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Occurrence of haemoprotozoa in dairy cattle across different animal husbandry setups in Haryana, India

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ABSTRACT

The present study was conducted over a period of 1.5 years from March 2022 to August 2023 in different animal husbandry setups [gaushala (cattle shelter), organized and unorganized farm] of cattle in Haryana, covering two different agro - climatic zones, i.e., North Eastern (NE) and South Western (SW). A total of 200 apparently healthy lactating animals (2nd to 6th lactation) were randomly included in this study. From the study, it was revealed that out of 200 samples collected from different zones and animal husbandry setups in Haryana, 100 samples (50%) tested positive for the presence of haemoprotozoa. The overall occurrence of different haemoprotozoa detected in this study was 34% for *Theileria* spp. and 11% for *Anaplasma marginale*. Chi-square analysis revealed a significantly higher positivity rate of *Theileria* spp. and *A. marginale* in the NE zone as compared to the SW zone. Wald Chi-square analysis revealed a higher positivity rate of *Anaplasma marginale* in organized farms and a higher positivity rate of *Theileria* spp. in gaushala. As a measure of animal welfare, this is the first study reporting positivity of haemoprotozoan diseases and may be useful in understanding disease prevalence, pathogenesis, treatment and prognosis.

Keywords: Dairy cattle, Haemoprotozoa, Haryana, Positivity rate

India has approximately 193.46 million cattle and 109.85 million buffaloes, which account for a significant portion of the world's livestock population. The state of Haryana is home to the best buffalo population, with 4.4 million buffaloes and 1.93 million cattle (20th Livestock Census 2019). Cattle are susceptible to three primary tick-borne haemoparasitic diseases, i.e., theileriosis, babesiosis and anaplasmosis. The dairy business suffers large financial losses due to these haemoprotozoa, which are mostly found in tropical and subtropical locations, particularly in developing countries. An estimated US$ 13.9 to 18.7 billion is lost globally each year as a result of tick-borne haemoparasitic diseases, whereas India incurs US$ 787.63 million cumulative loss due to ticks and tick-borne diseases (Minjauw and McLeod 2003, Singh *et al*. 2022). In addition to transmitting diseases, these tick-borne haemoparasitic diseases also inflict considerable harm to livestock production and health, making them a major concern in the area of animal welfare.

Anaplasma marginale, an obligatory intraerythrocytic rickettsial microorganism, is the causative agent of anaplasmosis, and *Rhipicephalus (Boophilus) microplus* is the main agent of transmission (Kumar and Sangwan 2010, Aubry and Geale 2011, Ganguly *et al*. 2017). The disease is mainly characterized by fever, hemolytic anemia, jaundice, decreased milk production, hyper excitability, abortion and in rare situations, rapid death (Richey and Palmer 1990, Sharma *et al*. 2013). As *A. marginale* lacks a typical cell wall and is unable to synthesize lipopolysaccharide and peptidoglycan, it is more closely connected to protozoa (Brayton *et al.* 2005). Cattle are susceptible to tropical theileriosis, which is caused by *Theileria annulata* and *Hyalomma spp.* of tick is the most important transmitting agent (Tuli *et al.* 2015). The symptoms of theileriosis include splenomegaly, lymphadenopathy, anemia, fever, weight loss and weakness (Maharana *et al*. 2016). Babesiosis poses a substantial threat to cattle globally, leading to notable morbidity and mortality. The protozoan parasites responsible for cattle babesiosis, namely *Babesia bigemina* and *Babesia bovis*, are transmitted by the Ixodid ticks, specifically *Rhipicephalus (Boophilus) microplus*. This tick species is prevalent in numerous tropical and subtropical regions worldwide (OIE 2005, Ganguly *et al*. 2017). Anorexia, high fever and dark brown coloured urine are the characteristic symptoms of babesiosis (Ganguly *et al*. 2018). The gold standard test for confirming the presence of haemoprotozoa involves the microscopic examination of a Giemsa-stained blood smear (Ganguly *et al*. 2018, Debbarma *et al*. 2020).

With the aim of assessing cattle health status for

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animal welfare, our study was designed to investigate the occurrence and association of haemoprotozoa with various animal husbandry setups in Haryana. This initiative stems from the observation that, to the best of our knowledge, no comparative study of this nature had been previously undertaken or reported.

MATERIALS AND METHODS

Ethical approval: The study was conducted in accordance with the ethical standards of the Institutional Animal Ethics Committee (IAEC) of Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar, Haryana, India (approval no. VCC/IAEC/2022/1624 + 51).

Study area and design: The present study was conducted over a period of 1.5 years from March, 2022 to August, 2023 in different animal husbandry setups [gaushala (cattle shelter), organized and unorganized farm] of cattle in Haryana covering two different agro - climatic zones (Fig. 1) i.e. north eastern (NE) zone (lies between 30° and 31°N and 76°50' to 77°30'E) and south western zone (SW) (lies between 27°50' and 30°N and 74°30' and 76°45'E) (Narwal *et al*. 2005). In the NE zone, different animal husbandry setups in districts Karnal, Kurukshetra, Jind, Sonipat and Gurugram were included, whereas in SW zone, the study incorporated districts Fatehabad, Hisar and Mahendragarh (Fig. 1). A total of 200 apparently healthy lactating animals in 2nd to 6th lactation were randomly included in this study from different animal husbandry setups (Table 1). The sample size was made with a meticulous consideration of the total population, estimated at approximately 100,0000 animals, applying a 10% margin of error and a 99% confidence interval for sample size calculation using the Raosoft sample size calculator (http://www.raosoft.com/samplesize.html).

Sample collection and peripheral blood smear examination: Five mL of blood was collected aseptically using EDTA (ethylenediamine tetra acetic acid) powder coated sterile vials from the jugular vein of the apparently healthy animals in different animal husbandry setups for peripheral blood smear examination.

For the presence of haemoprotozoa, a thin blood smear was prepared on clean and grease-free glass slides. A drop of blood was placed in the middle, near one end of the slide. The spreader slide was used at an angle of about 45° and allowed the blood to run its edges and pushed the spreader firmly and steadily along the slides by keeping it at an angle of about 30°. The margins were not extended to the sides of the slide. The smear was dried and labeled. The air-dried smear was fixed with methanol for two minutes. The smear was again dried and stained using diluted Giemsa stain (1:10) with distilled water/buffered water (pH 6.8). A sufficient amount of diluted stain on the slide was poured to cover the smear. The stain was allowed to act for about 45 min. The excess stain was discarded and the smear was washed with buffered water. The smear was air dried and examined under microscope using an oil-immersion lens (×1000) using a drop of immersion oil on the smear.

Statistical analyses

Association of different zones and animal husbandry setups of Haryana with positivity rate of haemoprotozoa: To investigate the relationship between different zones and animal husbandry setups in Haryana with positivity rate

Table 1. Different animal husbandry setups in Haryana State India

Northeastern (NE) zone (N= 103)	Southwestern (SW) zone (N= 97)
Gaushala (N= 43)	Gaushala (N= 34)
Organized Farm (N= 30)	Organized Farm (N= 31)
Unorganized Farm (N= 30)	Unorganized Farm (N= 32)

N = Number of animals

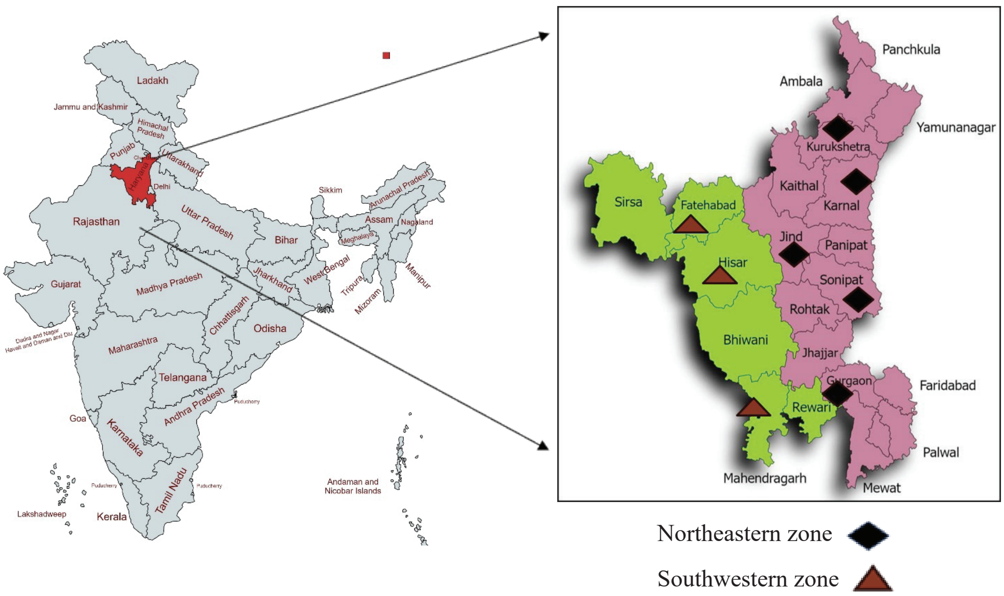

Fig. 1. Haryana state political map

Table 2. Chi- square analysis of different zones with Positivity rate of Haemoprotozoa

Anaplasma marginale	Haemoprotozoa (HP)							Total	Chi square value
	Anaplasma marginale + *Babesia bigemina*	*Anaplasma marginale* + *Theileria* spp.	*Babesia bigemina*	Negative	*Theileria* spp.	Positive for HP			
NE	21 (20.3%)	1 (0.97%)	5 (4.85%)	1 (0.97%)	35 (33.9%)	40 (38.8%)	68 (66.01%)	103	31.6**
SW	1 (1.03%)	0	3 (3.09%)	0	65 (67.01%)	28 (28.86%)	32 (32.98%)	97	
Total	22 (11%)	1 (0.5%)	8 (4%)	1 (0.5%)	100 (50%)	68 (34%)	100 (50%)	200	

**$p<0.01$, Highly Significance at 1%, NE: Northeastern, SW: Southwestern

of haemoprotozoa, the number of affected and unaffected animals was calculated. The relationship was calculated using Pearson's chi-square χ2 test (Snedecor and Cochran 1994) as follows:

$$\chi^2 = \sum (\text{Observed} - \text{Expected})^2 / \text{Expected}$$

Using the maximum likelihood method of the LOGISTIC procedure of the Statistical Analysis System for Windows, dichotomous Logistic regression models were used to examine the effect of different zones and animal husbandry setups of Haryana on the incidence of *A. marginale* and *Theileria* spp. (SAS Version 9.3; SAS Institute, Inc., Cary, NC, 2001).

Logistic regression approach: Logistic regressions work with odds rather than proportions. The odds are the ratio of the proportions for the two possible mutually exclusive outcomes. If p is the proportion for one outcome, then (1 – p) is the proportion for the alternate outcome:

$$\text{ODDS} = p / (1 - p)$$

Dichotomous Logistic Regression Model:

$$\ln\left[\frac{p}{1-p}\right] = \beta_0 + \sum_{j=1}^{c} \beta_j X_j$$

Here marginal one unit increase in X_j brings about an increase in Log $[p_i/1 - p_i]$ by β_1

p : Probability of affected animals

B_0 : Intercept

B_j X_J : Partial régression coefficients

RESULTS AND DISCUSSION

Association of different zones of Haryana with incidence of haemoprotozoa: Chi-square analysis revealed that different zones of Haryana had significant association ($p<0.01$) with the positivity rate of various haemoprotozoa as mentioned in Table 2. From the study, it was revealed that out of 200 samples from different zones and animal husbandry settings of Haryana, 100 samples (50%) tested positive for the presence of haemoprotozoa. (Table 2). The overall occurrence (Table 2) of different haemoprotozoa detected in this study was 34% for *Theileria* spp. (Fig. 2)*;* 11% for *A. marginale* (Fig. 3); 4% *A. marginale* and *Theileria* spp. mixed infection; 0.5% for *B. bigemina* (Fig. 4) and 0.5% for a mixed infection of *A. marginale* and *B. bigemina*. Table 2 illustrates the significantly higher ($p<0.01$) positivity rate of *Theileria* spp. (38.8%) and *A. marginale* (20.3%) in the NE zone of Haryana compared to the SW zone (28.86 % for *Theileria* spp. and 1.03 % for *A. marginale*) in the current study. The positivity rate of *A. marginale* and *Theileria* spp. mixed infection in NE and SW zone was 4.85% and 3.09%, respectively. The NE zone was the sole location where one sample tested positive for both *B. bigemina* (0.97%) and mixed infection of *A. marginale* and *B. bigemina* (0.97%).

In NE zone, positivity rate of *A. marginale* was

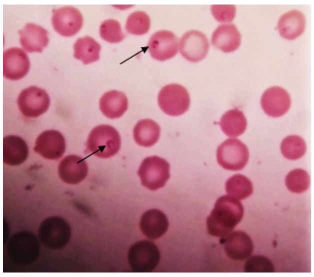

Fig. 2. Intra erythrocytic *Theileria* spp. piroplasm under oil immersion lens (1000X)

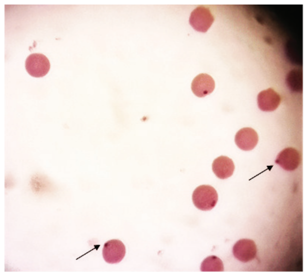

Fig. 3. *Anaplasma marginale* inclusion bodies in the red blood cell peripheries under oil immersion lens (1000X)

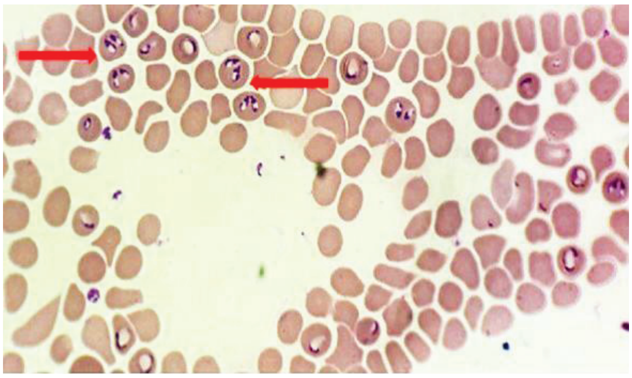

Fig. 4. Intra erythrocytic *Babesia bigemina* piroplasm under oil immersion lens (1000X)

significantly higher in organized (33.3%) and unorganized farm (30%) as compared to gaushala (4.65%) whereas positivity rate of *Theileria* spp. was significantly higher in gaushala (76.74%) as compared to other 2 setups (13.3% in organized and 10% in unorganized farm) (Table 3). The positivity rate of *A. marginale* and *Theileria* spp. mixed infection was only 6.66% in unorganized farms; 6.66% in organized farms and 2.32% in gaushalas. Only one sample was positive for *B. bigemina* (2.32%) and one for *A. marginale* and *B. bigemina* mixed infection (2.32%), i.e., in gaushala of the NE zone (Table 3).

In SW zone, the positivity rate of *Theileria* spp. was higher in organized farms (35.4%) and lower in gaushala (23.5%), whereas there was only one sample positive for *A. marginale* in organized farms (3.22%) in this zone out of all 3 setups. The positivity rate of *A. marginale* and *Theileria* spp. mixed infection was 5.88% in gaushala and 3.12% in unorganized farm. (Table 3).

Association of different animal husbandry setups of Haryana with incidence of haemoprotozoa: Chi-square analysis revealed that different animal husbandry setups in Haryana had a significant association ($p<0.01$) with the positivity rate of various haemoprotozoa. The overall positivity rate of different haemoprotozoa in Gaushala was 62.33%; 45.9% in organized farms and 38.7% in unorganized farms (Table 4). *A. marginale* had a higher positivity rate in organized farms (18.03%) followed by unorganized farms (14.5%) and gaushalas (2.59%) whereas positivity rate of *Theileria* spp. was higher in gaushalas (53.2%) followed by organized (24.59%) and unorganized farms (19.35%) as shown in Table 4.

Wald Chi square analysis revealed that positivity rate of *A. marginale* and *Theileria* spp. was significantly ($p<0.01$) associated with different setups of Haryana with higher positivity rate of *A. marginale* in organized farm as odd ratio was higher (2.898) compared to gaushalas (1.00) and unorganized farms (0.904) (Table 5). Positivity rate of *Theileria* spp. was higher in gaushalas as odd ratio was higher (1.00) as compared to organized (0.204) and unorganized farm (0.751) as shown in Table 5.

The two most common haemoprotozoan diseases that cattle contract are theileriosis and babesiosis, which

Table 3. Positivity rate of haemoprotozoa in different zones of Haryana

Anaplasma marginale		Haemoprotozoa					
		Anaplasma marginale + *Babesia bigemina*	*Anaplasma marginale* + *Theileria* spp.	*Babesia bigemina*	*Theileria* spp.	Negative	
NE	Gaushala (N = 43)	2 (4.65%)	1 (2.32%)	1 (2.32%)	1 (2.32%)	33 (76.74%)	5 (11.62%)
	Organized Farm (N = 30)	10 (33.3%)	0	2 (6.66%)	0	4 (13.3%)	14 (46.6%)
	UO (N = 30)	9 (30%)	0	2 (6.66%)	0	3 (10%)	16 (53.3%)
SW	Gaushala (N = 34)	0	0	2 (5.88%)	0	8 (23.5%)	24 (70.5%)
	Organized Farm (N = 31)	1 (3.22%)	0	0	0	11 (35.4%)	19 (61.2%)
	UO (N = 32)	0	0	1 (3.12%)	0	9 (28.1%)	22 (68.7)

N: Number of animals, UO: Unorganized farm, NE: Northeastern, SW: Southwestern

Table 4. Chi-square analysis of different setups with positivity rate of haemoprotozoa

	Haemoprotozoa (HP)							Total sample	Chi square value
	Anaplasma marginale	*Anaplasma marginale* + *Babesia bigemina*	*Anaplasma marginale* + *Theileria* spp.	*Babesia bigemina*	*Theileria* spp.	Negative	Positive for HP		
Gaushala	2 (2.59%)	1 (1.29%)	3 (3.89%)	1 (1.29%)	41 (53.2%)	29 (37.66%)	48 (62.33%)	77	
Organized Farm	11 (18.03%)	0	2 (3.27%)	0	15 (24.59%)	33 (54.09%)	28 (45.9%)	61	29.7**
Unorganized Farm	9 (14.5%)	0	3 (4.83%)	0	12 (19.35%)	38 (61.2%)	24 (38.7%)	62	
Total	22	1	8	1	68	100	100	200	

**$p<0.01$, Highly Significance at 1%

Table 5. Logistic regression of different setups with respect to positivity rate of *Anaplasma marginale* and *Theileria* spp.

Effect	Estimate ± S.E.	Wald chi square	Odds ratio	95% C.I.
		Anaplasma marginale		
Gaushala		5.43	1.00	
Organized farm	1.064 ± 0.533	3.97	2.898	1.019 – 8.243
Unorganized farm	-.101 ± 0.449	0.05	0.904	0.375 – 2.180
		Theileria spp.		
Gaushala		20.986	1.00	
Organized farm	-1.589 ± 0.395	16.177	0.204	0.094 -0.443
Unorganized farm	-0.286 ± 0.438	0.427	0.751	0.318 – 1.773

are caused by *Theileria* and *Babesia* spp., respectively and anaplasmosis, a rickettsial disease that is caused by *Anaplasma spp*. The present study showed that the overall occurrence of haemoprotozoa was 50%, with *Theileria* spp. (34%) as the most prevalent haemoprotozoa followed by *A. marginale* (11%) and other mixed infections of various haemoprotozoa. Analogous to our investigation, reports of tick-borne haemoprotozoa have also been documented from both local and international origins.

According to Ganguly *et al*. (2017), the overall prevalence of haemoprotozoa was observed to be 41.33%, with *T. annulata* accounting for the majority of these infections (33.32%) in Haryana. Swami *et al*. (2019) also reported 35.2% overall prevalence of haemoprotozoa in and around Junagadh, Gujarat. These results are consistent with our study findings. In contrast to these reports, lower overall prevalence of tick-borne haemoprotozoa was also reported by Velusamy *et al*. (2014)- 16.64% in Tamilnadu, India, and Bhatnagar *et al*. (2015)- 9% in Rajasthan cattle population. These results were likewise lower than the current findings. Variations in the research area's climate (high temperature and humidity) and geography, abundance of tick vectors, as well as animal husbandry techniques and methods used, could account for discrepancies observed between the current and prior findings by the aforementioned workers. Various previous research conducted in Northern India, such as in Haryana (Chaudhri *et al*. 2013) and Punjab (Aulakh *et al*. 2005); Northern Kerala (Nair *et al*. 2011) and Gujarat (Vohra *et al*. 2015) on crossbred cattle indicates that haemoprotozoan diseases were highly prevalent in large populations of dairy animals, which has a negative impact on milk production to a greater extent. High summer and rainy season tick vector activity may be associated with a higher frequency of haemoparasitic illnesses (Ananda *et al*. 2009, Vohra *et al*. 2015, Kumar *et al*. 2016).

The current investigation observed a higher incidence of *Theileria* spp. (38.8%) in the NE zone (particularly in gaushalas) followed by *A. marginale* (20.3%) (particularly in organized and unorganized farms that had the maximum crossbred population) and other mixed infections, whereas in SW zone, the incidence of *Theileria* spp. was 28.86% (almost similar incidence in all three different setups in this zone) which was significantly lower than the NE zone and only one sample was positive for *A. marginale* (1.03%) which was significantly very low as compared to NE zone. Reports from Ganguly *et al*. (2017) also revealed a higher prevalence of *T. annulata* in NE zone of Haryana, but contrary to present findings, the prevalence of anaplasmosis was only 1.67%. Ganguly *et al*. (2018) also noted 10.5% of samples tested positive for *A. marginale* by PCR analysis, compared to 7.5% of samples that tested positive under a microscope. Only one sample was positive for *B. bigemina* in the whole study and it was in the gaushala of NE zone. In contrast to this, a high prevalence of *B. bigemina* was observed by various authors (Bhikane *et al*. 2001, Shekhar and Haque 2007, Velusamy *et al*. 2014, Ganguly *et al*. 2017).

The study revealed that the incidence of haemoprotozoa has a significant association with different animal husbandry setups in Haryana, i.e., the incidence of *A. marginale* is more in organized farms whereas the incidence of *Theileria* spp. was more in gaushala. Overall incidence of haemoprotozoa was higher in gaushala, followed by organized and unorganized farms. Similar to our findings, Kumar and Sangwan (2010) also reported a higher prevalence of *A. marginale* in large organized dairy farms as compared to gaushala and other animal husbandry setups. The prevalence of ticks in an area is highly influenced by its macro as well as micro-climate and thus, the epidemiological pattern of ticks may be different in various agro-climatic zones, which influences the prevalence of different haemoparasitic species. Relatively higher temperature and lower rainfall favors the development of *Hyalomma anatolicum anatolicum*. Researchers from the neighboring state of Haryana and Punjab have reported similar findings earlier (Sangwan *et al*. 2000, Haque *et al*. 2011). Studies indicate that *Rhipicephalus (Boophilus) microplus* prefers a hot and humid environment whereas, arid and semi-arid conditions suit better for *H. anatolicum anatolicum*. Our research findings also fall in the same line. In gaushalas, the microclimate is a bit different compared to well-organized farms, which favor the growth and propagation of *H. anatolicum* compared to *Rhipicephalus (Boophilus) microplus*. Additionally, adaptation of an enhanced management system is detrimental to the survival of multi-host ticks. So, the chances of survival of multi-host ticks are higher in gaushala compared to organized farms. Additionally, age and breed of the host also play a key role in disease occurrence. In gaushalas, usually indigenous breeds are given shelter, whereas, in organized farms, exotic high-producing animals are kept for commercial purposes. It is well cited by several researchers that indigenous breeds are more resistant to *Anaplasma* infection compared to exotic breeds (Sarangi *et al*. 2021). This difference in incidence of *A. marginale* was also demonstrated in the current study. As stated by Chhillar *et al*. (2014), *H. anatolicum*, the hard tick species that spreads *T. annulata*, is highly prevalent throughout north India, including Haryana and is most active in the summer and after the rainy season. The prevalence and parasitaemia in the various animal husbandry management systems, as well as within the same management system at different locations, may have varied as a result of various epidemiological variables (breeds, nutritional status, population size, feeding practices, herd system, and use of acaricides) related to host, vector and environment (Kumar and Sangwan 2010).

The current investigation unveiled a widespread positivity rate of haemoprotozoa among cattle across the entire study area. A significant association of different agro climatic zones and animal husbandry setups in Haryana with the positivity rate of haemoprotozoa was also revealed in the study. The epidemiological insights gained from this study, serving as a crucial instrument in advancing animal welfare, should be harnessed to develop strategic control programs for cattle in Haryana, India.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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