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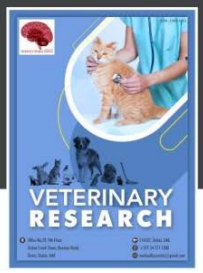

Studies on Incidence of Babesiosis in Stray and Pet Dogs in and Around Bhubaneswar, Odisha: By Simple Microscopy

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Key words: Canine, *Babesia canis*, *Babesia gibsoni*, Bhubaneswar, Odisha

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Page No.: 9-14
Volume: 9, Issue 3, 2016
ISSN: 1993-5412
Veterinary Research

Abstract: Babesiosis is caused by tick borne protozoal parasite prevalent in the canines *Babesia canis* and *B. gibsoni* worldwide. In the current study, a total of 198 blood samples from both stray and pet dogs with history of fever (102-106^{0}F), anorexia, anaemia, hemoglobinuria, enlarged lymph nodes and tick infestation were collected and examined at teaching Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, OUAT, Bhubaneswar, Odisha during a period of one year (August 2014 to June 2015). Examination of blood smears revealed an overall incidence of 8.07% (16/198) with 3.53% (7/198) positive for *B. canis* and 4.54% (9/198) positive for *B. gibsoni*. The younger males (<1 year of age) of both stray and pet dogs belongs to Labrador breed were found to be most infected with the babesiosis infection during summer followed by rainy and winter season. The percentage of incidence among pet dogs was higher (9%) than stray dogs (7.14%).

INTRODUCTION

Canine babesiosis is an emerging tick-borne life threatening protozoal hemoparasitic disease caused by the intra-erythrocytic protozoan parasites under the genus Babesia in many parts of the world including India. The identification of species has traditionally been based on the host specificity and on the morphology of the intra-erythrocytic forms (piroplasms). *Babesia canis* (Large, 3.0-5.0 μm) and *B. gibsoni* (Small, 1.5-2.5 μm) are recognized as the two species that cause canine babesiosis worldwide and are transmitted by *Dermacentor reticulatus* in Europe, *Rhipicephalus sanguineus* in tropical and subtropical regions and *Haemaphysalis leachi* in South Africa (Uilenberg, 2006). *B. canis* usually occurs as a single pear-shaped piroplasm or in pairs of merozoites divided by binary fission within the erythrocyte and *B. gibsoni* appears as small pleomorphic organism.

Besides individual factors like age, sex, immunity, genetic makeup of different breeds, adaptability to prevailing environment, inter current diseases, other stress causing factors such as pregnancy, lactation, transportation are also important which can have direct impact on babesiosis infection (Brandao *et al*., 2003; Irwin, 2009). Clinically canine babesiosis has been found to result in a wide range of presentations, from subclinical disease to serious and even fatal illness characterized by fever, pallor, jaundice, splenomegaly, weakness and collapse associated with intra and extra-vascular haemolysis, hypoxic injury, systemic inflammation, thrombocytopenia and pigmenturia (Irwin, 2009).

Direct microscopic examination is the conventional method for detecting *Babesia* spp. in animal blood samples. This is a conclusive, feasible and low cost diagnostic method. Though large surveys on canine babesiosis are scanty, a number of reports suggest that the parasite infects dogs worldwide. In Indian content, there have been sporadic reports of canine babesiosis based on conventional diagnostic methods (Chaudhary, 2006; Singh *et al*., 2012; Sahu *et al*., 2014; Das *et al*., 2015; Kumar *et al*., 2015).The present study was conducted to find out the status of canine babesiosis in stray and pet dogs in and around Bhubaneswar by microscopic study.

MATERIALS AND METHODS

Ethical approval: The experiment was done in accordance with the guidelines provided by the institutional ethical committee.

Study area: The stray dogs from different regions of Bhubaneswar (Fig.1) and it's periphery brought to the Veterinary Dispensary, Saheed Nagar, Bhubaneswar for Animal Birth Control (ABC) programme (carried out jointly by the Fisheries and Animal Resources Development Department, Government of Odisha and Bhubaneswar Municipal Corporation under the direct supervision of the Animal Welfare Board of India and CPCSEA for controlling the population of stray dogs) and the pet dogs presented to the Teaching Veterinary Clinical Complex of the College of Veterinary Science and Animal Husbandry, OUAT, Bhubaneswar were included in the present study. Bhubaneswar is located between 20° 14' 0" North, 85° 50' 0" East with an average relative humidity of 70% and annual rainfall of 1,542 mm (61 in). It is in the eastern coastal plains, along the axis of the Eastern ghats mountains. The city has an average altitude of 45 m (148 ft) above sea level.

Population size: A total of 198 (100 pet and 98 stray) dogs (82 in summer, 43 in rainy and 73 in winter season) having clinical signs like anorexia, weakness, lethargy, fever (102-106 ^{0}F), anaemia, pale mucous membrane, haemoglobinuria and with the history of tick infestation were examined for babesiosis infection for a period of nearly 1 year (August 2014 to June 2015). The study included 125 males and 73 females belonging to two age groups (below 1 year age and above 1 year age) who were examined for the presence of babesiosis.

Samples collection and examination: About 2 mL of blood samples were collected from all these dogs in vials containing EDTA (Ethylene Diamine Tetra-acetic Acid) as anticoagulant. To make a thin blood film, a drop of blood was placed on a clean glass slide drawn into a smear; air dried, fixed in methanol, stained with Giemsa strain and examined under light microscope by using the oil immersion objective (100X) was performed within 4-6 h according to standard protocol described by Benjamin (2007).

Statistical analysis: Statistical analysis was carried out by Statistical Package for Social Science (SPSS) using chi-square test.

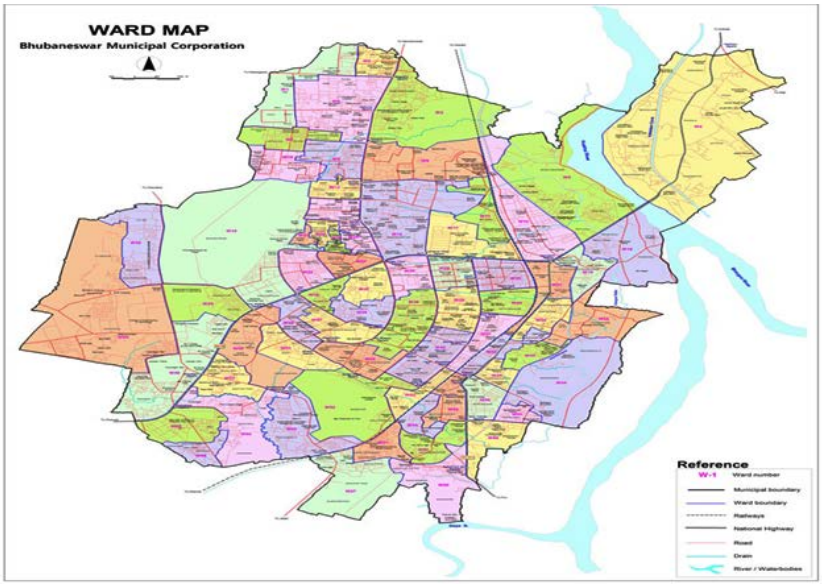

Fig. 1: Ward map for Bhubaneswar municipal corporation

RESULTS

In the present study, examination of blood smear from 198 cases revealed an overall incidence of 8.07% (16/198) with 3.53% (7/198) positive for *B. canis* and 4.54% (9/198) positive for *B. gibsoni* (Fig. 2).

Age wise incidence of babesiosis infection among stray and pet dogs has been illustrated in Table 1. Perusal of the table reveals that the overall incidence of babesiosis infection was higher in both stray and pet dogs <1 year of age, while the variation in the percentage of incidence in different age groups was not statistically significant.

The data on sex wise incidence of babesiosis in dogs have been summarized in Table 2. Perusal of the table reveals the overall percentage of incidence was found to be higher (9.6%) among males than females (5.47%) in both stray and pet dogs and the variation due to sex was statistically not significant.

Analysis of data on the influence of season on incidence of babesiosis infection in dogs distributed over three seasons (Table 3) revealed highest percentage (10.97%) of incidence during summer season followed by rainy (6.97%) and lowest (5.47%) in winter, and influence of season on incidence of babesiosis infection was not significant ($p>0.05$).

Breed wise incidence of babesiosis infection in dogs is described in Table 4. The overall incidence of babesiosis was higher in Labrador (17.85%) followed by German shepherd (15.38%), spitz (12.5%), pug (10%), rottwiller (9.09%), mixed breed (8.33%), great dane (5.55%) and non-descript (4.08%).

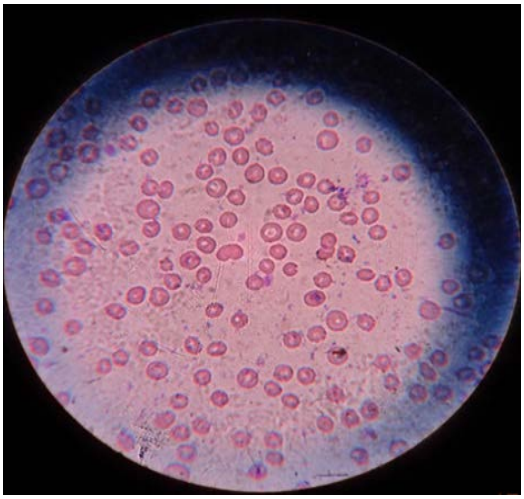

Fig. 2: *B. canis* and *B. gibsoni* within RBC in Giemsa stained blood smear of an infected dog (100X)

Table 1: Age-wise incidence of babesiosis infection in dogs

	Pet dogs			Stray dogs			Total		
Age	No. of dogs examined	No. found positive	Age (%)	No. of dogs examined	No. found positive	Age (%)	No. of dogs examined	No. found positive	Age (%)
<1 year	25	3	12	20	2	10	45	5	11.11
>1 year	75	6	8	78	5	6.41	153	11	7.18
Total	100	9	9	98	7	7.14	198	16	8.08
χ^2	0.545028			0.578128			0.396163		

N.B.**:Highly Significant, p<0.01; *:Significant, p<0.05; NS: Non Significant, p>0.05

Table 2: Sex-wise incidence of babesiosis infection in dogs

	Pet dogs			Stray dogs			Total		
Sex	No. of dogs examined	No. found positive	Age (%)	No. of dogs examined	No. found positive	Age (%)	No. of dogs examined	No. found positive	Age (%)
Male	65	7	10	60	5	8.33	125	12	9.6
Female	35	2	5.71	38	2	5.26	73	4	5.47
Total	100	9	9	98	7	7.14	198	16	8.08
χ^2	0.399513			0.565286			0.304713		

N.B.**: Highly Significant, p<0.01; *: Significant, p<0.05; NS: Non Significant, p>0.05

Table 3: Season-wise incidence of babesiosis infection in dogs

	Pet dogs			Stray dogs			Total		
Season	No. of dogs examined	No. found positive	Age (%)	No. of dogs examined	No. found positive	Age (%)	No. of dogs examined	No. found positive	Age (%)
Rainy	22	2	9.09	21	1	4.76	43	3	6.97
Winter	38	3	7.89	35	1	2.85	73	4	5.47
Summer	40	4	10	42	5	11.9	82	9	10.9
χ^2	0.948502			0.274636			0.43589		

N.B. **: Highly Significant, p<0.01; *: Significant, p<0.05; NS: Non Significant, p>0.05

Table 4: Breed-wise incidence of babesiosis infection in dogs

Breed	Total No. of dogs examined	Total No. of infected dogs	Percentage
Labrador	28	5	17.85
Mixed	12	1	8.33
German shepherd	13	2	15.38
Spitz	8	1	12.5
Pug	10	1	10
Non descript	98	4	4.08
Great dane	18	1	5.55
Rottwiller	11	1	9.09
Total	198	16	8.08

DISCUSSION

As per our investigation, examination of blood smear from 198 cases revealed an overall incidence of 8.07% (16/198) with 3.53% (7/198) positive for *B. canis* and 4.54 % (9/198) positive for *B. gibsoni*. Previously, from the same region, prevalence of 1.66% & 4.81% for *B. canis* and *B. gibsoni* respectively was reported (Sahu *et al*. 2014). The relatively lower incidence could be due to inability of microscopic study to detect the organisms in chronic cases and in low parasitemia. Similar findings were recorded in earlier studies at Punjab (Singh *et al*., 2012) and from North Western region of India (Eljadar, 2010) revealing the prevalence of *B. gibsoni* and *B. canis* in the range of 0.65-8.26 and 1.43-4.51%, respectively while reports from South India have cited 3.9% and 84.9% prevalence of *B. canis* and *B. gibsoni* respectively. The prevalence of canine babesiosis from various parts of Northern India has been reported to be ranging from 0.66-8.9% (Chaudhary, 2006; Kumar *et al*., 2015; Singh *et al*. 2014; Varshney and Dey, 1998). The lower prevalence of babesiosis in dogs recorded could be attributed to a substantial improvement in the management practices which has reduced the exposure of canines to tick vector, thus, leading to a decrease in the cases of canine babesiosis. The prevalence of canine babesiosis in Assam, however, has been reported to be as high as 48.57 and 39.63%. The higher prevalence in this area might be due to favourable climate with moderate temperature, high humidity and unabated movement of dogs making it highly enzootic for canine babesiosis (Bhattacharjee and Sarmah, 2013; Laha *et al*., 2014).

The results of the present study indicate that dogs <1 years of age were more likely infected with *Babesia species* than the dogs having age >1 year. *Babesia* spp. cause disease mostly in young dogs, although, dogs of all ages can be affected (Schoeman, 2009) while some researchers have reported that more prevalence is seen in older dogs (>3 years) (Kumar *et al*., 2015, Shrivastava and Shukla, 2013). This could be due to an increased susceptibility to infection or less immunity. It has also been reported that age do not have any influence on the animals' susceptibility to the disease (Martinod *et al*., 1986).

The male dogs of either sex or age groups (<1year or >1 year) showed higher percentage of incidence of babesiosis among all the dogs examined which corroborated the findings in Jalandhar where prevalence of the babesiosis was comparatively higher in males (6.47%) than female dogs (3.52%)(Kumar *et al*., 2015). In Pakistan similar observation was reported where 3.39% of males and 1.32% were females were affected by canine babesiosis (Bashir *et al*., 2009). Investigation from different parts of the world also reported that the prevalence of babesiosis was higher in males than females (Taboada *et al*., 1992; Sonq *et al*., 2004; Mathe *et al*., 2006; Yao *et al*., 2014). However, other studies have showed that disease was more prevalent in females than males (Das *et al*., 2015; Laha *et al*., 2014; Gadahi *et al*., 2008). Few researchers have opined that there were no significant differences among sexes in relation to the disease (Eljadar, 2010) and no association between the occurrence of the disease and sex of host (Singh *et al*., 2014). Higher incidence rate in males during our study might be due to their frequent roaming to look for mates and establishing territories there by picking the ticks which act as vector for this disease. The aggressiveness and hormonal status of male dogs could also be a contributory factor.

The present study revealed that the incidence of babesiosis infection in dogs was highest during summer (10.97%) followed by rainy (6.97%) and lowest in winter (5.47%) which agrees to the findings recorded by other workers (Das *et al*., 2015; Collet 2000; Jacobson, 2006). As regards to seasonality of infection, variable observations by different researchers are available (Bashir *et al*., 2009; Mathe *et al*., 2006; Yao *et al*., 2014; Vatsya *et al*., 2010) which could be due to variation in relative humidity, temperature and number of samples at different places. The highest rate of incidence in summer recorded during present study followed by rainy season could be attributed to the seasonal activity of the brown dog tick, *Rhipicephalus sanguineus* which is in its abundance in hot and humid period of the year.

Our study revealed that the incidence of babesiosis infection was higher in Labrador breed (17.85%) followed by German shepherd (15.38%) and other breeds (49.55%). In South India, investigation revealed higher prevalence in German shepherd due to their heavy hair coat (Banu *et al*., 2006). The higher prevalence in Labrador in our study might be due to the preference of most of people to keep this breed as pet. Most of the police dogs belonging to this breed were involved in different patrolling and trafficking activities, so, there might be chances of exposure to tick infestations. However, some workers have also described that non-descript dogs were found to be most commonly affected due to their scavenging habits and exposure to vectors (Selvaraj *et al*., 2010; Bansal *et al*., 1990).

CONCLUSION

The present study revealed the incidence of canine babesiosis, though not of alarming rate in Bhubaneswar, Odisha which might be due to the hot and humid environmental condition which favours the survival of tick vectors. Though microscopic examination may not be very revealing in the detection at low parasitemia, it still remains the most rapid confirmatory method. Use of molecular techniques as well as serosurveillance with more sample and data collection will help in the better understanding of the disease as well as pave way for formulating efficient control strategies against the disease.

ACKNOWLEDGEMENTS

The researchers acknowledge the cooperation extended by the in-charge of Animal Birth Control Program, Saheed Nagar, Bhubaneswar for collection of samples, the Dean, C. V. Sc. & A.H., OUAT, Odhisa, India for providing necessary financial and administrative facilities to undertake the study.

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