



Prevalence of Blood Protozoa and Impact of Chemotherapy in Exotic Cattle, With Special Reference to Haematological Parameters

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Abstract

THIS study investigated the prevalence of haemoprotozoan infections in exotic cattle, their effects on Haematological parameters, and the comparative efficacy of different therapeutic agents. A total of 200 blood samples were collected from exotic cattle on private farms in Dera Ismail Khan, Pakistan, between April 13 and September 13, 2024. Samples were examined microscopically using Giemsa stain, and the haematological parameters [red blood cell count (RBCs), white blood cell count (WBCs), platelet counts, packed cell volume (PCV), and haemoglobin concentration (Hb) concentration] were determined with a veterinary haematology analyzer. Haemoprotozoan-positive cattle were divided into four groups: Group A (*Anaplasma* spp.), Group B (*Anaplasma* spp. + *Babesia* spp.), Group C (*Babesia* spp.), and Group D (*Theileria* spp.). Group A and B were treated with imidocarb dipropionate (2.5 mL/100 kg), Group C with diminazine aceturate (10 mL/300 kg), and Group D with buparvaquone (1 mL/20 kg). Overall prevalence of haemoprotozoan infection was 21.5%, including *Anaplasmosis* (7%), *Babesiosis* (5.5%), mixed infection of *Anaplasma* spp. + *Babesia* spp. (4.5%), and *Theileriosis* (4.5%). Therapeutic trials indicated highest efficacy against *Babesiosis* (81.8%), followed by *Theileriosis* (77.7%), mixed infection (77.7%), and *Anaplasmosis* (71.4%). Haematological analysis showed significant post-treatment improvement in RBC counts in Groups A, C, and D at day 7. Additionally, the study evaluated tick infestation rates in relation to different flooring types to identify possible predisposing factors for haemoprotozoan infections. In conclusion, haemoprotozoan infections are prevalent in exotic cattle in Dera Ismail Khan, adversely affecting Haematological values. Imidocarb dipropionate, diminazine aceturate, and buparvaquone demonstrated effective therapeutic efficacy under local field conditions and can be recommended for effective management of these infections.

Keywords: Haemoprotozoan infections, *Anaplasmosis*, *Babesiosis*, *Theileriosis*, Chemotherapy, Haematological parameters, Exotic cattle.

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Introduction

Livestock sector plays a pivotal role in the national economy of Pakistan that shares 61.9% to Gross Domestic Product (GDP) in agriculture, while in national GDP its contribution is 15% [1]. Unfortunately, livestock sector is prone to financial losses by ticks infestations and haemoparasitism [2]. The climatic conditions of tropical and subtropical areas are very conducive for multiplication of ticks, predisposing the animals to different haemoprotozoan diseases like *Theileriosis* caused by *Theileria* spp., *Babesiosis* caused by *Babesia* spp., and *Anaplasmosis* caused by *Anaplasma* spp. are three major tick-borne diseases of bovines, resulting in low production [3]. They cause significant mortality in exotic cattle which may reach up to 90%, while in non-descript breeds it ranges up to 5% [4]. Haemoprotozoan diseases (HPD) are considered as major handicap for productive performance of cattle [5]. Globally, it has been estimated that HPD causes 13.9 to 18.7 billion US dollars losses per annum [6]. Ticks serve as vector for transmission of various tick borne diseases [7]. Haemoprotozoan diseases like *Theileriosis*, *Babesiosis* and *Anaplasmosis* are three major tick borne diseases of bovines [8]. These haemoprotozoan parasites have great economic value and serve as formidable barrier to survival of exotic cattle in Pakistan. Majority of haemoprotozoan parasites cause anemia by induction of erythrophagocytosis. The major clinical manifestations of tick borne diseases are pyrexia, anemia, progressive weight loss, declined milk production and abortion in pregnant cattle [9]. Haemoprotozoan infections have been reported in bovines of different parts of Pakistan [10]. However, true status of haemoprotozoan infection has not yet been explored in exotic cattle of Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan. Therefore, keeping in view the foregoing facts, the current study was designed to determine the prevalence of haemoprotozoan infections, evaluate the comparative efficacy of different therapeutic agents, and assess their impact on haematological parameters in exotic cattle of Dera Ismail Khan, Pakistan. The study further aimed to identify tick genera associated with haemoprotozoan infections to understand their role in disease transmission. Additionally, the study assessed the relationship between tick infestation rates and different housing floor types as a potential risk factor influencing haemoprotozoan prevalence.

Materials and Methods

Ethical approval

The study design was approved by the Ethics Committee, Faculty of Veterinary and Animal Sciences (FVAS), Quaid-e-azam Campus, Gomal University, Dera Ismail Khan.

Study Design

The present study was carried out on exotic cattle of various ages that were privately owned and operated by forward-thinking dairy producers in the Dera Ismail Khan area of Khyber Pakhtunkhwa, Pakistan. For this purpose, a total no. of 200, apparently healthy cattle of various age groups, i.e., calves < 1 year (50), heifers > 1-3 years (50), adult cows > 3 years (50) and 50 animals of diverse age groups were screened for presence of haemoprotozoan infection during April to September 2024.

Collection of blood samples

After proper disinfection with alcohol swab, blood samples were collected from ear veins of 200 animals randomly for preparation of thin smears. Three to five milliliters of blood were drawn in the morning hours from the jugular veins of the afflicted animals and placed in EDTA-coated vacutainers for the haematological test.

Smear preparation

Thin blood smear was prepared from aseptically collected blood on pre-cleaned labelled glass slides. Briefly, a 2-3mm blood drop was placed at a distance of 1cm from the edge of glass slide. Then another spreader slide was held at 45 degree angle, pushed forward quickly and smoothly to prepare a thin smear [11].

Giemsa staining

Smears were air-dried and fixed in absolute methanol for 2–3 minutes before staining with Giemsa stain [12] and was examined under oil immersion lens 100X of microscope for identification of *Theileria* spp, *Anaplasma* spp and *Babesia* spp according to the morphological characteristics as described previously [13].

Haematological profile

ExigoTMH400 veterinary haematology analyzer (Boule Medical, Sweden) was used to determine haematological parameters, viz, RBCs, WBCs, PCV, Hb concentration and platelet count.

Therapeutic regimen

On the basis of clinical (including assessment of body temperature, mucous membrane color, appetite, respiration, and general body condition) and blood smear examination at day 0, animals positive for haemoparasites were divided into four groups, i.e., A; *Anaplasmosis*, B; *Anaplasmosis* + *Babesiosis* (mixed infection), C; *Babesiosis* and D; *Theileriosis*. At day 0, animals of group A and B were treated intramuscularly with imidocarb dipropionate (Imizol; Schering-Plough Animal Health) at a dose of 1.2 mg/kg body weight, group C intramuscularly with diminazine aceturate (Pronil; Selmore Pharmaceutical Pvt. Ltd) at a dose of 3.5 mg/kg body weight, and group D intramuscularly with

buparvaquone (Butalex; ICI Pakistan) at a dose of 2.5 mg/kg body weight.

Post treatment examination

After 48 h, blood from all treated animals was re-examined and if any animals were found positive for haemoparasites, a repeated dose was administered and again examined on day 7 for positive or negative samples.

Tick infestation assessment

During the study, each animal was examined visually for the presence of ticks, particularly around the neck, udder, perineum, and tail regions. The number of ticks was recorded, and infestation rates were compared among animals reared on different floor types (brick, cemented, and kutcha floors) to evaluate possible associations with haemoprotozoan infections. Ticks collected from infested cattle were preserved in 70% ethanol and identified under a stereomicroscope based on morphological characteristics such as scutum shape and festoon pattern, following standard identification keys.

Prevalence and drug efficacy

The prevalence was calculated by the formula already reported [14].

$$P = d/n \times 100$$

Where P = prevalence, d = no. of animals found positive, and n = total no. of animals sampled

The drug efficacy was measured by the formulas as described previously by Asmaa *et al.*, [15].

$$\text{Efficacy} = \frac{\text{no. of animals cured}}{\text{total no. of animals treated}} \times 100$$

Statistical Analysis

Haematological parameters were assessed through Sampled Paired T-test. Data are presented as mean and standard deviation. Values were considered significantly different where $P < 0.05$. Statistical analysis was executed using SPSS software version-21.

Results

Microscopic examination through Giemsa-stained blood smears as shown in (Figure 1) revealed that among 200 cattle, 43 were found positive for various haemoprotozoan infection, resulting 21.5% prevalence of haemoprotozoan infection in exotic cattle of District Dera Ismail Khan, Pakistan. Moreover, significantly higher prevalence of *Anaplasmosis* (7%) was observed, followed by *Babesiosis* (5.5%), *Anaplasmosis* and *Babesiosis* (4.5%) and *Theileriosis* (4.5%) (Figure. 2)

As for as therapeutic efficacy of drugs is concerned, imidocarb dipropionate was found 71.4% effective against *Anaplasmosis*. The same drug was used in 9 animals against mixed infection

(*Anaplasma* spp. + *Babesia* spp.) and 7 animals recovered on 7th day post-treatment contributing 77.7% efficacy. On the other hand, diminazine aceturate administered in 11 animals at day zero showed recovery of 9 animals at day 7 and efficacy was noticed 81.8%. While buparvaquone exhibited complete recovery of 7 animals out of 9 on day 7 post-treatment with efficacy of 77.7% (Figure 3).

The mean RBC count in group A was $5.04 \pm 0.88 \times 10^6 \mu\text{L}$ at day zero and $6.37 \pm 1.70 \times 10^6 \mu\text{L}$ at day seven post-treatment, demonstrating a significant difference, according to change to hematological analysis. The mean hemoglobin levels in group B were $5.83 \pm 0.79 \text{ g/dl}$ at day zero and $8.63 \pm 1.98 \text{ g/dl}$ following therapy, indicating a substantial change. Before treatment, the mean RBC count in group C was $5.40 \pm 0.95 \times 10^6 \mu\text{L}$, and one week later, it was $7.0 \pm 1.5 \times 10^6 \mu\text{L}$. Significant results ($p < 0.05$) were obtained with a mean hemoglobin value of $5.08 \pm 1.5 \text{ g/dl}$ prior to therapy and $9.5 \pm 2.82 \text{ g/dl}$ on day 7 after treatment. On day seven after treatment, the mean RBC count in group D was $6.12 \pm 2.0 \times 10^6 \mu\text{L}$ and $6.44 \pm 1.90 \times 10^6 \mu\text{L}$, showing a statistically significant difference ($p < 0.05$) (Figure 4).

In a study of 200 cattle, tick infestation rates differed according to flooring type. Cattle with brick floors had the greatest infection incidence (64.2%), followed by those with kutcha flooring (40.1%) and cemented floors (41.9%) as shown in (Figure 5). Of the 200 cattle, 91 were determined to be tick-infested, for an overall infestation rate of 45.5%.

Discussion

Forty-three of the 200 exotic cattle whose blood smears were analyzed in this investigation tested positive for different haemoprotozoan diseases. Overall, 21.5% of cattle were infected with hemoprotozoan parasites. This result was in line with the findings of Shit *et al.* [16], who observed an overall prevalence of 52.52% in crossbred cattle in west Bengal, India, during the summer, and the assertion that seasonality has a significant impact on the frequency of hemoparasitic illnesses. *Theileriosis* (4.5%), *Babesiosis* (5.5%), *Anaplasmosis* and *Babesiosis* (4.5%), and *Anaplasmosis* (7%) were also shown to be substantially more common (Figure 2).

Among haemoprotozoan infections, the highest prevalence of *Anaplasmosis* was found to be 7 % and 4.5 % for mixed infection, it varied a little bit 10.84 % with the result of Shaukat *et al.* [17] in cattle in Faisalabad districts of Pakistan and 2 % found by Hasan *et al.* [18] in district Lahore, Pakistan. The fact that *Anaplasma* infection can be transmitted by numerous means (biologically by ticks, mechanically by biting flies, through blood-contaminated fomites and shearing instruments) could have been the reason for our high finding. The state of livestock (host), other infectious agents, vectors, and the environment

that makes up socio-cultural and socioeconomic areas, as well as the weather and geographic conditions, all have a significant impact on the prevalence of both single and mixed infections [19].

Babesiosis prevalence in the current study was 5.5%, which was lower than the findings of Ahmad *et al.* [20], who reported a prevalence of 37.2%, and Siddique *et al.* [21], who worked with cattle in Pakistan's Punjab Province and found a prevalence of 22.75%. They found that the summer months had the highest prevalence of *Babesiosis*. This could be because there are more vectors throughout the summer than during other times of the year.

In Our study area the lowest prevalence was found for *Theileriosis* (4.5%), Atif *et al.* [14] reported 6.8%, Kebzai *et al.* [22] had reported 31.37 % while Saleem *et al.* [23] reported 10.7 % while working in different provinces of Pakistan in crossbred cattle and Khan *et al.*, [24] found 14.32% prevalence of *Theileria* parasite in cattle in Dera ismail khan, Khyber pakhtunkhwa province, Pakistan. This variation might be due to changing climatic conditions.

Geographical location affects the prevalence of haemoprotozoan illnesses, which are influenced by climate factors as temperature, humidity, and precipitation [25,32]. This study found a higher number of illnesses with *Anaplasmosis*, *Babesiosis*, and *Theileriosis*. This could be because the vector (tick) is involved in the spread of haemoprotozoan diseases.

As per the results of tick identification only at genus level the ticks have observed as shown in (Figure 6). The *Boophilus* (20%) was the mostly observed ticks followed by Mixed species of *Boophilus* and *Hyalomma* (14.5%) while the lowest was seen for *Hyalomma* (11%). Exotic crossbred cattle are comparatively naturally less resistant against ticks as compared to indigenous cattle, possibly due to a lack of their extra muscles in their musculature and innate immunity. As per the results of our finding cattle reared on bricks floor were highly susceptible (64.2) to ticks infestation as compared to muddy floor while our results are not in agreement with the finding of Lovelu *et al.* [26] who reported higher prevalence of higher prevalence of tick infestation in cattle reared on muddy floor.

In this study Imidocarb (I-IMIZOL® International Pharma PVT Ltd. Pak) was found 71.4% efficacious against *Anaplasmosis* and 77.7 % effective against mixed infection. The Drug efficacy of Diminazine aceturate (I-PRONIL® International Pharma PVT Ltd. Pak) against *Babesiosis* was 81.8% in present trial it is in consonant with the findings of Rashid *et al.* [27] who recorded 80-90 % in horses. Buparvaquone (I-BUTALEX® International Pharma PVT Ltd. Pak) was found to be 77.7% effective against *Theileriosis* in the current trial. In Sudan,

Ibrahim *et al.* [28] found that buparvaquone was 81.3%, 57.1%, and 66.7% effective in pregnant cows that were six, seven, and eight months along. However, Saleem *et al.* [23] reported that buparvaquone treatment resulted in 90% recovery of crossbred cattle affected with *theileriosis* in the Faisalabad district.

In current research to determine the effect of hemoparasites on blood components such as red blood cells, white blood cells, HB, PLTs, and haematocrit, to value haematological investigations were conducted. Overall, there was a difference between the values at day zero and day seven after treatment, but Group A's RBC count showed a significant increase ($p < 0.05$), while Group B's WBC, HB, PLT, and and haematocrit values showed no significant change ($p > 0.05$). Conversely, Hb exhibited a highly significant improvement ($p < 0.01$) following treatment, showing 5.83 ± 79 gm/dl at day zero and 8.63 ± 1.98 at the seventh day. It contradicts the results of Qayyum *et al.* [29], who found that *Theileriosis*-affected animals had lower levels of RBCs, Hb, PCV, and WBCs. While researching dairy crossbred cattle in Punjab, India, Ashuma *et al.* [30] noted similar results.

In Group B, the RBCs (5.04 ± 0.95 - 7.0 ± 1.5 10^6 μ l), Hb (5.8 ± 1.5 - 9.5 ± 2.82 10^6 / μ l), and Haematocrit value (23.2 ± 6.4 - 36.2 ± 9.3 %) all showed a highly significant difference of roughly three values. This is consistent with the findings of Niazi *et al.* [31], who found that the mean values of RBCs increased on the seventh day after treatment in a trial of diminazine aceturate in crossbred cattle with reference to Group-C. The values of RBCs, Hb, and PCV were 6.12 ± 2.0 , 6.46 ± 1.85 , and 24.12 ± 5.0 before the treatment, which is consistent with the findings of Khan *et al.* [24], who found that the RBC count, hemoglobin level, and packed cell volume were all decreased. There was a highly significant difference between the values of RBCs count (6.12 ± 2.0 - 6.44 ± 1.90 10^6 / μ l) before and after the treatment ($p < 0.05$), while the other parameters were non-significant ($p > 0.05$).

Conclusion

The geographical location of Pakistan is in the warm climate zone (WCZS) of the world, along with large scaled crossbreeding programmes has made it endemic area for haemoprotozoan diseases, haemoprotozoan diseases are more prevalent in District Dera Ismail Khan in summer months. So, the above said drugs are not only therapeutically effective against the haemoprotozoan diseases but also has a remarkable impact on haematological values. The future needs for farmers to control on haemoprotozoan diseases are to run appropriate tick eradication programme to reduce both prevalence and incidence of the diseases, controlled crossbreeding, and thoroughly use of these effective

drugs against these diseases in the initial stages to maximize its results.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this article.

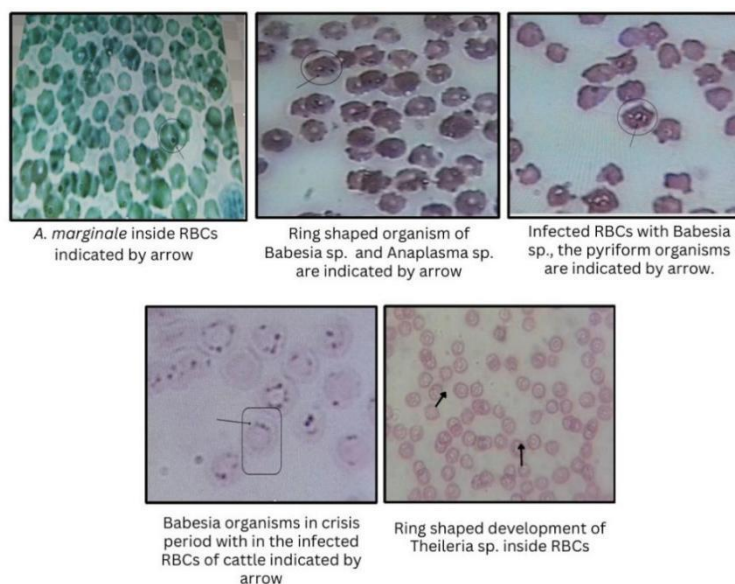


Fig. 1. Microscopic examination through Giemsa-stained blood smears

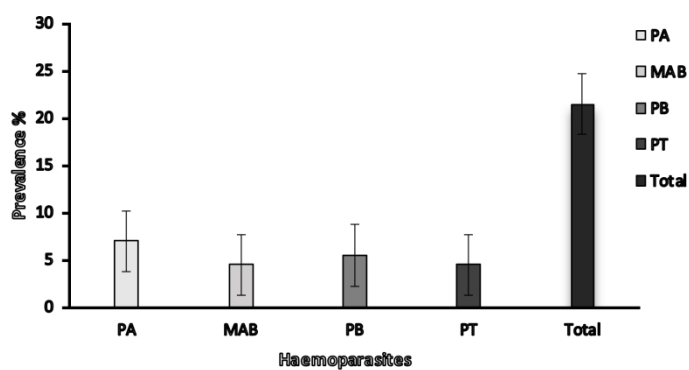


Fig. 2. Prevalence percentage of haemoparasites in cattle

:Abbreviations = PA = Anaplasma spp., MABM= ixed infection of Anaplasma spp. + Babesia spp., PB Babesia spp., PT = Theileria spp

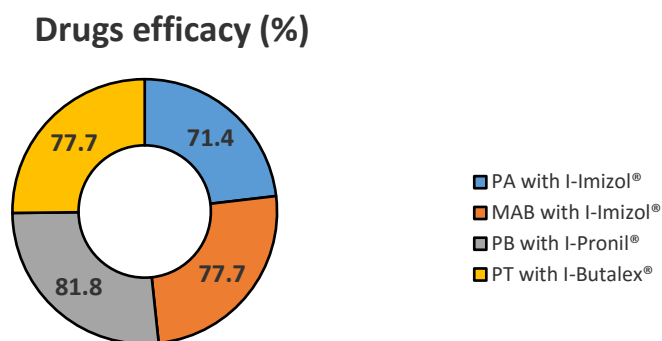


Fig. 3. Drug's efficacy percentage against haemoparasites

:Abbreviations = PA = Anaplasma spp., MABM= ixed infection of Anaplasma spp. + Babesia spp., PB Babesia spp., PT = Theileria spp

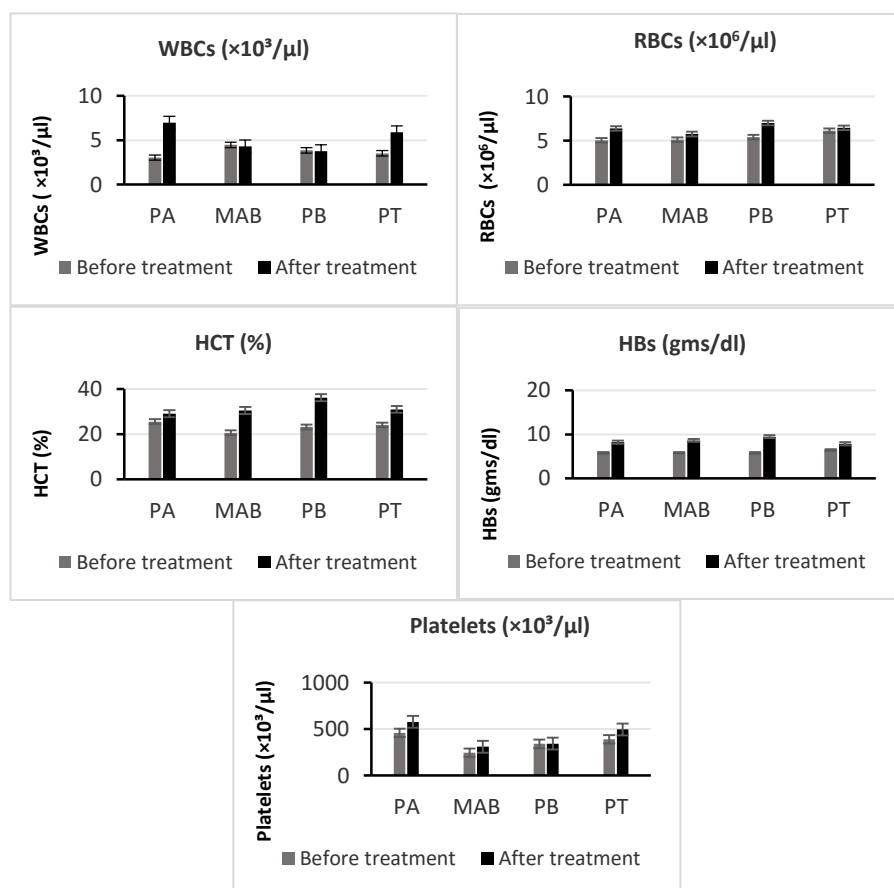


Fig. 4. Haematological values of Groups before and after their treatment

:Abbreviations = PA = Anaplasma spp., MABM= Anaplasma spp. + Babesia spp., PB ixed infection of Babesia spp., PT = Theileria spp

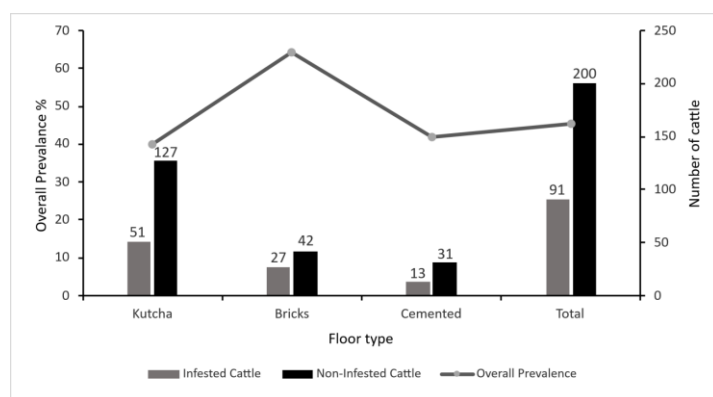


Fig. 5. Prevalence of ticks in association with floor condition of the cattle houses

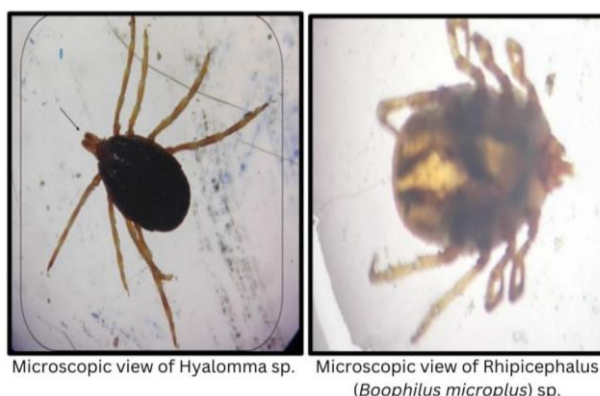


Fig. 6. Microscopic view of different types of ticks observed during study

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