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Clinical occurrence of canine babesiosis in around Indore Madhya Pradesh in India associated with epidemiological implications

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Abstract
The present study "Clinical occurrence of canine babesiosis in around Indore Madhya Pradesh in India associated with epidemiological implications" was conducted on 4304 dogs brought at the Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, Mhow (M.P.) and in privet veterinary clinics across Indore. The duration of study was one year from March 01, 2021 to February 28, 2022. The collection of blood samples from the dogs showing the symptoms of high rise temperature, poor general condition, weakness, pale mucus membrane and anorexia were considered for primary screening. The collected blood samples were screened for the presence of babesiosis thin blood smear examination after Giemsa stain. The incidence in relation to breed, age, gender, season and month was studied based on the results of blood smear examination. A total of 54 cases were babesiosis positive and overall prevalence of canine babesiosis diseases was 1.25 percent. The highest incidence was observed 0.185% in the month of August followed by 0.162% in the month of September. The higher incidence of 0.79% was recorded in dogs of age group above 18 months and gender wise male had 0.91% higher incidence of babesia as compared to the female 0.35%. Breed wise incidence was higher observed in German shepherd 0.51% followed by Labrador 0.23% and none descript 0.09%.

Keywords: Dog, canine babesiosis, incidence

Introduction
Deforestation has changed the natural habitation of vectors and introduced new vectors from wildlife into rural and urban areas. Babesiosis was initially described in cattle with red water (hemoglobinuric) fever in 19th Century 1888, when Dr. Victor Babes, a Romanian microbiologist, observed inclusions within bovine erythrocytes (Mihalca *et al.,* 2010) [13]. Canine babesiosis (or piroplasmosis) is a significant and potentially life threatening tick-borne protozoan disease of dog populations, worldwide. The disease is caused by intraerythrocytic parasites of the genus Babesia, order *Piroplasmida, phylum Apicomplexa* (Irwin, 2009) [9]. Tick-borne protozoan infections with *Babesia gibsoni* and *Hepatozoon canis* are more frequent than filarial infections with *D. immitis* and *A. reconditum* (Sarma *et al.,* 2019) [19]. The dimensions of the Babesia parasite in relation to the size of the erythrocyte (approximately 7 µm) serve as a suitable resource for figuring out the shape of *Babesia spp.* inside the affected canine. The purpose of the study was to evaluate the incidence of canine babesiosis in and around Indore.

Materials and Methods
Location of work: The study was carried out at department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry Mhow, Indore (Madhya Pradesh).

Source of animals: The dogs were presented at veterinary clinical complex (VCC) Veterinary College Mhow, and in and around Indore (M.P.).

Duration of work: The study was conducted from the month of March 2021 to February 2022.

Research methodology and experimental design
Selection of animals: Dogs having history of high rise of body temperature and not

responding to antibiotics treatment were selected for study.

Method of blood collection: Blood samples were collected aseptically from cephalic vein or from tip of ear as well as were obtained using needles (22G) in the blood collecting vial containing EDTA @ 2mg/ml of blood.

Methods of blood smear examination
a. Thin smears: Thin film smears of the fresh blood sample were made by placing a drop of well mixed blood on a clean grease free slide at about 2cm from the right end. The drop of blood is touched with three edge of another slide and held at an angle of 30 degree and pushed in a quick but gently move such that a head, body and were made.

b. Staining with Giemsa stain
1. The dried thin blood film was fixed with pure methanol for 1 minute and allowed to dry.
2. The Giemsa stain used is diluted 1:10 in buffered distilled water to a pH of 7.2.
3. The stain was poured over the film on the slide and left to stand for 50 minutes.
4. The stained slide was then flushed in a gentle flow of distilled water.
5. After which it was placed in an upright position to drain and dry.
6. The stained film was examined under oil-immersion lens.

c. Examination of stained smears: The blood smears were examined under 100x oil emersion fields (OIFs), Results were interpreted as babesiosis when piroplasm Babesia or developing stages will be found in at least one cell of erythrocytes. The blood smears were examined for *Babesia*.

Incidence
In the present study, age, breed, season and gender wise incidence of canine babesiosis were calculated by using following formula.

$$Incidence\ (\%) = \frac{Total\ number\ of\ dogs\ positive\ for\ babesiosis\ during\ one\ year}{Total\ number\ of\ dogs\ surveyed}$$

Results and Discussion
The present investigation was undertaken to study clinical occurrence of canine babesiosis in around Indore Madhya Pradesh in India associated with epidemiological implications. A total of 4304 dogs brought at the Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, Mhow (M.P.) and in privet veterinary clinics across Indore were observed for high rise temperature, poor general condition, weakness, pale mucus membrane and anorexia. A total of 534 dogs of different age, breeds, seasons and gender were selected for microscopic blood smear examination Giemsa stain. A total of 54 dogs blood samples were positive for babesiosis showing the presences of pear shaped merozoites (in pairs) (Fig. 1) and (in tetared form) (Fig. 2) in erythrocytes microscopically after Giemsa stain. The overall incidence of canine babesiosis diseases was 1.25 percent (54/4304).

Incidence of Canine Babesiosis
A total of 4304 dogs were treated under the medicine section at VCC, Veterinary College, Mhow for various diseases and disorders during March 01, 2021 to February 28, 2022.

1. Month wise Incidence
In the present study, the month wise Incidence (%) of canine babesiosis from March 2021 to February 2022 was 5 (1.16%), 2 (1.35%), 4 (1.75%), 5 (1.30%), 6 (1.26%), 8 (2.06%), 7 (2.01%), 6 (1.55%), 4 (1.13%), 3 (0.81%), 2 (0.51%) and 2 (0.51%) respectively. The overall Incidence (%) of canine babesiosis was 54 (1.25%) (Table 1).
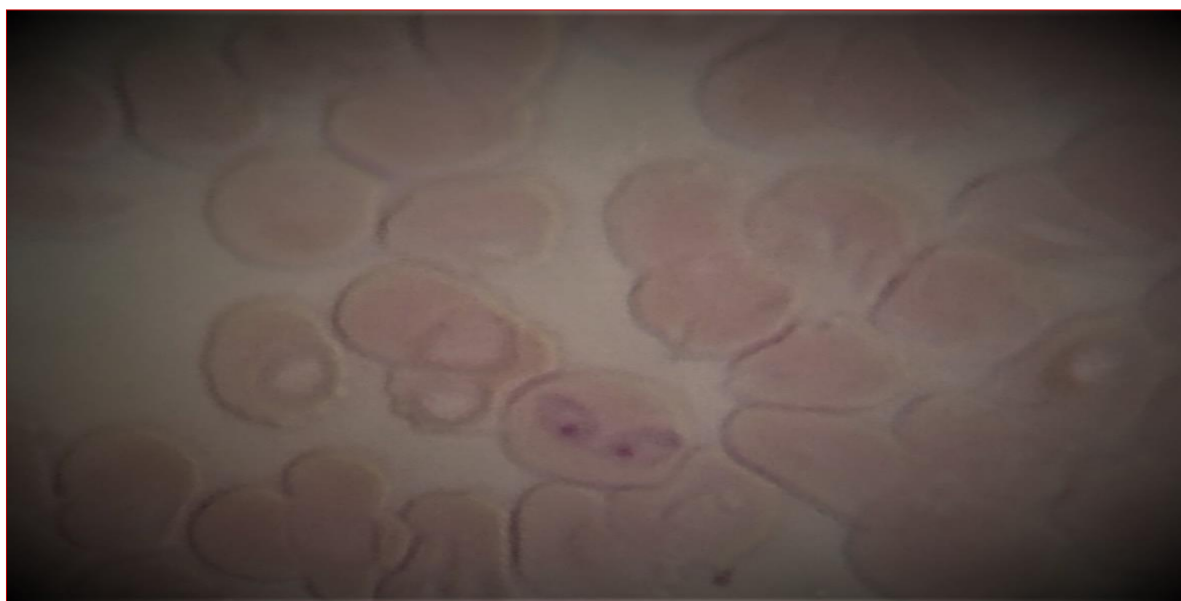

Fig 1: Blood smear (Giemsa stained) from babesiosis infected dog reveled presences of pear shaped merozoites (in pairs) in erythrocytes.

Fig 2: Blood smear (Giemsa stained) from babesiosis infected dog reveled presences of pear shaped merozoites (in tetared form) in erythrocytes.

Table 1: Month wise Incidence of Babesiosis in dogs

S. No.	Months	No. of dogs observed	No. of dogs positive for Babesiosis (%Incidence)	Incidence % Over total cases (n=4304)
1.	March -2021	430	5 (1.16%)	0.116%
2.	April -2021	148	2 (1.35%)	0.046%
3.	May -2021	229	4 (1.75%)	0.093%
4.	June -2021	385	5 (1.30%)	0.116%
5.	July -2021	476	6 (1.26%)	0.139%
6.	August -2021	389	8 (2.06%)	0.185%
7.	September -2021	348	7 (2.01%)	0.162%
8.	October -2021	386	6 (1.55%)	0.139%
9.	November -2021	355	4 (1.13%)	0.092%
10.	December -2021	372	3 (0.81%)	0.069%
11.	January -2022	391	2 (0.51%)	0.046%
12.	February -2022	395	2 (0.51%)	0.046%
	Overall Incidence	4304	54 (1.25%)	1.254%

The overall incidence of canine babesiosis was recorded more during month of August 8 (2.06) and September 7(2.01).

The highest incidence was recorded in the month of August (2.06%) and in September (2.01%) and lowest incidence (0.51%) in January and February (Table 4) similar finding was observed by Obeta et al. (2020) [14], Mahima et al. (2020) [12], Konto et al. (2014) [8] and Gray et al. (2013) [5] reported high tick infestations in dogs during the months of wet season than the dry season months. Arong et al. (2011) [1] the reason could be due to availability of vegetation cover which provides favourable environment for ticks during rainy season, as ticks are noted to drop off the host to moult and quest on blades and attach on hosts that come in contact with them during movement. Konto et al. (2014) [8] additionally suggested that most suitable temperature and high humidity can also inspire excessive fecundity in ticks.

2. Seasons wise Incidence
The incidence of canine babesiosis infections during summer season (March to June) was 1.34% (16/1192), during rainy season (July- October) 1.68% (27/1599) and during winter season (November- February) 0.73% (11/1513) (Table 2).

Among all three seasons, the incidence (%) of canine babesia was higher during the rainy season 27(0.63%), followed by summer 16(0.37%) and least in winter 11(0.25%) season. Besides this, the overall incidence of positive cases among suspected cases was similarly found high during rainy season 27(0.63%) in comparison with other seasons.

The more number of canine babesia positive cases were observed during rainy seasons could be a correlation with the seasonal activity of *Rhipicephalus sanguineus* (brown dog ticks) which is most abundant in wet and humid period of the year, thus resulting to higher transmission rate and thus higher prevalence of *B. canis* infections in the season (Soulsby 1982) [23]. Senthil and Chakravarthi (2021) [20], Obeta et al. (2020) [14] and Sharma et al. (2011) [21] have reported similar findings in rainy season at Abuja, Chennai and Mathura, respectively. Most of the animals suffering during rainy seasons, which might be due to more number of ticks in rainy seasons that had developed during summer. Dissimilar to the result also reported by Sahu et al. (2014) [21], Das et al. (2015) [3], and Badawi and Yousif (2020) [2] who recorded that disease is spread by tick, incidence of which high during sumer.

Table 2: Seasons wise Incidence of Babesiosis in dogs

S. No.	Seasons	No. of dogs observed	No. of dogs positive for Babesiosis (%Incidence)	Incidence % Over total cases (n=4304)
1.	Summer (March – June)	1192	16 (1.34%)	0.37%
2.	Rainy (July- October)	1599	27 (1.68%)	0.63%
3.	Winter (November- February)	1513	11 (0.73%)	0.25%
	Overall Incidence	4304	54 (1.25%)	1.25%

3 Age wise Incidence

The out of 4304 suspected cases, 54 were positive for canine babesiosis by blood smear examination. The highest incidence was noted in the above 18 month (1.77%) followed by 7-18 months (1.37%) and 0-6 months (0.49%) age groups. However, the overall prevalence of cases found positive among the suspected was observed higher during >18 months age group (1.77%).

Age wise highest (%) of incidence was observed above 18 months 34/1920 (1.77%), 7-18 months 13/949 (1.37%) and 0-6 months of pups respectively.

Age wise incidence of canine babesiosis was calculated in 54 cases. The higher incidence as 0.79% (34/4304) was recorded in dogs above 18 months followed by 0.30% (13/4304) in dogs between 7 to 18 months and minimum as 0.16% (7/4304) in 0-6 months of pupsc (Table 3).

Table 3: Age wise Incidence of babesiosis in dogs

S. No.	Age	No. of dogs observed (n=4304)	No. Of dogs positive for babesiosis (%Incidence)	Incidence % Over total cases (n=4304)
1.	0 - 6 Months	1435	7(0.49%)	7(0.16%)
2.	7 - 18 Months	949	13(1.37%)	13(0.30%)
3.	Above 18 Months	1920	34(1.77%)	34(0.79%)
4.	Total	4304	54(1.254%)	54(1.254%)

Age wise incidence of canine babesiosis was calculated in 54 cases. The higher incidence as 0.79% (34/4304) was recorded in dogs above 18 months followed by 0.30% (13/4304) in dogs between 7 to 18 months and minimum as 0.16% (7/4304) in 0-6 months of pupsc (Table 3).

The age wise high incidence of canine babesiosis was observed above 18 months dogs reported by Mahalingaiah *et al.* (2017) [10], Leica *et al.* (2019) [9], Obeta *et al.* (2020) [14] and Mahima *et al.* (2020) [12] and least was recorded in 0 - 6 months age group which was similar to present findings. The higher rate of *Babesia* infection in the age ranges of 1–3 years, may probably be a reflection of lowered maternal immunity/resistance associated with older dogs as well as frequent exposures to tick bites Egege *et al.* (2008) [4]. Hornok *et al.* (2006) [6] observed that the canine babesiosis increase with age.

Arong *et al.* (2011) [1], Vishwakarma and Nandini (2019) [25] and Badawi and Yousif (2020) [2] also observed higher prevalence of babesiosis in young dogs. Ogo *et al.* (2011) [15] declare that younger dogs were more susceptible to *Babesia canis* infection than the adult dogs, due to their underdeveloped immune system.

4. Gender wise Incidence

A total of 4304 suspected cases of canine babesiosis comprising of 2815 (65.40%) males and 1489 (34.60%) females. The incidence of canine babesiosis infection was recorded 1.39% in males and 1.01% in females. Among the suspected cases, 39 (0.91%) males 15 (0.35%) females were confirmed positive for presence of babesiosis infection (Table 4).

Table 4: Gender wise Incidence of babesiosis in dogs

S. No.	Gender	No. of dogs Observed (n=4304)	No. of dogs positive for babesiosis (%Incidence)	Incidence % Over total cases (n=4304)
1.	Male	2815(65.40%)	39(1.39%)	39(0.91%)
2.	Female	1489(34.60%)	15(1.01%)	15(0.35%)

The present study revealed that males were more susceptible to canine babesiosis infection than that of females. The present finding was corroborated with Sahu *et al.* (2014) [21], Leica *et al.* (2019) [9], Mahima *et al.* (2020) [12] and Obeta *et al.* (2020) [14], the higher incidence can be attributed to the hormonal factors or frequent roaming behaviour of males in search of mates and territory as reported by Tsegay *et al.* (2016) [24]. Dissimilarly to the current study Shrivastava and Shukla (2013) [23], Opara *et al.* (2017) [16] and Obeta *et al.* (2020) [14] reported the higher incidence of babesiosis female dogs and reasoned that female dogs are commonly greater sedentary in particular throughout nursing in their offspring, which predisposes them to increase infestation with tick vector.

5. Breed wise Incidence

During the present study various breeds of dog were presented with similar clinical signs of canine babesiosis.

Breed wise Incidence of babesiosis in dogs (n=4304)

Breed wise highest incidence (%) in babesia infected dogs under (n=4304) was German Shepherd 22(0.51), Labrador 10(0.23), Non descript 4 (0.09), Pomeranian 3 (0.07), Saint Bernard 3 (0.07), Doberman 2 (0.05), Golden Retriever 2 (0.05), Beagle 2 (0.05), Pug 1 (0.02), Rottweiler 1 (0.02), Great dane 1 (0.02), Schitzu 1 (0.02), Siberian Husky 1 (0.02), and Bull dog 1(0.02) consequently (Table 5).

Table 5: Breed wise Incidence of babesiosis in dogs

S. No.	Breed	No. of dogs	No. of dogs positive for Babesiosis (%Incidence)	Total No. positive cases (n=54) (%Incidence)	Over total cases (n=4304) (%Incidence)
1.	German Shepherd	1125	22 (1.96%)	22(40.74%)	0.51%
2.	Labrador	1012	10 (0.99%)	10(18.52%)	0.23%
3.	Non descript	775	4 (0.52%)	4(7.41%)	0.09%
4.	Pomeranian	450	3 (0.67%)	3(5.56%)	0.07%
5.	Saint Bernard	124	3 (2.42%)	3(5.56%)	0.07%
6.	Doberman	98	2 (2.04%)	2(3.70%)	0.05%
7.	Pug	97	1 (1.03%)	1(1.85%)	0.02%
8.	Golden Retriever	84	2 (2.38%)	2(3.70%)	0.05%
9.	Beagle	52	2 (3.70%)	2(3.70%)	0.05%
10.	Rottweiler	46	1 (2.17%)	1(1.85%)	0.02%
11.	Great dane	45	1 (2.22%)	1(1.85%)	0.02%
12.	Schitzu	35	1 (2.86%)	1(1.85%)	0.02%
13.	Siberian Husky	26	1 (3.85%)	1(1.85%)	0.02%
14.	Bull dog	15	1 (6.67%)	1(1.85%)	0.02%
15.	Other exotic Breed	321	0 (0%)	0 (0%)	0%

Similar judgment were mention by Shrivastava and Shukla (2013) [23], Mahima *et al.* (2020) [12] and Obeta *et al.* (2020) [14], recorded a high incidence of canine babesiosis in hairy breed dogs, which may be due to more tick infestation and difficulties during tick control in hairy breed because of their long hair coat. Prevalence of antibodies to *B. canis* was significantly higher among German Shepherds suggestion of a genetic predisposition of German shepherd dogs to chronic babesiosis (carrier status) with long-term maintenance of their seropositivity (Sandor *et al.*, 2006) [18].

Conclusion
In the present investigation, the overall incidence of canine babesiosis was 1.25% (54/4304). The incidence (%) of canine babesia was higher during the rainy season 27(0.63%) and lower in winter 11(0.25%) season. The highest incidence was noted in the above 18 month (1.77%) while lowest incidence 0-6 months (0.49%) of pups. The present study revealed that males were more susceptible to canine babesiosis infection than that of females. The highest incidence (0.51) was seen in German Shepherd breed of dogs while the lowest (0.02) was observed in Pug, Rottweiler, Great dane, Schitzu, Siberian Husky, and Bull dog.

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