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Preliminary study on the susceptibility of jersey and Holstein Friesian cattle breeds to the infestation of *Haemaphysalis* and *Rhipicephalus* (Boophilus) Ixodid ticks in Bugudanahalli Village, Tumkur, Karnataka

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Abstract
Ticks are vectors and, more interesting, ectoparasites that cause significant economic loss to livestock and adversely affect livestock hosts in several ways. The study was taken out in the Bugudanahalli village of Tumkur district, Karnataka, from February to April. It was found that the tick load is increased because of the availability of favorable conditions (an agro-climatic region with an annual rainfall of 592.9 to 900mm), which led to embryonic development and increased larval activity for the infestation in cattle. The study was carried out to assess the infestation of ticks on different anatomical regions of these two breeds. The necessary data collected during the survey is recorded separately for each week to analyze the result statistically using datasheets. For the ticks per animal, the factors considered were tick genera, cattle breed, cattle anatomy region, the severity of ticks, etc. In the present study, 1324 ticks were collected from both the cattle breeds, and the two identified tick genera are *Haemaphysalis* and *Rephicephalus* (Boophilus). The severity of tick load in various anatomical regions of these two breeds varied significantly. Holstein Friesian is more infested when compared to Jersey breed due to its less resistance to the infestation of ticks. This study suggests the farmers and livestock owners in the selecting the tick-resistant cattle; age-wise, breed-wise, and season-wise.

Keywords: tick infestation, susceptibility, Holstein Friesian, jersey, Ixodidae ticks, *Haemaphysalis*, and *Rephicephalus* (Boophilus)

1. Introduction
Ticks and mites are the groups of organisms that belong to the family Arthropoda and subclass Acari. So, the study of these organisms is termed "Acarology." Arthropods mainly include the members of crustaceans, chelicerates, myriapods, and insects. Chelicerates include spiders, mites and ticks, scorpions, and relatives [52]. Ticks are exciting, obligate, and one of the most familiar groups of ectoparasites and evoke different reactions causing physical damage to their hosts by sucking blood and injuring the skin. Still, many of those tick species can also transmit a greater extent of infectious agents than any other arthropod group [33].
Worldwide, nearly 900 tick species have been identified, out of which more than 700 species belong to the Ixodidae (hard ticks) and remainder to the Argasidae (soft ticks). Although it is common to consider domestic animals as the preferred host of ticks, most species occur in the wild. Several would not complete their life stages without small wild mammals or birds as hosts for their immature stages. These species are among the primary vectors of human and animal diseases caused by protozoa rickettsia, bacteria, viruses, and Helminthes [33].
Tick causes significant economic losses to livestock and adversely affects livestock hosts in several ways [41]. Most importantly, at the livestock interface, the transfer of tick-borne pathogens frequently occurs and poses a risk to livestock farming and development. Failure to control ticks effectively is a significant factor limiting livestock production [15]. The tick load in any region depends upon certain factors such as predators, competitor species, availability of other hosts. Furthermore, they are also influenced by vegetation, rainfall, temperature, and other ecological factors [21]. So, the selection of tick-resistant animals can reduce the devastating effect of tick infestation. This selection method is not harmful to the environment,

entails no additional costs, and can be a viable solution for the livestock sector [45].

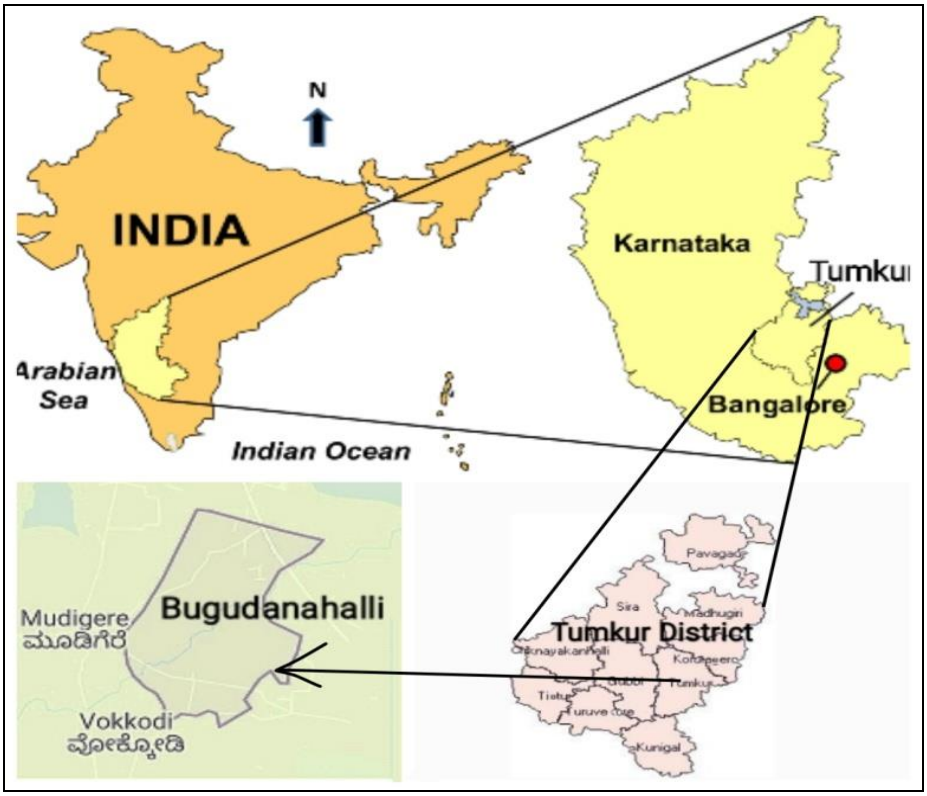
After considering the global economic deprivation due to tick infestation, the present preliminary study was undertaken in Karnataka state, particularly in rural areas of the Tumkur district. This research was carried out to assess the infestation of different tick species in the environment and the influence of breeds on the infestation. There is no known published checklist about the infestation of ticks in other cattle breeds in the Tumkur district.

2. Materials and Methods

2.1 Study Area

Bugudanahalli is a village panchayat located in the Tumkur district of Karnataka State, India. It is situated 8km away from Tumkur, which is both district & sub-district headquarter of Bugudanahalli village. The total geographical area of the Bugudanahalli village is 258.72 hectares which are situated at an altitude of 894.6 meters in the latitude 13.377120 N and longitude 77.0531060 E. It is the 146th biggest village by area in the sub-district.

The period from February to April is one of continuous rise in temperature (34 ^{0}C, 35 ^{0}C, 39 ^{0}C). April is usually the hottest month when the maximum temperature may reach 40 ^{0}C. The study area comprises the Agro-climatic region (central dry zone) with different types of vegetation: banana, coconut, areca nut, betel vine, paddy, pulses, etc. The animals that were reared in that particular area are sheep, goats, hens, and buffaloes. The cattle infected with ticks were selected from different sites of Bugudanahalli.



Showing Google map view of Bugudanahalli village (Source; googleimages.com)

2.2 Selection of Animals

A total of 8 cattle, 4 Holstein Friesian (Two cows and two calves), and 4 Jersey (Two cows and two calves) were selected randomly to serve as a study sample. The chosen animals were reared and raised in the same pastures.

2.3 Collection of Ticks

The sampling of ticks was carried out weekly on each cattle for four weeks from February to March 2020, a dry season period. The animals were kept in barns with good physical environment (sunlight, aeration etc.) to collect ticks in the anatomical regions. Before collecting ticks, attempts were made to collect almost all the ticks from each area of the animal body, determine the degree of the infestation, and note their predilection sites of attachment of each species of ticks. In the light infestation, the ticks were individually collected as far as possible and particular attention was paid for searching the presence of ticks in the six predefined parts; Head (Including ears and neck), Back (Croup), Abdomen, Teats, Tail (including ventral-genital) and legs, etc. In moderate to heavy infestation, a scoring system was used to assess the tick population on the host body. For proper assessment, six predefined parts on both sides of the body were carefully examined. The total number of ticks occurring at the sites were counted, and cattle were given a score as follows:

1. No ticks recorded: 0 (no infestation)
2. 1-50 ticks recorded: 1 (light infestation)
3. 51-100 ticks recorded: 2 (moderate infestation)
4. More than 100 ticks recorded: 3 (heavy infestation)

The ticks were quickly removed by grasping them between fingers and pulled away from the skin very gently (manual plucking). For some of the ticks, the forceps method was used as described by Walker *et al*. (2003) [53] by gripping the capitulum firmly with the help of a steel forceps and turned the ticks over on its back and then pulled out. Acaricides were not used till the two weeks before the experiment and during its duration. The dermatological lesions and the good physical environment (Sunlight, aeration, etc.) to collect ticks in their anatomical regions, the other clinical signs observed on the

animals were noted. Still, we did not make the case in this study.

2.4 Conservation of Ticks
Ticks were collected manually by using delicate pincers from the six defined parts of each animal by a team. The collected ticks were stored in seven vials containing 70% ethyl alcohol and labeled according to the predefined part of each animal. The information was marked on each vial using a black permanent marker that includes the date of collection, animal identification number, week number, and relevant data. Ticks are satisfactorily preserved in 70% ethyl alcohol to prevent the specimens from drying out completely.

2.5 Preparation of Permanent Slide of Tick
To proper identification of the ticks, good cleaning was done during the preparation procedure. The purpose of clearing is to macerate and dissolve internal tissues, especially muscle. Potassium hydroxide (10%) is used to soften the cuticle for dissection and maceration purposes.

The ticks were removed from the preservative, 70% ethyl alcohol, placed in water for one hour, and then in a beaker containing 10% KOH for at least 24 hours until it is suitably cleared (i.e., light honey color). Potassium hydroxide made the exoskeleton more transparent and dissolved the internal tissues. The ticks were pierced with a fine entomological pin at several places on the ventral surface before placing into a 10% KOH (to facilitate the destruction of internal and soft tissues) solution without damaging the points of identification. The dissolved body contents remained within, were further removed by applying slight pressure from outside by using a fine needle or brush, taking care not to damage or disorient the genitalia. After maceration, the ticks were washed thoroughly in distilled water to remove potassium hydroxide. Then the specimens were dehydrated by placing in 80%, 90%, and absolute alcohol for 10 minutes each. The dehydrated material should be placed in the clove oil alone for 2 minutes. Excessive clove oil is removed by placing the specimen on a clean, white filter paper and pressing dorso-ventrally. After the samples are cleared, they are mounted in Canada Balsam medium on glass slides (7.5 cm x 2.5 cm). Then the coverslip is placed over the specimen on a glass slide, and it is slightly pressed to flatten it. Then the slide was dried for 4-5 days at room temperature. Then the tick was identified and labeled.

2.6 Examination and Identification of Ticks
The ticks were identified as far as possible up to the genus level with the help of identification keys elaborated by Walker *et al*. (2003) [53]. The preserved specimens are placed on a slide and examined under a stereoscopic binocular microscope using direct light to determine the large-sized adult ticks. For identification of the immature stages (nymph, larvae), permanent mounts are examined for finer details under a research microscope. The maximum use of orientation and correct lighting is essential for an appreciation of the diagnostic characters.

2.7 Statistical analysis
The data collected during the study is recorded separately for each week in the datasheets. It includes all the data necessary for the analysis of the result statistically. For the sum of ticks per animal, the factors of variables considered were tick genera, cattle breed, cattle anatomy region, the severity of ticks, etc.

3. Results
From February to April at Bugudanahalli, it was found that the tick load is increased because of the availability of favourable conditions. This good condition (an agro-climatic region with an annual rainfall of 592.9 to 900mm) leads to embryonic development and increased larval activity for the infestation of cattle. The tick infestation was found to be influenced by temperature, rainfall, and relative humidity. In addition, for some tick species, dense humid vegetation is beneficial to tick survival and could lead to an increase in tick numbers as well. Such vegetation may also benefit pathogen-reservoir hosts such as cattle (Jaenson *et al*.,2012).

The overall tick index computed during the study was 1,324 ticks that are infested to the two cattle breeds. The maximum number of infestations is recorded in the Holstein Friesian breed, i.e., 1,176 ticks and minimum in Jersey breed 147 ticks. The effect of tick infestation in cattle breeds showed that the Holstein Friesian is more infested by the ticks when compare to the Jersey breed; this is because Holstein Friesian has less resistance to the infestation of ticks and in jersey has more resistance to the infestation of ticks.

The week-wise prevalence of ticks throughout the four weeks was presented in table 1 and graph 1; both indicate that in week two and week four, ticks' infestation more compared to the other two weeks. This is due to the change in the cattle grazing field and shows that the variation in the numbering of ticks from week to week is expected to ecological factors that vary in a particular week.

Table 1: Shows the graphical representation of data of

Week	Cattle	Breed	Head	Abdomen	Leg	Teats	Tail	Back
Week 1	Cow	Jersey 1	2	-	-	-	-	5
		Jersey 2	3	-	-	-	1	-
		HF 1	82	50	-	1	6	-
		HF 2	84	4	-	-	-	-
	Calf	Jersey 1	-	-	-	-	1	-
		Jersey 2	2	-	-	-	3	-
		HF 1	-	-	-	-	1	-
		HF 2	7	-	-	-	-	-
Week 2	Cow	Jersey 1	-	-	-	-	-	-
		Jersey 2	-	1	-	-	-	-
		HF 1	61	-	38	13	3	-
		HF 2	90	55	40	9	-	-
	Calf	Jersey 1	-	-	-	-	-	-
		Jersey 2	-	-	-	-	-	-
		HF 1	-	-	-	-	-	-
		HF 2	56	5	-	-	1	-

Week 3	Cow	Jersey 1	23	-	-	-	-	-
		Jersey 2	-	-	-	-	-	
		HF 1	35	20	35	20	4	33
		HF 2	8	51	66	20	-	-
	Calf	Jersey 1	-	-	-	-	-	-
		Jersey 2	-	-	-	-	-	-
		HF 1	-	-	-	-	-	-
		HF 2	2	-	-	-	-	-
Week 4	Cow	Jersey 1	-	-	-	-	-	-
		Jersey 2	1	8	25	66	-	-
		HF 1	49	79	15	19	-	-
		HF 2	21	30	26	1	-	-
	Calf	Jersey 1	1	-	-	-	-	-
		Jersey 2	-	-	-	-	-	-
		HF 1	1	-	-	-	-	-
		HF 2	12	5	-	17	-	-

The rise in the number of ticks from week to week is seen in cows. This research also revealed the influence of animal age on Ixodidae tick infestation. When only the cattle age is considered in the study, it was seen that there was a gradual increase of tick load in all four cows from week to week. Here the calves are less infested than cows. This increase of ticks on cows is also due to the rise in temperature, which is the main factor influencing the development and survival of ticks. These results were the same as those obtained by some researchers, which show that young cattle were more resistant to ticks than older ones. It should even be noted that the management of young cattle can contribute to the reduction of infestation. Sometimes, they were kept in a stall or shed, reducing the probability of their exposure to the infestation of ticks in pastures.

Ticks may infest any part of the host's body, but they prefer an area with soft skin in general. The infestation level in Holstein Friesian is high in the head region, i.e., 508 (heavy infestation), followed by 299 in the abdomen region (heavy infestation), 220 in the leg region (heavy infestation), 100 in the teat region (moderate infestation), 33 in the back part (light infestation) and a minimum number of infestations in the tail region, i.e., of 22 (light infestation. The infestation level in jersey is high in the teat region, i.e., 70 (moderate infestation) in number followed by 38 in the head region (light infestation), 25 in the leg region (light infestation), 9 in the abdomen region (light infestation), 6 in the tail region (light infestation) and a minimum number of infestations in the back part, i.e., of 5 in number (light infestation). The overall percentage of infestation is high in the head region compare to other anatomical areas of both the cattle breed. Infestation of ticks to the cattle varies in the different anatomical regions due to the presence or absence of other alternative ectoparasites present in the particular environmental condition.

1. 2.

Fig 1, 2: Genus: *Haemaphysalis*

3. 4.

Fig 3, 4: Genus: *Rhipicephalus*

Based on morphological studies, the collected ticks are identified as Ixodid ticks and are examined for identification up to the genus level. The two identified genera are *Haemaphysalis* (Figure 1 and 2) and *Rephicephalus* (*Boophilus*) (Figure 3 and 4). However, the abundance of infestation by *Haemaphysalis* ticks is more when compare to *Rephicephalus* (*Boophilus*). This is due to the environmental factors which influence the survival of the ticks. Thus, the present study also confirms Ixodidae ticks as the predominant parasite of cattle breeds.

Upon completing the study, it was recorded that the infestation of ticks is mainly attributed to the vegetation, animal activity, host availability, and other climatic factors in the study area. Many species of ticks are adapted to seasonal variations in climate within their geographical range. Ecological factors, particularly temperature, rainfall, relative humidity, and vegetation of a particular environment, influence the survival and development of ticks.

4. Conclusion

Ticks are the most important vectors due to their direct impact, and the diseases they transmit are significant constraints to the development of cattle breeding in India. The study carried out in the Bugudanahalli village of Tumkur district, Karnataka, on the infestation of cattle by ticks allowed us to identify ticks of genera *Haemaphysalis* and *Rephicephalus* (*Boophilus*) ticks. *Haemaphysalis* is more abundant in both the cattle breeds, whereas *Rephicephalus* (*Boophilus*) was found only in thin-skinned regions. The effect of tick infestation on cattle breeds showed that Holstein Friesian (HF) is more infested due to its less resistance than Jersey. This research also revealed the influence of animal age on Ixodidae tick infestation. Calves are less infested than cows, and it also noted that the management of young cattle could contribute to the reduction of infestation. Taking into account, the information obtained from our present study suggests the farmers and livestock owners in the selection of

tick-resistant cattle; age-wise, breed-wise, Season-wise. This study will support future researchers or students, as this study will contribute information suitable for the research in acarology.

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