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Prevalence of Tick-borne Feline Haemoparasitic Diseases in and around Kolkata, West Bengal, India

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Abstract

Haemoparasitic diseases are important vector-borne infections affecting domestic cats and are associated with anaemia, pyrexia, lethargy, weight loss, and other systemic disorders that adversely influence feline health. Early diagnosis of these infections is essential for effective clinical management and control. A total of 42 domestic cats of different age groups and both sexes were included in the study. Among the 42 cats examined, 9 (21.43%) were positive for haemoparasitic infection by microscopic examination. *Mycoplasma* spp. was the most frequently detected haemoparasite, with an overall prevalence of 14.28% (6/42), followed by *Cytauxzoon* spp. at 7.14% (3/42). Sex-wise analysis showed that 5 of the 27 male cats (18.52%) and 4 of the 15 female cats (26.67%) were positive for haemoparasitic infection. Although female cats exhibited a numerically higher prevalence than males, the difference was not statistically significant ($P > 0.05$). Age-wise analysis revealed that cats between 7 months and 2 years of age had the highest prevalence (25.00%), followed by cats older than 2 years (15.38%).

Keywords: Haemoparasitic diseases, Cats, *Cytauxzoon* spp., Haemotropic *Mycoplasma*, *Hepatozoon* spp., Blood smear microscopy, Prevalence

Introduction

Cats are among the most popular companion animals worldwide, and their population has increased considerably in both urban and rural areas. Along with this growing population, the occurrence of infectious diseases affecting feline health has also increased. Among these, haemoparasitic diseases are of particular importance because they infect blood cells, leading to a wide range of clinical manifestations including anaemia, pyrexia, lethargy, anorexia, weight loss, jaundice, and, in severe cases, death (McCosker, 1975) [6]. These diseases not only compromise the health and welfare of domestic cats but also pose significant diagnostic and therapeutic challenges for veterinarians (Böse *et al.*, 1995) [1]. Feline haemoparasitic infections are caused by a diverse group of blood-borne pathogens, including protozoa and haemotropic bacteria. Among these, *Cytauxzoon* spp., *Hepatozoon* spp., and haemotropic *Mycoplasma* spp. are considered clinically important pathogens in many parts of the world. Their distribution varies according to geographical location, climate, vector abundance, and management practices. These organisms are primarily transmitted through arthropod vectors such as ticks and fleas, although other routes including blood transfusion, bite wounds, and vertical transmission have also been reported for some haemoplasma species. *Cytauxzoon* spp. are intraerythrocytic protozoan parasites belonging to the phylum Apicomplexa. *Cytauxzoonosis* is an emerging tick-borne disease of domestic and wild felids (Carvalho *et al.*, 2024) [2]. The disease is characterized by high fever, depression, anorexia, icterus, anaemia, and multiple organ dysfunction. While *Cytauxzoon felis* has been extensively reported from North America, *Cytauxzoon*-like organisms have also been documented in Europe, Asia, and India, indicating a wider geographical distribution than previously recognized (Saleh *et al.*, 2019) [8].

Hepatozoon spp. Are apicomplexan parasites that infect leukocytes and various tissues of felids. Unlike most tick-borne pathogens, transmission generally occurs following ingestion of an infected tick rather than through tick bites. Infected cats may remain asymptomatic or

develop fever, lethargy, anaemia, weight loss, and muscular abnormalities depending on the parasite burden and concurrent illnesses. Although feline hepatozoonosis has been reported less frequently than canine infections, increasing molecular evidence suggests that the disease may be underdiagnosed.

Haemotropic *Mycoplasma* spp., formerly classified as *Haemobartonella*, are epicellular bacteria that attach to the surface of erythrocytes. The major species infecting cats include *Mycoplasma haemofelis*, *Candidatus Mycoplasma haemominutum*, and *Candidatus Mycoplasma turicensis*. These organisms may cause haemolytic anaemia of varying severity, particularly in immunocompromised animals or those concurrently infected with retroviruses such as feline immunodeficiency virus (FIV) and feline leukaemia virus (FeLV). Subclinical infections are also common, making diagnosis challenging.

The clinical manifestations of feline haemoparasitic diseases are often nonspecific and overlap with several other infectious and non-infectious disorders. Common clinical signs include fever, pale mucous membranes, anorexia, lethargy, dehydration, splenomegaly, lymphadenopathy, haemoglobinuria, and weight loss. Haematological abnormalities such as regenerative or non-regenerative anaemia, thrombocytopenia, leucocytosis, and alterations in biochemical parameters including liver enzymes and serum proteins are frequently observed. Therefore, laboratory diagnosis plays a crucial role in confirming infection and guiding appropriate treatment (Carvalho *et al.*, 2024) [2]. Microscopic examination of Giemsa- or May-Grünwald-Giemsa-stained peripheral blood smears remains one of the most commonly employed diagnostic methods in veterinary practice because it is rapid, inexpensive, and readily available. It is particularly useful during acute infections when parasitemia is high. However, microscopy has relatively low sensitivity in chronic or carrier infections due to intermittent or low parasite loads and may fail to differentiate morphologically similar organisms. The epidemiology of feline haemoparasitic diseases is influenced by several factors including age, sex, breed, housing system, outdoor access, vector exposure, seasonal variations, and geographical location. Cats allowed to roam outdoors are more likely to encounter ticks, fleas, and infected animals, thereby increasing their risk of acquiring haemoparasitic infections. Climatic conditions favouring vector survival further contribute to disease transmission, particularly in tropical and subtropical regions (Horn *et al.*, 2011) [4].

India possesses climatic conditions favourable for the maintenance of arthropod vectors throughout much of the year. Despite this, information regarding the prevalence and distribution of feline haemoparasitic diseases remains limited, especially in eastern India. West Bengal, characterized by a warm and humid climate, supports abundant populations of ticks and fleas that may facilitate transmission of these pathogens. Moreover, the increasing population of pet and free-roaming cats in and around Kolkata necessitates systematic investigation of haemoparasitic infections in this region. Although sporadic reports of feline haemoparasites have been published from different parts of India, comprehensive studies integrating microscopic diagnosis, molecular detection, and evaluation of haematological and biochemical alterations are scarce. Such information is essential for understanding disease epidemiology, improving diagnostic accuracy, and developing effective preventive and therapeutic strategies (Hanmer *et al.*, 2017) [3].

Keeping these facts in view, the present investigation was

undertaken to determine the prevalence of haemoparasitic diseases caused by *Cytauxzoon* spp., *Hepatozoon* spp., and *haemotropic Mycoplasma* spp. in cats from selected regions in and around Kolkata, West Bengal. The study also aimed to evaluate the prevalence of tick borne prevalence in

Materials and Methods

Study Area

The present investigation was conducted to determine the prevalence of feline haemoparasitic diseases in and around Kolkata, West Bengal, India. Five different locations, namely Mohanpur (Nadia), Belgachia (Kolkata), Sodhpur (North 24 Parganas), Sonarpur (South 24 Parganas), and Salkia (Howrah), were selected for the study. These regions were chosen because of their comparatively large pet cat population and easy accessibility for clinical investigation. The study was carried out over a period of six months from July 2024 to December 2024. Most of the cats included in the study had outdoor access during the daytime and were housed indoors or in shelters during the night.

Selection of Animals

A total of 42 cats of different age groups and both sexes were included in the study. Animals were selected randomly from the study areas based on the presence of clinical signs suggestive of haemoparasitic infection. The commonly observed clinical signs included anorexia, pyrexia, lethargy, vomiting, pale mucous membranes, anaemia, haematuria, weakness, tick infestation (where present). Each animal underwent a detailed clinical examination. Rectal temperature, pulse rate, respiratory status, and colour of the visible mucous membranes were recorded. Particular attention was given to the presence of ectoparasites, especially ticks. Information regarding age, sex, geographical location, management practices, housing system, and clinical history was recorded for each animal before sample collection.

Categorization of Animals

The examined cats were categorized according to age as follows

- Baby cats: Less than 7 months of age
- Junior cats: 7 months to 2 years of age
- Adult cats: More than 2 years of age

Based on microscopic examination of blood smears, animals were also categorized into iInfected and Non-infected animals.

Collection of Blood Samples

Blood samples were collected from all 42 selected cats for microscopic examination and molecular diagnosis. Whenever possible, peripheral blood smears were prepared immediately using blood obtained from the scapha of the ear pinna. When immediate smear preparation was not feasible, approximately 1-2 mL of whole blood was collected aseptically by saphenous venipuncture into sterile EDTA vacutainer tubes. Blood smears were subsequently prepared in the laboratory from the collected samples.

Blood samples were collected from cats belonging to Mohanpur (Nadia), Belgachia (Kolkata), Sodhpur (North 24 Parganas), Sonarpur (South 24 Parganas), and Salkia (Howrah).

Preparation of Blood Smears

Thin blood smears were prepared according to the method described by McCosker (1975) [6]. A small drop of fresh

blood was placed near one end of a clean, grease-free glass slide. A spreader slide was held at an angle of approximately 30-45° and drawn backward to allow the blood to spread evenly along its edge. The spreader was then pushed smoothly forward to obtain a thin monolayer smear. The prepared smears were allowed to air dry completely before fixation. Air-dried blood smears were fixed in absolute methanol for 2-3 minutes using a Coplin jar. Each slide was properly labelled with animal identification, collection site, date of collection of samples.

The fixed smears were stained using Giemsa stain (Himedia Laboratories, India). The working Giemsa solution was prepared by mixing one part Giemsa stain with nine parts distilled water (1:9 dilution) immediately before use. The blood smears were flooded with the stain and allowed to stain for 60 minutes. Thereafter, excess stain was gently washed off under running tap water. Slides were air-dried completely before microscopic examination and subsequently stored in labelled slide boxes. Selected blood smears were also stained using May-Grünwald-Giemsa (MGG) stain to improve visualization of haemoparasites and associated cellular morphology.

Microscopic Examination

All stained blood smears were examined under a compound light microscope (Olympus®, India). Initially, the smears were scanned under low and medium magnifications to assess smear quality. Detailed examination for haemoparasites was carried out under 1000× magnification using the oil immersion objective. The blood smears were examined for the presence of *Cytauxzoon spp.*, *Hepatozoon spp.*, And Haemotropic *Mycoplasma spp.* Parasite identification was based on their characteristic morphology as described by Soulsby (1982) [10].

Estimation of Prevalence

The prevalence of haemoparasitic infection was calculated separately for each parasite detected by microscopy as per the protocol described by Thru field (2007) [12].

The percentage prevalence was calculated using the following formula:

$$\text{Percent Prevalence of CHM (On Microscopy \%)} = \frac{\text{No of animals positive for CHM (on microscope)}}{\text{No of total animals examined}} \times 100$$

The prevalence was further analysed according to age, sex, and study area.

Statistical Analysis

The data generated during the study were compiled in Microsoft Excel 2007 and analysed using IBM SPSS Statistics Version 21.0. Descriptive statistics were used to summarize the prevalence of haemoparasitic infections. The association between haemoparasitic infection status (infected versus non-infected) and categorical variables such as age, sex, study area, and haematological or biochemical abnormalities was analysed using the Chi-square (χ^2) test of independence. Where the assumptions of the Chi-square test were not fulfilled because of small expected frequencies, Fisher's Exact Test was applied. Differences were considered statistically significant at a 95% confidence level ($P < 0.05$).

Results

The present study aimed to determine the prevalence of haemoparasitic diseases in cats and their impact on various hematological and biochemical parameters. It also focused on diagnosing these conditions through blood smear analysis and molecular detection methods. The study investigated haemoparasite and hemoplasma infections in cats from the following five districts in and around Kolkata, West Bengal, i.e., Nadia, Kolkata, North 24 Parganas, South 24 Parganas, and Howrah. 42 (Forty-two) cats were selected based on clinical symptoms like anorexia, fever, anemia, and smears taken. Giemsa and May-Grunwald Giemsa-stained blood smears were examined under a light microscope.

Prevalence of Cats by Age, Sex, and Areas

The study involved forty-two cats exhibiting signs of illness, comprising 27 males (64.28%) and 15 females (35.72%). These cats were further categorized by age: one cat (2.38%) was less than 7 months old, 28 cats (66.66%) were between 7 months and 2 years old, and 13 cats (30.96%) were over 2 years of age (Table 1; Figure 1).

Table 1: Showing overall age and sex-wise cats examined

Study Area	Total no. of Animals Examined	Sex		Age		
		Male	Female	<7 months	7 months- 2 Years	>2 Years
Nadia	8	6	2	1	7	0
Kolkata	12	7	5	0	8	4
North 24 PGS	10	8	2	0	7	3
South 24 PGS	6	2	4	0	2	4
Howrah	6	4	2	0	4	2
Total	42	27(64.28%)	15(35.72%)	1(2.38%)	28(66.66%)	13(30.96%)

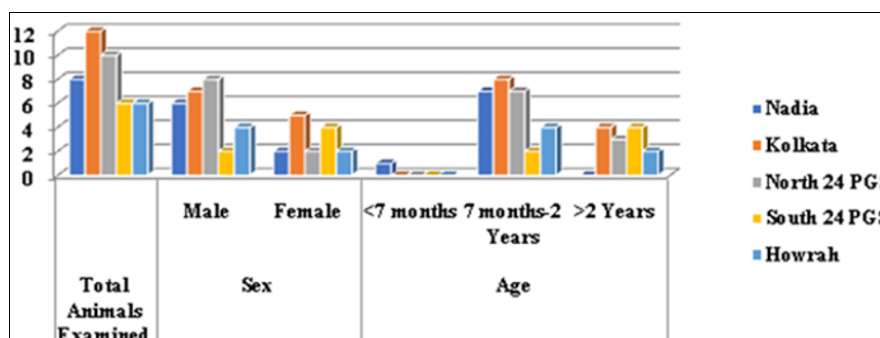


Fig 1: The Overall Age and Sex-Wise Cat Prevalence Study

Prevalence of Haemoparasitic Infection in Cats on Morphological Identification

Through blood smear-based tests, all 42 of the study's animals were checked for the presence of hemoparasites. Therefore, 42 thin blood smears were prepared from each animal, stained with Giemsa and May-Grunwald Giemsa, and examined under a microscope at 100X magnification. The organisms that could be detected in the peripheral blood smear of cats were piroplasms of *Cytauxzoon* and haemotropic mycoplasmas. Microscopical examination of blood smears is

one of the diagnostic tools for detecting parasites in acute disease conditions. It is a rapid, cheap, and feasible method for on-the-spot diagnosis (Bose *et al.*, 1995) [1]. The study results were also presented in Tables (3,4,5 and 6) and Figure (1) below according to the animals' age group, sex, and study region. Among the parasites examined, the prevalence of *Mycoplasma* species was significantly higher (14.28%) compared to *Cytauxzoon* species (7.14%) and *Hepatozoon* sp. (0%).

Table 2: Overall Prevalence of CHM in Cats in West Bengal

Total no. of Cats Examined	Total no. of Cats CHM Positive	Prevalence of CHM			
		<i>Cytauxzoon</i> sp.	<i>Hepatozoon</i> sp.	<i>Mycoplasma</i> sp.	Mixed Infection
42	9 (11.1%)	3 (7.14%)	0	6 (14.28%)	0

Sex Wise Prevalence of Haemoparasitic Diseases in Cats

As mentioned earlier, the present study comprised 27 male and 15 female cats, a total of 42 animals. Five out of those 27 males (18.51%) and four out of those 15 females (26.66%) were positive for haemoparasites on microscopy. Among the females, the prevalence of *Mycoplasma* sp. (20%) was found to be higher than *Cytauxzoon* sp. (6.66%).

Similarly, in males, the infection rate of *Mycoplasma* sp.

(11.11%) was higher compared to *Cytauxzoon* sp. (7.40%). Among the different haemoparasites, the infection rate of *Cytauxzoon* sp. was found higher in males (7.40%) as compared to females (6.66%). However, an opposite trend was recorded in the case of *Mycoplasma* sp. A greater number of females (20%) carried *Mycoplasma* sp. than males (11.11%).

Table 3: Sex Wise Prevalence of CHM in Cats in West Bengal, India

CHMs Sex	Prevalence of CHM				Total
	<i>Cytauxzoon</i> sp.	<i>Hepatozoon</i> sp.	<i>Mycoplasma</i> sp.	Mixed Infection	
Male	2 (7.40%)	0	3 (11.11%)	0	5 (18.51%)
Female	1 (6.66%)	0	3 (20%)	0	4 (26.66%)
Chi-square Calculated Value	0.225				
Chi-square Critical Value at 1d.f. at $P < 0.05$	3.841				
Significance ($P < 0.05$)	0.635256 (Not significant)				

Age-Wise Prevalence of CHM in Cats

The study revealed that the infection rate of animals 7 months - 2 Years old was higher than the other two age groups, i.e., >2 years and <7 months. 25% (7 out of 28) of the animals aged 7 months - 2 Years old were found to harbor infection, compared to 15.38% (2 out of 13) of the animals aged >2 years and one animal examined below 7 months and found negative for haemoparasitic infection.

This age-wise study further demonstrated that the infection

rate of *Mycoplasma* sp. was highest between 7 months - 2-year-old of age animals (17.85%), followed by > 2-year-old (7.7%) and <7-month-old (0%) animals. On the other hand, the prevalence of *Cytauxzoon* sp. was found slightly higher in animals aged >2 years (7.7%) than between 7 months - 2 years of age (7.14%) and <7 months (0%) animals. Mixed infection from both *Mycoplasma* sp. and *Cytauxzoon* sp. Was found negative (0%).

Table 4: Showing Age-Wise Prevalence with Morphological Identification of CHM in Cats

CHMs Age	Prevalence of CHM				Total
	<i>Cytauxzoon</i> sp.	<i>Hepatozoon</i> sp.	<i>Mycoplasma</i> sp.	Mixed Infection	
<7 months	0	0	0	0	0
7 months - 2 Years	2 (7.14%)	0	5 (17.85%)	0	7 (25%)
>2 Years	1 (7.7%)	0	1 (7.7%)	0	2 (15.38%)
Total	3	0	6	0	9
Chi-square Calculated Value	0.3214				
Chi-square Critical Value at 2 d.f. at $P < 0.05$	5.991				
Significance ($P < 0.05$)	0.57075 (Not significant)				

Area-Wise Prevalence of CHM with morphological identification in Cats

In the present study, 9 animals (21.42%) were tested positive for hemoparasites among the 42 screened. Out of which, the highest infection rate was observed in cats of Nadia (37.5%), Kolkata (25%), North 24 PGS (20%), Howrah (16.66%), and

South 24 PGS (0%) in decreasing order.

Among areas, the prevalence of *Mycoplasma* sp. was higher in Nadia (25%) compared to North 24 PGS (20%), Kolkata (16.67%), South 24 PGS (0%), and Howrah (0%).

In the case of *Cytauxzoon* sp., comparatively higher prevalence was noted in Howrah (16.67%) than Nadia

(12.5%), Kolkata (8.33%), North 24 PGS (0%) and South 24 PGS (0%). Mixed infections in the study areas were negative (0%). No prevalence of *Hepatozoon* sp. was detected on microscopic examination.

Discussion

The present study found that haemoprotozoan parasites in cats were prevalent in West Bengal during the year. The high prevalence of haemoparasitic infection in cats may be attributed to the agroclimatic conditions in West Bengal, as this is quite appropriate for adequate survival and propagation of different ticks Vectors. Several studies carried out from various regions of the country and around the world revealed the prevalent incidence of haemoprotozoan parasite diseases. Variation in the present findings concerning prevalence and infection level might be ascribed to the varying climatic and geographical situations and the animal management and diagnostic methods practised by the study population. Additionally, there were no instances of mixed infections in the population. Comparatively similar result found in Senthil *et al.* (2014) [9], where blood smear examination detected only 9 positive cases of haemotropic *Mycoplasmas*, and 14 positive cases of haemotropic *Mycoplasmas* were detected by molecular diagnosis. However, microscopic examination of blood smears revealed piroplasms of *Cytauxzoon* species. Previously, signet-ring and safety pin-shaped piroplasms resembling *C. felis* were identified in a cat from Gujarat (Varshney *et al.*, 2009) [13].

As such, it is unclear why male cats had a higher prevalence of disease compared to female cats in our study and others. A larger sample size as well as more complete patient background information would have been helpful in more thoroughly evaluating acute cytauxzoonosis risk factors; however, we were limited by the information provided in the case records. Overall, our findings regarding acute cytauxzoonosis seasonality and feline age/sex predilections in Kansas provide additional support for age and sex being important risk factors. It has also been reported previously (Carvalho *et al.*, 2024; Horn *et al.*, 2011; Hanmer. *et al.*, 2017) [2, 4, 3]. Saleh *et al.* (2019) [8] reported that young cats in their first and second year of life were more likely to have samples submitted and diagnosed with acute cytauxzoonosis. Our finding that young cats had a predisposition for acute cytauxzoonosis diagnosis supports previous data regarding age distribution for cats diagnosed with acute cytauxzoonosis. There is no significant difference between the age groups. However, the current study found no significant predisposition related to gender or age groups. The findings of this study align with the data reported by Inokuma *et al.* (2004), who also found no significant association between haemoplasma infection and factors such as age, gender, or the overall health condition of the cats. In contrast, Tasker *et al.* (2003) [11] reported differing results, indicating that older male cats were significantly more likely to be infected with haemotropic mycoplasmas than juvenile cats.

Table 5: Showing Area Wise Prevalence of CHM in Cats

Area	Total no. of Cats Examined	Total no. of Cats CHM Positive	Prevalence of CHM				Chi-square Calculated Value	Chi-square Critical Value at 3 d.f. at $P<0.05$	Significance ($P<0.05$)
			<i>Cytauxzoon</i> sp.	<i>Hepatozoon</i> sp.	<i>Mycoplasma</i> sp.	Mixed Infection			
Nadia	8	3 (37.5%)	1 (12.5%)	0 (0%)	2 (25%)	0 (0%)	1.13	7.815	0.2878
Kolkata	12	3 (25%)	1 (8.33%)	0 (0%)	2 (16.67%)	0 (0%)			
North 24 PGS	10	2 (20%)	0 (0%)	0 (0%)	2 (20%)	0 (0%)			
South 24 PGS	6	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)			
Howrah	6	1 (16.6%)	1 (16.67%)	0 (0%)	0 (0%)	0 (0%)			
Total	42	9 (19.15%)	3 (7.14%)	0 (0%)	6 (14.28%)	0 (0%)			

Conclusion

The age factor that age of cat is important in the occurrence of such infections, and animals are found most susceptible to such diseases in an age-specific manner, which can be used to plan appropriate measures for control of such infections. The study offers baseline data that could potentially be utilized to control tick transmission. More in-depth research, including utilizing modern technology, is warranted for the exact carrier status identification and programmes for the effective management of the tick populations to control the spread of haemoprotozoan parasites in cats within the affected region.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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