



Lane 1 100bp DNA ladder
 Lane 2 Positive Control
 Lane 3 Negative Control
 Lane 4 No template Control
 Lane 5 Negative sample
 Lane 6, 7, 8,9 and 10 Positive Samples

Plate 1: Screening of blood samples using PCR

In the present study out of 64 positive cases, 13 cases (20.31%) were less than 1 year of age, 30 cases (46.87%) belonged to 1 to 3 years old dogs, 8 cases (12.5%) belonged to 3 – 6 years of age, 9 cases (14.06%) were between 6 to 9

years of age, 3 cases (4.68%) were between 9 to 12 years old and 1 case (1.56%) was more than 12 years of age (Table 4).

Table 4: Age – wise incidence of *B. gibsoni* in dogs

Age group	Number of infected dogs	Percent incidence
< 1 year	13	20.31
1 – 3 years	30	46.87
3 – 6 years	8	12.5
6 – 9 years	9	14.06
9 – 12 years	3	4.68
>12 years	1	1.56
Total	64	100

The breed wise incidence of *B. gibsoni* was found to be highest in Labrador Retriever with 15 cases (23.43%) followed by 13 (20.31%) cases of Golden Retriever, 5 (7.81%) cases of schitzu, 5(7.81%) cases of German shepherd, 5 (7.81%) cases of cocker spaniel, 5 (7.81%) of Non descript, 4 (6.25%) cases of Pomeranian, 4 (6.25%) cases of Rottweiler, 2 (3.12%) cases of Husky, 2 (3.12%) cases of Pitbull, 2 (3.12%) cases of Beagle, 1 (1.56%) case of Saint Bernard, 1 (1.56%) of Daschund (Table 5).

Table 5: Breed – wise incidence of *B. gibsoni* in dogs

Breed	Number of infected animals	Percent incidence
Labrador Retriever	15	23.43
Golden Retriever	13	20.31
Schitzu	5	7.81
German shepherd	5	7.81
Cocker spaniel	5	7.81
Non descript	5	7.81
Pomeranian	4	6.25
Rottweiler	4	6.25
Husky	2	3.12
Pitbull	2	3.12
Beagle	2	3.12
Saint Bernard	1	1.56
Daschund	1	1.56

Out of 64 cases positive for *B. gibsoni*, 43 (67.18%) cases were male dogs and 21 (32.81%) cases were female dogs (Table 6).

Table 6: Gender – wise incidence of *B. gibsoni* in dogs

Gender	Number of infected dogs	Percent incidence
Male	43	67.18
Female	21	32.81
Total	64	100

The incidence of *B. gibsoni* was recorded in different seasons. The highest incidence of *B. gibsoni* were recorded in Summer season with 25 cases (39.06%), followed by South –West Monsoon with 21 cases (32.81%), Winter season with 11 cases (17.18%), North – East Monsoon with 7 cases (10.93%) (Table 7).

Table 7: Season – wise incidence of *B. gibsoni* in dogs

Season	Number of infected dogs	Percent incidence
Winter	11	17.18
Summer	25	39.06
South – West Monsoon	21	32.81
North – East Monsoon	07	10.93

Discussion

In this present study, a total of 136 dogs presented with the clinical signs suggestive of canine babesiosis presented to Veterinary College Hospital, Bengaluru were PCR to know the incidence of *B. gibsoni* in dogs during the period from July 2022 to August 2023. Of 136 cases examined 64 cases were positive for *B. gibsoni* accounting to an incidence of 47.05 percent.

This finding is in accordance with the reports of Mahalingaiah *et al.* (2017) [18], Bhattacharjee and Samarah (2013) [4], Tuska *et al.* (2021) [29] and Jain *et al.* (2017) [13] who detected 49 percent, 47.16 percent, 40.5 percent and 47.3 percent of *B. gibsoni* incidence respectively.

However, in contrast to our study reports, a lower incidence was observed by Chandra *et al.* (2018) [7], Gonde *et al.* (2017) [9], Vipani *et al.* (2015) [33] and Manoj *et al.* (2020) [19] who reported a incidence of 13.2 percent, 8.26 percent, 7.84 percent, 3.84 percent and 0.4 percent respectively.

Higher incidence of *B. gibsoni* was reported by Kumar *et al.* (2009) [14], Selvaraj *et al.* (2010) [23] and Vairamuthu *et al.* (2012) [30] who observed 84.9 percent, 93.5 percent, 56.65 percent respectively.

The differences in the incidence of *B. gibsoni* could be attributed to the immune status of the host, tick population, seasonal variation and the climatic condition in different geographical locations and the type of diagnostic method involved.

Age wise incidence of *B. gibsoni*

In this study, dogs in the age group of 1 to 3 years had highest incidence of *B. gibsoni* which is in agreement with Vipani *et al.* (2015) [33], Mahalingaiah *et al.* (2017) [18], Obeta (2020) [21] and Kunwar *et al.* (2021) [15]. Higher incidence of *B. gibsoni* in 1 to 3 years of age in our study could be due to over presentation of dogs of this age group. In contrast to our study Ma *et al.* (2021) [17], Senthil and chakravarthi (2023) [24] found higher incidence in adults, though the difference was not statistically significant. Therefore it could opined that age is not the criteria for canine babesiosis and occurrence of infection depends on the transmitting vector, pathogen load and the immune status of the host.

Breed wise incidence of *B. gibsoni*

The results of the present study showed that highest incidence of *B. gibsoni* was in Labrador Retrievers.

This is in agreement with the findings of Mahalingaiah *et al.* (2017) [18], Bilwal and Mandali (2020) [5] who reported higher prevalence in Labrador retriever breed of dog. On contrary, Bastos *et al.* (2004) [3], Shrivastava *et al.* (2014) [25], Kunwar

et al. (2021) [15] who recorded maximum prevalence in German shepherd dogs and Yeagley *et al.* (2009) [16] who observed higher prevalence in pit bull terriers.

The variations in breed wise occurrence of *B. gibsoni* in dogs could be attributed to the variations in the population of different breeds, variation in the dog breed presented to the veterinary hospital, preference of owner to different breeds of dogs in different areas, variation in the number of dogs screened per breed, genetic and immunological status of the dogs.

Gender - wise incidence of *B. gibsoni* in dogs (n=64)

Out of 64 cases positive for *B. gibsoni*, 43 (67.18%) cases were male dogs and 21 (32.81%) cases were female dogs. The results of our study revealed highest incidence of *B. gibsoni* infection in male dogs. Higher occurrence of *B. gibsoni* in dogs has also been reported by Bashir *et al.* (2009) [2], Ilie *et al.* (2010) [10], Vipani *et al.* (2015) [33], Mahalingaiah *et al.* (2017) [18], Kunwar *et al.* (2021) [15], whereas Das *et al.* (2015) [8] observed highest occurrence in female dogs. This discrepancy in the increased occurrence in male dogs could be due to increased wandering behaviour, differences in environmental exposure, genetic or hormonal influences or due to the increased interest among owners to keep male dogs as companion.

Season- wise incidence of *B. gibsoni* in dogs (n=64)

The incidence of *B. gibsoni* was recorded in different seasons. The highest incidence of *B. gibsoni* were recorded in Summer season with 25 cases (39.06%), followed by South–West Monsoon with 21 cases (32.81%), Winter season with 11 cases (17.18%), North – East Monsoon with 7 cases (10.93%). The findings of the present study is in agreement with Varshney *et al.* (2003) [31], Singh *et al.* (2014) [26] and Azhar *et al.* (2023) [1] who observed higher incidence during summer. The highest incidence in summer season could be attributed to the seasonal activity of brown dog tick *Rhipicephalus sanguineous* during the hot and humid period of the year which provides conducive environment for its growth and development.

Conclusion

Overall incidence of canine babesiosis was 47.05 percent. Increased incidence in *B. gibsoni* could be attributed to the increased population of tick vectors and lower immune status of the host. Therefore, appropriate control measures for ectoparasites should be followed in dogs.

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