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Shakuntala Kage
Department of Parasitology, Veterinary College, Bengaluru, Karnataka, India

Mamatha GS
Department of Parasitology, Veterinary College, Bengaluru, Karnataka, India

Jaya Nagappa Lakkundi
Department of Parasitology, Veterinary College, Bengaluru, Karnataka, India

BP Shivashankar
Institute of Animal Health & Veterinary Biologicals Bengaluru, Karnataka, India.

Placid Eugene D'Souza
Department of Parasitology Veterinary College, Bengaluru Karnataka, India.

Correspondence
Mamatha GS
Department of Parasitology, Veterinary College, Bengaluru, Karnataka, India

Prevalence of ixodid ticks on *Babesia ovis* infected sheep and goats in Karnataka state

Shakuntala Kage, Mamatha GS, Jaya Nagappa Lakkundi, BP Shivashankar and Placid Eugene D'Souza

Abstract
A study was conducted to know the prevalence of ixodid ticks on *Babesia ovis* infected sheep and goats in Bengaluru Urban and Rural districts of Karnataka state. Out of 343 animals examined (225 sheep and 118 goats) from 44 flocks of both ailing and apparently healthy groups, 188 (83.55%) sheep and 96 (81.35%) goats were infested with ticks. An overall infestation rate was found to be 82.79 per cent. In a total of 253 and 99 ticks collected from infested sheep and goats, respectively 30.83 and 17.17 per cent were identified as *Haemaphysalis kutchensis*, 24.50 and 25.25 per cent as *Rhipicephalus sanguineus*, 20.94 and 28.28 per cent as *Haemaphysalis bispinosa*, 15.01 and 12.12 per cent as *Rhipicephalus haemaphysaloides* and 8.69 and 17.17 per cent as *Hyalomma marginatum isaaci*. A majority of the ticks were infested in the ears and least number of ticks were found on other parts of the body.

Keywords: *Babesia ovis*, Ixodid ticks, sheep, goats

Introduction
Small ruminants especially sheep and goats which contribute to the livelihood of millions of rural population are susceptible for majority of the tick borne infections. Among tick borne diseases, babesiosis is considered as an economically important haemoprotozoan disease in tropical and subtropical countries. The important pathogenic species include *Babesia motasi* and *Babesia ovis. Babesia crassa*, *Babesia foliata* and *Babesia taylori* are mild to non-pathogenic [1, 2].

The disease is transmitted by ticks belonging to the family Ixodidae such as *Rhipicephalus*, *Haemaphysalis* and *Hyalomma* spp. [3, 4]. *B. motasi* and *B. crassa* are transmitted by *Hyalomma anatolicum* and *Haemaphysalis punctata* [5] whereas *B. ovis* is transmitted by *Rhipicephalus bursa* and *R. turanicus. B. foliata* and *B. taylori* are transmitted by *H. Anatolicum* [6]. However, different species of ticks were reported by many authors throughout the world from *Babesia* spp. infected sheep and goats. *Haemaphysalis bispinosa*, *R. haemaphysaloides* and *Hyalomma savignyi* ticks were recorded in *B. motasi* infected sheep from Hyderabad [7, 8]. *H. intermedia* was identified from babesiosis infected sheep and goats from Karnataka [9]. In India, *Haemaphysalis* has been reported as a potential vector for transmission of theileriosis and babesiosis in sheep and goats [10]. In abroad, many authors have reported different ixodid ticks *viz.*, *R. bursa*, *R. sanguineus* and *R. turanicus* from *B. ovis* infected sheep and goats [11, 12]. However, *H. qinghaiensis*, *H. longicornis*, *H. bispinosa*, *R. microplus*, *R. sanguineus* and *D. silvarum* have been identified from *Babesia* sp. Xinjiang infected sheep and goats [13]. Hence, the present literature aims at collection, identification and probable role of ixodid ticks in transmission of *B. ovis* infected sheep and goats.

2. Materials and Methods
2.1 Study area
The ticks were collected from both sheep and goats with clinical symptoms *viz.*, fever, inappetance, weakness and from animals without any symptoms but infested with ticks, respectively belonging to different taluks of Bengaluru Urban (Bengaluru North, Bengaluru South, Bengaluru East and Anekal) and Bengaluru Rural (Devanahalli, Hosakote, Doddaballapura and Nelamangala) districts during the period from November 2017 to May 2018.

2.2 Collection, processing and identification of ticks

Ticks were collected manually from different locations on animal body like ears, head, axillae, abdomen and base of the tail in a well ventilated collection bottles. Then the samples were labelled and transported to the laboratory for further processing and identification. The adult unengorged male and female and nymphal ticks were processed and cleared by Phenol (Carbolic acid) [14]. Ticks collected from each flock were transferred to a separate labelled cavity blocks containing liquified phenol (Carbolic acid) and allowed to clear for three to eight hours or more by frequently observing under the stereozoom microscope. Fully engorged female and nymphal ticks were processed by clearing with 10% KOH [15]. Initially, the engorged ticks were punctured with fine needle without damaging their internal and external structures to remove the blood. Then the ticks were transferred to glass test tube containing 10 per cent KOH and boiled for 15 to 30 min until ticks become very transparent. Ticks were then transferred to petridish and thoroughly washed with distilled water to remove the traces of KOH. Later, they were dehydrated by placing in ascending grades of alcohol (50, 60, 70, 80, 90 per cent and absolute alcohol) for 15 min each. After dehydration, ticks were transferred to petridish containing a clearing agent containing mixture of clove oil and few drops of glacial acetic acid for 30 min. Later, ticks cleared by Phenol and 10% KOH were mounted on a glass slides (7.5 cm x 2.5 cm) by using phenol balsam mountant (1:1) and the cover slip was applied by pressing the ticks dorsoventrally. Ticks were observed under 10x and 40x objective of the compound microscope and were identified as per the standard keys [16-18].

2.3 Statistical analysis

The statistical analysis of data was carried out by Chi-square test using graph pad prism software, version 5.01.

3. Results

During this study, out of 343 (225 sheep and 118 goats) animals examined, 188 (83.55%) sheep and 96 (81.35%) goats were infested with ticks, with an overall prevalence rate of 82.79 (284/343) per cent. The processed ticks were identified based on the standard morphological keys and the morphological characters of five different species of ticks are described (Figures 1, 2, 3, 4, 5, 6). Out of 352 ticks collected from infested animals, 51.77 (131/253) and 45.45 (45/99) per cent were identified as *Haemaphysalis* spp., 39.52 (100/253) and 37.37 (37/99) per cent of as *Rhipicephalus* and 8.69 (22/253) and 17.17 (17/99) per cent as *Hyalomma* spp. in sheep and goats, respectively. In *Haemaphysalis* spp., 30.83 and 17.17 per cent of ticks were identified as *H. kutchensis*, 20.94 and 28.28 per cent as *H. bispinosa*, whereas in *Rhipicephalus* spp., 24.50 and 25.25 per cent of ticks were identified as *R. sanguineus*, 15.01 and 12.12 per cent as *R. haemaphysaloides* in sheep and goats, respectively. However, 8.69 per cent of ticks in sheep and 17.17 per cent in goats were found to be *H. m. issaci*. The statistical analysis of data between the tick infestation in sheep and goats was found to be non-significant ($p<0.05$). In a total of eight taluks, heavy infestation of *H. kutchensis* was recorded in flocks located in Bengaluru North followed by Anekal and Doddaballapura. *H. bispinosa* was recorded maximum from Bengaluru South, Anekal, Doddaballapura followed by Bengaluru North. *R. sanguineus* was recorded maximum from Bengaluru East, Devanahalli and Hosakote followed by Anekal and Doddaballapura. *R. haemaphysaloides* was recorded from Bengaluru East and Hosakote taluks. However, *H. m. isaaci* was recorded only from Nelamangala taluk (Table 1). The heavy tick infestation was observed in sheep compared to goats and more numbers of ticks were collected during the period from January 2018 to May 2018. A majority of the ticks were infested in the ears and least number of ticks were found on abdomen, near eyelids, axillae, around perineum and other parts of the body.

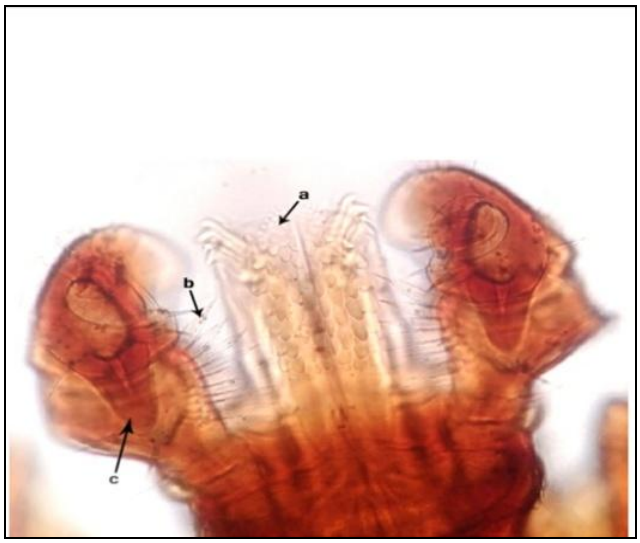

Fig 1: Morphological features of *Haemaphysalis kutchensis* a) Hypostome b) Infrainternal setae c) Retroventral spur

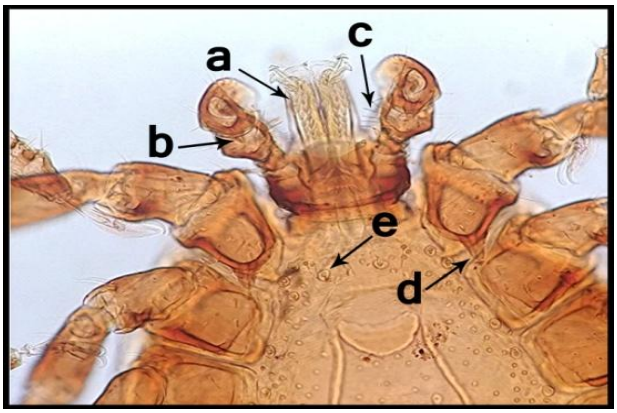

Fig 2: Morphological features of *Haemaphysalis bispinosa* a) Hypostome b) Retroventral spur c) Infrainternal setae d) Coxal spur e) Punctations

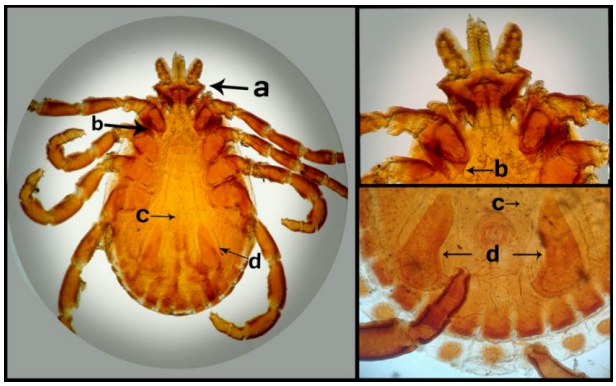

Fig 3: Morphological features of male *Rhipicephalus haemaphysaloides* tick a) Hexagonal basis capitulum b) Bifid coxa c) Punctations d) Adanal shields

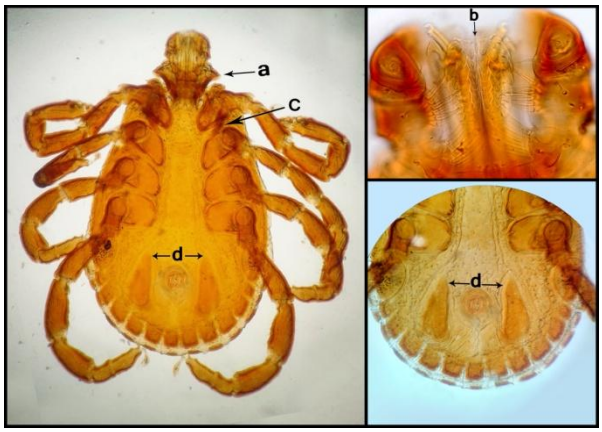

Fig 4: Morphological features of male *Rhipicephalus sanguineus* tick a) Hexagonal basis capitulum b) Hypostome c) Bifid coxa d) Adanal shields

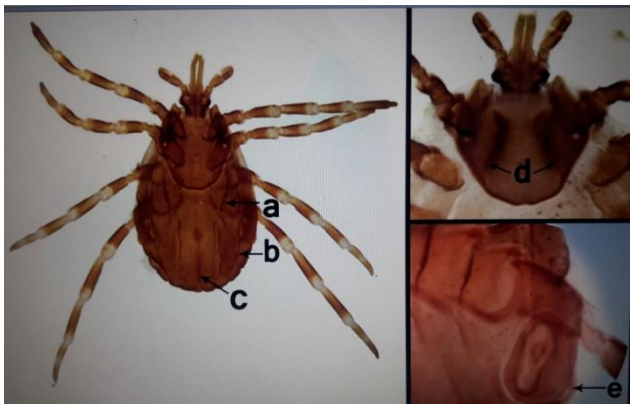

Fig 5: Morphological features of female *Hyalomma marginatum isaaci* tick a. External genital groove b. Lateral groove c. Median groove d. Cervical groove e. Spiracle

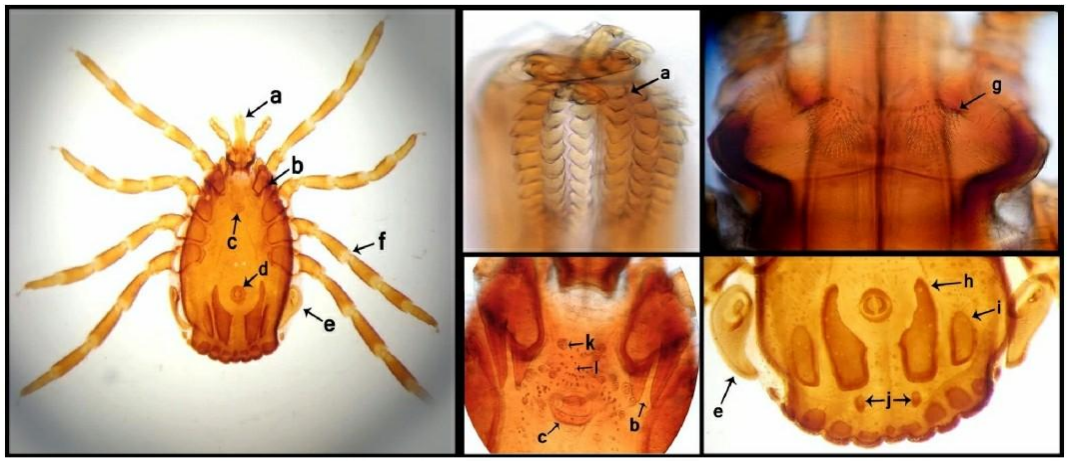

Fig 6: Morphological features of male *Hyalomma marginatum isaaci* tick a. Hypostome b. Coxa c. Genital aperture d. Anal opening e. Spiracle f. Whitish rings on joints g. Porose area h. Adenal shields i. Subadenal shields j. Subanal shields k. Large punctuations l. Small punctuations

Table 1: Taluk wise distribution of ticks in sheep and goats

Sl. No.	Districts	Taluks	Tick species
1	Bengaluru Urban	Bengaluru North	*H. kutchensis, H. bispinosa*
		Bengaluru South	*H. bispinosa, H. kutchensis*
		Bengaluru East	*R. sanguineus, R. haemaphysaloides*
		Anekal	*H. bispinosa, H. Kutchensis, R. sanguineus*
2	Bengaluru Rural	Devanahalli	*R. sanguineus*
		Hosakote	*R. sanguineus, R. haemaphysaloides*
		Doddaballapura	*H. bispinosa, H. kutchensis, R. sanguineus, R. haemaphysaloides*
		Nelamangala	*H. m. issaci*

4. Discussion

Babesiosis caused by *B. ovis* confirmed by our previous studies on parasitological and molecular diagnostic methods has been considered as an emerging haemoprotozoan disease of small ruminants in Karnataka state [19]. In a total of 343 animals examined, 188 (83.55%) sheep and 96 (81.35%) goats were infested with ticks. Out of 352 ticks collected from infested animals, 51.77 and 45.45 per cent of ticks from sheep (131/253) and goats (45/99) were identified as *Haemaphysalis* spp., 39.52 (100/253) and 37.37 (37/99) per cent of ticks as *Rhipicephalus*. The *Hyalomma* spp. was found to be 8.69 (22/253) and 17.17 (17/99) per cent in sheep and goats, respectively. The present findings confirmed that the ixodid ticks are commonly prevalent on both sheep and goats in South India. Similarly, the prevalence of ixodid ticks in small ruminants has been reported by many authors in India [9, 20, 21, 22].

During this study, the different tick species encountered in *B. ovis* infected sheep and goats were *viz.*, *Haemaphysalis kutchensis* (30.83 & 17.17%), *Rhipicephalus sanguineus* (24.50 & 25.25%), *Haemaphysalis bispinosa* (20.94 & 28.28%), *Rhipicephalus haemaphysaloides* (15.01 & 12.12%) and *Hyalomma marginatum isaaci* (8.69 & 17.17%), respectively. In general in Karnataka state, *Haemaphysalis* spp. has been reported as the most predominant tick species in sheep and goats [7, 9, 23, 24]. Similar to the present findings, many authors have reported *Haemaphysalis* spp. as a principle tick species in sheep and goats from other parts of India [20, 22, 25, 26].

In the present study, the tick infestation was found to be higher in Anekal and Doddaballapura taluks followed by Bengaluru South, Nelamangala, Bengaluru North, Hosakote,

Devanahalli and Bengaluru East. The severe tick infestation observed in the flocks located in these taluks may be probably due to the free range grazing system observed in the majority of the flocks located in Anekal, Doddaballapura, Bengaluru South and Nelamangala taluks, in which the animals were allowed to graze in hilly and forest areas, where the flora might have harboured different species of Ixodid ticks. Similarly, the high occurrence of *H. bispinosa* in grazing animals has been reported [28]. However, in other taluks very few ticks were found which could be probably due to intensive farming practices adopted in these flocks which inturn restrict the movement of animals. In addition, hygienic management practices, regular dipping with acaricides and good nutritional management probably resulted in reduced tick infestation [20]. The higher rate of tick infestation observed in sheep (83.55%) than goats (81.35%) during this study is in concurrence with findings from Egypt who reported higher prevalence of *H. anatolicum* tick infestation in sheep (32.26%) than goats (18.52%) [27]. However, in contrast to the present findings, higher tick prevalence has been reported in goats (97.66%) compared to sheep (64.66%) from Tamil Nadu (India) [28]. More number of ticks were collected during the period from January 2018 to May 2018. Since, during this period in Southern districts of Karnataka state the temperature will be around 25 to 35 °C with an average rainfall of 1068.8 mm per annum which is favourable for development of ticks [20]. Most of the ticks collected during this study were infested on ears (>70%) but very few were observed on other parts of body like neck, on eyelids, axillae, anus, tail and around genitalia, which might be attributed to the fact that the attachment of tick is dependent on the temperature and the thickness of the skin of the animals because the temperature of the skin covering the body (35 °C) will be higher than the ear (25 °C) and the easiness for ticks to acquire blood for nourishment [29, 30].

5. Conclusion

H. kutchensis and *H. bispinosa* were found to be the most predominant ticks in sheep and goats, respectively followed by *R. sanguineus*. The present findings suggested that these ticks may play an important role as a vector in transmission of *B. ovis.* Hence, there is a need for considering the contributing formative factor of these ticks in the epidemiology of babesiosis in Karnataka. However, further research work is needed to know the prevalence of ticks in other regions and their probable role as a vector in transmission of *Babesia* spp., in India.

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