Research Article:

MORPHOMETRY-BASED IDENTIFICATION OF TICK SPECIES INFECTING RABBITS AT THE AGRICULTURE AND FORESTRY UNIVERSITY LIVESTOCK FARM

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Received date: 26 January 2024, Accepted date: 28 March 2025
DOI: https://doi.org/10.3126/jafu.v6i1.79093

ABSTRACT

Rabbits (*Oryctolagus cuniculus*) are valuable as laboratory animals and farm assets due to their adaptability and rapid reproduction. They can host ticks that feed on blood and transmit various pathogens. Ticks and blood samples were collected from the AFU livestock farm from September 2023 to December 2023. The size of morphologic features was recorded, and the species was identified using taxonomic keys. An automatic hematology analyzer was used for hematological analysis of blood samples. A Giemsa-stained blood smear was observed with 1000x magnification using a binocular compound microscope for haemoparasites. About 32 tick specimens were collected from 16 rabbits, comprising 37.50% adult males, 25.00% adult females, 25.00% larvae, and 12.50% nymphs. All adults and nymphs were identified as *Haemaphysalis howletti*. The rabbits had a higher prevalence of ticks during early autumn (100%) than in late autumn (0%). There was a significant decrease in Total Erythrocyte Count (TEC), Hemoglobin (HGB), Hematocrit (HCT), and Platelet count (PLT), whereas an increase in Total Leucocyte Count (TLC) and granulocyte count occurred during the tick-infested state in rabbits ($P<0.05$). In addition, the overall prevalence of *Anaplasma* spp. was 31.25% in rabbits. Here, tick infestation showed a major role in hematological alterations and reservoirs for tick-borne pathogens. Thus, ultimately affects the health of rabbits, adding potential risk of zoonosis, and a challenge to public health.

Key words: *Anaplasma*, ectoparasites, experimental animal, *Haemaphysalis howletti*, zoonoses

INTRODUCTION

Rabbits are multipurpose mammals that have considerable economic significance and have secured a valuable asset in the developing world as versatile laboratory animals and farm settings (Fontanesi, 2021). However, some ectoparasitism, like tick infestations in the rabbits, could hamper their overall performance. Ticks are ectoparasitic arachnids that feed on the blood of most domesticated and wild animals worldwide, mainly in tropical and subtropical areas, including Nepal (Anderson & Magnarelli, 2008). They are vectors of several viral, protozoal, rickettsial, and bacterial diseases in domestic and livestock animals, including humans, carrying the greatest variety of infections of any arthropod vector (Dantas-Torres et al., 2012). Feeding by large numbers of ticks causes a reduction in growth and anemia among rabbits and domestic animals, while tick bites also reduce the quality of hides (Hurtado et al., 2018; Rechav et al., 1980). The effects of tick infestation on hematological parameters in various livestock have been documented, but limited data are available about its effects on rabbits (Kaur et al., 2017; Ragulraj et al., 2023; Rechav et al., 1980).

In research settings, rabbits have been utilized for experiments, such as maintaining tick colonies (Almazán et al., 2018). However, in natural conditions, there are limited publications about the distribution and prevalence of ticks in wild and domestic rabbits in different world locations, including Nepal. Globally, rabbits serve as important hosts for a range of tick species, for example, *Ixodes dentatus* in North America and *Hyalomma lusitanicum, Rhipicephalus pusillus, Rhipicephalus turanicus*, and *Ixodes gibbous* in Europe and they predominantly support the immature tick life stages (Napoli et al., 2021; Taylor et al., 2020). Similarly, studies in Pakistan show rabbits are infested with species such as Amblyomma variegatum, *Amblyomma americanum, Dermacentor reticulatus, Hyalomma anatolicum, Hyalomma excavatum, Ixodes ricinus, Rhipicephalus microplus*, and *Rhipicephalus sanguineus* (Baloch et al., 2023; Jamil et al., 2022). The close contact of tick-infested rabbits with humans in some areas could represent a threat to human health and sympatric domestic animals as studies show that rabbits may serve as a reservoir host for important vector-borne diseases, such as *Francisella tularensis, Rickettsia* spp., *Leishmania* spp. *Babesia* spp., *Borrelia burgdorferi*, and *Anaplasma phagocytophilum* (Napoli et al., 2021; Yabsley et al., 2006).

Thus, this study aimed at conducting a comprehensive morphometric analysis to identify tick species parasitizing rabbits at the Agriculture and Forestry University (AFU) livestock farm, investigate their seasonal dynamics and prevalence, elucidate the hematological alterations induced by tick infestations, and quantify the prevalence of haemoparasites.

MATERIALS AND METHODS

Study period and location

This study was conducted from September 2023 to December 2023 at the Agriculture and Forestry University livestock farm, Rampur, Nepal, located at 27°39'49"N-84°21'06" E.

Ticks and blood collection

Ticks and blood samples were collected and observed in two phases from 16 rabbits, with the first round of collection taking place in the last week of September. The rabbits were tagged with a colored ribbon for their identification in the second round. The ticks were picked using forceps and then preserved in 70% ethanol. Blood samples were collected from the ear vein using a sterile syringe and immediately transferred to a sterile vacutainer (EDTA Tube).

Identification of ticks and morphometry

The ticks were identified by observation of ticks under a Stereo/Dissecting microscope and compound microscope in the lab of the Department of Veterinary Microbiology and Parasitology under Agriculture and Forestry University (AFU) by running through identification keys (Geevarghese & Mishra, 2011; Soulsby, 1968). Species, sex, stage, and morphometry were recorded for each specimen. The measurements of palp, basis capituli, scutum, and spiracles of all ticks were recorded. All measurements were taken in millimeters at their longest or the widest points.

Determination of hematological parameters

The major hematological parameters were determined in Spincell 3 Automatic hematology analyzer Ref. 5006101 at Central Biotechnology Lab, AFU.

Preparation and examination of blood smear

A thin blood smear was prepared as soon as possible after the blood collection, fixed with methanol, and stained with Giemsa. The blood smear slides were observed under high-power magnification (10X ocular and 100X objective lens) with the help of immersion oil using a binocular compound microscope.

Statistical analysis

Data was entered in Microsoft Excel 2019 and analyzed using IBM SPSS Version 22. Data regarding hematology were analyzed by using a student's t-test. The P-value was considered statistically significant ($p<0.05$).

RESULTS

Tick identification and its morphometry

A total of 32 ticks were collected from 16 rabbits. Among which 37.50 % (n = 12) were adult males, followed by 25 % (n = 8) adult females, 25 % (n = 8) larvae, and 12.50 % (n = 4) nymphs. Based on the morphological features and taxonomic keys as described by Geevarghese & Mishra, (2011), the collected ticks were found to be *Haemaphysalis howlletti*.

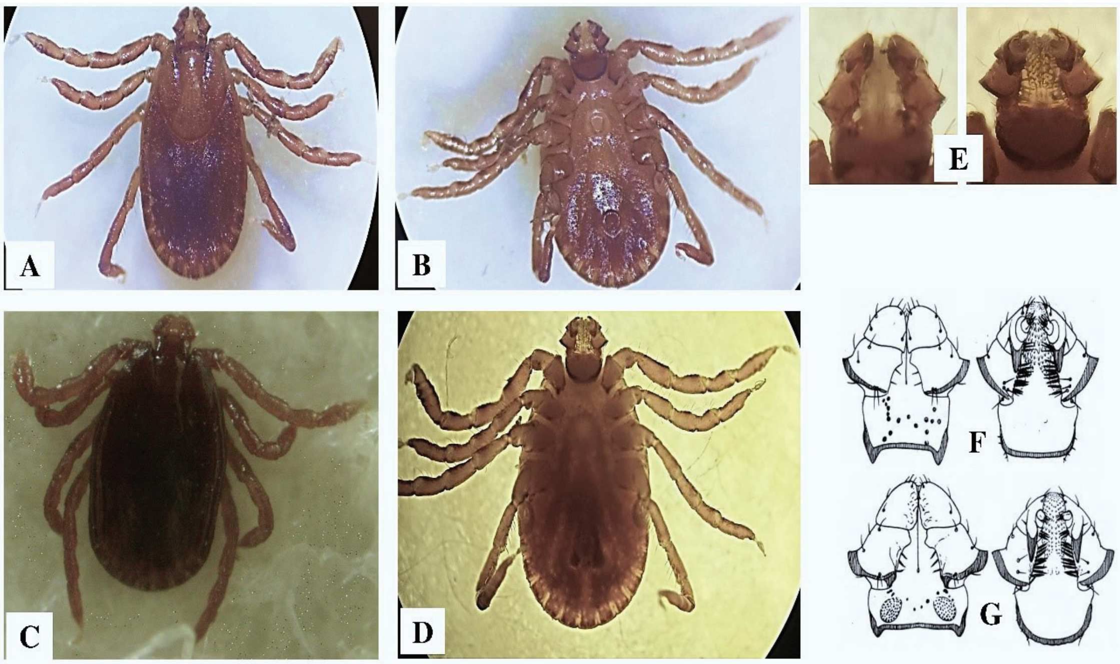

Fig. 1. Representing the *Haemophysalis howletti*. **A**. Male dorsal, **B**. Male ventral view, **C.** Female dorsal, **D.** Female ventral view, **E.** Basis capitula (dorsal and ventral), **F.** Male basis capitula, **G.** Female basis capitula. [F and G drawings adapted and modified from Geevarghese & Mishra, (2011)].

Identification of male *Haemophysalis howletti*

The scutum was ovoid and slightly posterior to the level of coxa IV. Lateral grooves were prominent, long, and extended from the level of coxa III anteriorly to enclose the first festoon posteriorly. Cervical grooves were moderate, deep, and narrow anteriorly. The festoons were long, clearly marked, and numbered 11 (Fig. 1A). The basolateral margin of palpal segment II was convex or rounded. The ventral spur of palpal segment III was broadly triangular, overlapping the anterior one-third of palpal segment II (Fig. 1B). Coxal spurs were small and blunt, decreasing in size progressively from coxa I to IV (Fig. 1B). Infra-internal setae were short, feathery, and closely spaced, numbering eight.

The morphological measurements of adult male specimens, with length and breadth recorded in millimeters for different body parts. The palp measures 0.13–0.16 mm in length (mean: 0.15 mm), the basis capituli ranges from 0.09–0.11 mm in length (mean: 0.1 mm) and 0.14–0.17 mm in breadth (mean: 0.15 mm), the scutum is 0.82–1.05 mm long (mean: 0.95 mm) and 0.59–0.66 mm broad (mean: 0.63 mm), the total body length is 1.05–1.32 mm (mean: 1.2 mm) with a breadth of 0.59–0.66 mm (mean: 0.63 mm), and the spiracles measure 0.04–0.06 mm in length (mean: 0.05 mm) and 0.13–0.16 mm in breadth (mean: 0.15 mm) as presented in Table 1.

Table 1. Measurements of major body parts of male ticks infesting rabbits.

Characteristics	Adult Male			
	Length (mm)		Breadth (mm)	
	Range	Mean	Range	Mean
Palp	0.13 - 0.16	0.15		
Basis Capituli	0.09 - 0.11	0.1	0.14 -0.17	0.15
Scutum	0.82 - 1.05	0.95	0.59 - 0.66	0.63
Total	1.05 - 1.32	1.2	0.59 - 0.66	0.63
Spiracles	0.04 - 0.06	0.05	0.13 - 0.16	0.15

Identification of female *Haemophysalis howletti*

Cervical grooves were distinct, deep, and narrow anteriorly, becoming shallow and wide posteriorly, extending beyond one-third to half the scutum. Marginal grooves were distinct on the dorsal integuments, extending to the scutum anteriorly and enclosing one festoon on each side posteriorly. Clearly marked festoons were 11 in number (Fig. 1C).

Palps were very compact, with the basolateral margin of palpal segment II convex and slightly extending beyond the lateral margin of the basis capituli. The ventrobasal spur of palpal segment III was broadly triangular, overlapping half of palpal segment II (Fig. 1D). The coxal and trochanter spurs were short and blunt. Infra-internal setae were short, feathery, closely spaced, and numbered eight. Spiracular plates were subcircular, with a short, broad dorsal extension at the posterior end.

The palp of adult female was recorded 0.29–0.36 mm in length (mean: 0.33 mm), the basis capituli ranges from 0.14–0.16 mm in length (mean: 0.15 mm) and 0.36–0.40 mm in breadth (mean: 0.38 mm), the scutum is 0.79–0.92 mm long (mean: 0.85 mm) and 0.85–0.92 mm broad (mean: 0.88 mm), the total body length is 2.35–2.62 mm (mean: 2.50 mm) with a breadth of 1.30–1.44 mm (mean: 1.38 mm), and the spiracles measure 0.23–0.26 mm in length (mean: 0.25 mm) and 0.12–0.14 mm in breadth (mean: 0.13 mm) as presented in Table 2.

Table 2. Measurements of major body parts of female ticks infesting rabbits.

Characteristics	Adult Female			
	Length (mm)		Breadth (mm)	
	Range	Mean	Range	Mean
Palp	0.29 – 0.36	0.33		
Basis Capituli	0.14 – 0.16	0.15	0.36 – 0.40	0.38
Scutum	0.79 – 0.92	0.85	0.85 – 0.92	0.88
Total	2.35 – 2.62	2.50	1.30 – 1.44	1.38
Spiracles	0.23 – 0.26	0.25	0.12 – 0.14	0.13

Identification of larval and nymphal stages of *Haemophysalis howletti*

Larval stages of *H. howletti* had the characteristic basis capitulum as sub-rectangular ventrally, with a posterior edge that is convex; it is twice as wide as long dorsally, with no visible cornua. The corona is absent, the hypostome is small, and the dental formula is 2/2, with six or seven denticles. There were eleven different festoons on the dorsal integument (Fig. 2A and B). The basis capitulum of *H. howletti* was dorsally well developed, pointed, and each approximately as long as its base (Fig. 2C and D).

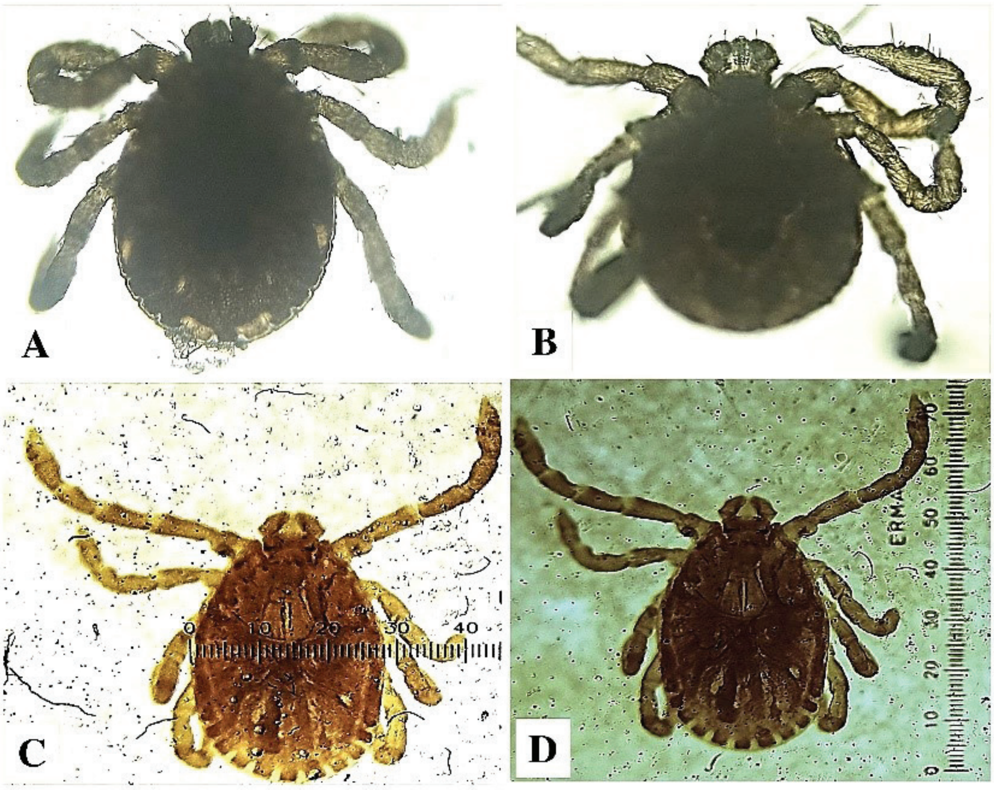

Fig. 2. Representing the *Haemophysalis howletti*. **A**. Larva dorsal, **B.** Larva ventral view, **C**. Nymph dorsal, **D**. Nymph ventral view.

The morphological measurements of larval specimens, with length and breadth recorded in millimeters for different body parts (Table 3). The palp measures 0.06–0.08 mm in length (mean: 0.07 mm), the basis capituli ranges from 0.04–0.05 mm in length (mean: 0.04 mm) and 0.11–0.13 mm in breadth (mean: 0.12 mm), the scutum is 0.23–0.28 mm long (mean: 0.25 mm) and 0.27–0.34 mm broad (mean: 0.30 mm), and the total body length is 0.53–0.62 mm (mean: 0.57 mm) with a breadth of 0.41–0.46 mm (mean: 0.43 mm).

Table 3. Measurements of major body parts of larval stage ticks infesting rabbits.

Characteristics	Larva			
	Length (mm)		Breadth (mm)	
	Range	Mean	Range	Mean
Palp	0.06 - 0.08	0.07		
Basis Capituli	0.04 - 0.05	0.04	0.11 - 0.13	0.12
Scutum	0.23 - 0.28	0.25	0.27 - 0.34	0.30
Total	0.53 - 0.62	0.57	0.41 - 0.46	0.43

The morphological measurements of nymphal specimens, with length and breadth recorded in millimeters for different body parts. The palp measures 0.11–0.14 mm in length (mean: 0.13 mm), the basis capituli ranges from 0.07–0.09 mm in length (mean: 0.08 mm) and 0.13–0.16 mm in breadth (mean: 0.14 mm), the scutum is 0.32–0.39 mm long (mean: 0.35 mm) and 0.33–0.40 mm broad (mean: 0.37 mm), and the total body length is 0.93–1.07 mm (mean: 0.98 mm) with a breadth of 0.51–0.58 mm (mean: 0.55 mm).

Table 4. Measurements of major body parts of the nymphal stage ticks infesting rabbits.

Characteristics	Nymph			
	Length (mm)		Breadth (mm)	
	Range	Mean	Range	Mean
Palp	0.11 - 0.14	0.13		
Basis Capituli	0.07 - 0.09	0.08	0.13 - 0.16	0.14
Scutum	0.32 - 0.39	0.35	0.33 - 0.40	0.37
Total	0.93 - 1.07	0.98	0.51 - 0.58	0.55

Variation in infestation of ticks within the autumn season

In the last week of September (early autumn), the prevalence of tick infestation in rabbits was found to be 100 % (n = 16), whereas in the last week of November (late autumn), the prevalence of ticks was nil.

Variation in Hematological parameters in infested and after-infestation-free

The mean values of these parameters were recorded during the naturally infested period during early autumn and late autumn, when they were tick-free (Table 5).

Table 5. Hematological alterations during tick-infested and tick-free states in rabbits.

Hematology Parameters	Ticks Present		Ticks Absent		t value	*p* value
	Mean±SE	Range	Mean±SE	Range		
TEC($*10^6/\mu L$)	5.25±0.33	3.59 - 7.16	6.8±0.45	4.32 - 9.68	-4.12	<0.01**
HGB(g/dL)	12.77±0.75	9.2 - 17.2	17.6±0.96	12.2 - 26.1	-5.84	<0.01**
HCT (%)	31.38±2.26	18.6 - 46.6	43.15±2.27	29.9 – 59	-5.57	<0.01**
MCV (fL)	60.68±0.63	57.2 - 65.1	60.30±0.58	55.8 – 64	0.46	0.65
MCH (pg)	25.02±1.28	13.39 - 34.53	26.63±1.42	27.14 - 42.05	-0.91	0.37
MCHC(g/dL)	42.24±2.32	25.11 - 57.85	41.53±1.77	28.40 - 54.30	0.25	0.8
PLT($*10^3/\mu L$)	155.69±24.07	35 – 398	226.94±37.35	46 -519	-3.02	<0.01**
TLC($*10^3/\mu L$)	10.34±0.4	8 - 13.2	9.56±0.59	5.8 - 13.7	2.47	0.03*
Lymho($*10^3/\mu L$)	4.03±0.25	3.1 - 6.3	4.13±0.25	2.6 - 6.2	-0.49	0.63
Gran($*10^3/\mu L$)	5.9±0.31	4.4 - 8.6	4.95±0.40	2.3 - 6.8	4.96	<0.01**
Mid($*10^3/\mu L$)	0.41±0.03	0.3 - 0.6	0.48±0.07	0.2 - 1.1	-0.85	0.41

Prevalence of haemoparasites

Out of 16 rabbit blood samples examined, 31.25% were detected positive for the presence of *Anaplasma*. The presence of dense, rounded, intraerythrocytic bodies located either at the center or near the margin of erythrocytes, as described by OIE (2015) confirmed the diagnosis of *Anaplasma* infection (Fig. 3).

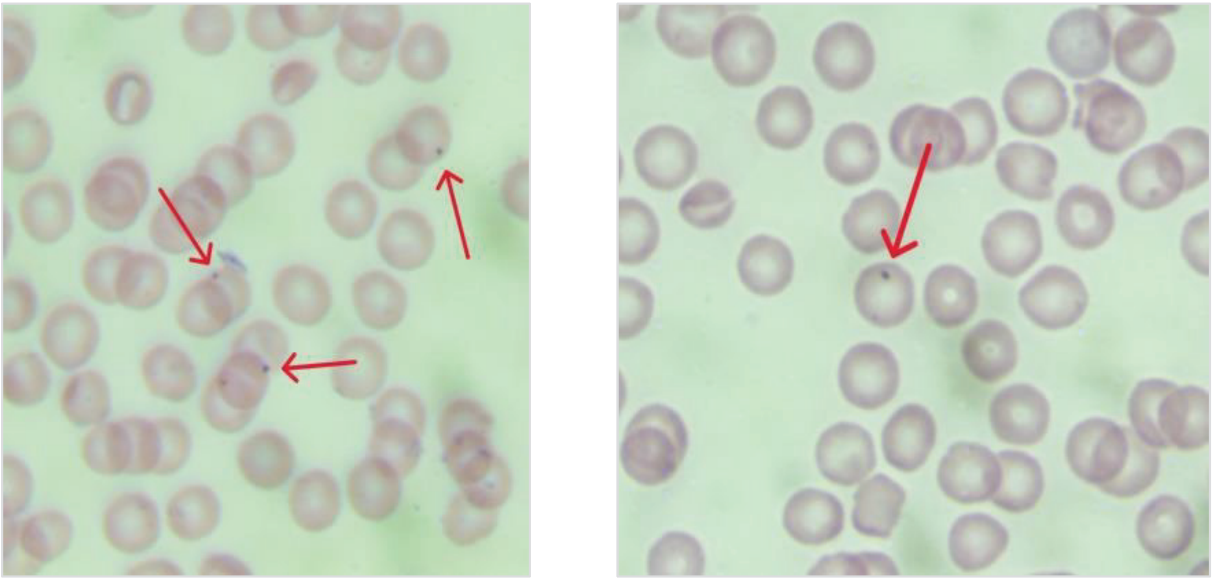

Fig. 3. Microscopic observation, Giemsa-stained blood smear. *Anaplasma*-infected red blood cells of rabbits are pointed with red arrows.

DISCUSSION

All adult and nymphal stage ticks were identified as Haemaphysalis howletti of subgenera Ornithophysalis. It was first collected and described from a hill pony in the Rawalpindi area of Pakistan by Warburton (1913). Subsequently, it was redescribed and collected from the Pune area of the Maharashtra state (Geevarghese & Mishra, 2011). This tick has not been described in lagomorphs yet, however, it has been reported in other small mammals, rodents, and birds such as *Bandicota bengalensis, Funambulus pennenti, Mus platythrix, Mus saxicola, Mus booduga, Rattus rattus rufescens*, and *Centropus sinensis* (Geevarghese & Mishra, 2011).

In this study, the measurement of the average total length and breadth of adult male *Haemaphysalis howletti* was 1.20 mm and 0.63 mm respectively with Basis capitula dorsally approximately 1.5 times as wide as long and the average total length and breadth of adult female *H. howletti* was 2.50 mm and 1.38 mm respectively with Basis capitula dorsally approximately 2.53 times as wide as long which is a similar result for adult female *H. howletti* in previous studies showing average total length and breadth of adult female *H. howletti* 2.53 mm and 1.40 mm respectively with Basis capitula dorsally approximately 2.6 times as wide as long (Dhanda, 1964). However, the adult male *H. howletti* were found larger in previous studies with measurements of average total length and breadth of adult male ticks 1.70 mm and 1.16 mm respectively, but with similar Basis capituli dorsally approximately 1.7 times as wide as long (Dhanda, 1964).

This study shows a higher prevalence of ticks during early autumn (100%) than in late autumn (0%). The outcomes of this investigation align with a report of other researchers with results and rationales regarding the high prevalence of tick infestations near the rainy season and low prevalence as the dry season approaches in various livestock (Kabir et al., 2011; Kumar et al., 2004; Vatsya et al., 2007). The elevated occurrence of tick infestation during the summer and early autumn seasons can be attributed to the heightened temperature and humidity resulting from heavy rains, characteristic of the rainy season, which creates favorable conditions for tick growth. Conversely, the reduced number of ticks in the late autumn and winter season can be attributed to lower temperatures, dry weather, and shorter days, which hinder the tick's reproductive cycle (Shoaib et al., 2022).

Variations in hematological parameters were observed between rabbits infested with ticks and those without infestations. When infestation-free, rabbits exhibited a significant increase ($p<0.05$) in TEC, HGB, HCT, and PLT compared to infested rabbits, similar to previous research (Kaur et al., 2017; Ragulraj et al., 2023; Vatsya et al., 2008). However, MCV, MCH, and MCHC showed non-significant variability between infested and non-infested rabbits. The lower levels of HGB, HCT, and TEC indicate anemia resulting from the blood-sucking and hemorrhage by ticks. In the present study, TLC and Granulocytes of the infested rabbits were found to be significantly ($p<0.05$) higher when compared to non-infested rabbits, which aligns with the research conducted in calves Kaur et al. (2017) which may be attributed to the inflammation triggered by tick bites, prompting the migration of white blood cells as a response to the tick bite.

31.25% of rabbits were positive for the presence of *Anaplasma* which suggests that the *Haemaphysalis howletti* might play a role as a vector for the transmission of *Anaplasma* supported by the previous findings as *Anaplasma* species were identified in *Haemaphysalis* species ticks collected from Jiaonan County in Eastern China, including *Anaplasma phagocytophilum*, A. capra, and *A. bovis* (Qin et al., 2018).

CONCLUSION

The study conclusively identified tick species of rabbits at AFU livestock farm as *Haemaphysalis howletti* and the morphometry of different developmental stages. The higher prevalence of ticks in early autumn than in late autumn resulted in a variation in various hematological parameters. Ticks may have played a role as vectors, resulting in the prevalence of *Anaplasma* sppplay a major role in the transmission of tick-borne pathogens affecting the health of rabbits and posing a potential risk of zoonosis.

DECLARATIONS

The rabbits involved in the tick and blood collection were performed according to the standardized protocol, which had prior approval by the Agriculture and Forestry University, Institutional Board for Research.

ACKNOWLEDGMENTS

The authors would like to acknowledge the Department of Veterinary Microbiology and Parasitology for the laboratory facility.

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