



Rhipicephalus (Boophilus) microplus Infestation and its Implications in *Anaplasma marginale* Infection among Cattle in South Gujarat, India

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ABSTRACT

Background: This study aims to identify risk factors for *Rhipicephalus (Boophilus) microplus* ticks' infestation in South Gujarat's cattle and explore its implications on *Anaplasma marginale*.

Methods: An approach was employed to retrieve the crucial insights from the owners and examinations of 2305 cattle (crossbred=1495, indigenous=810) of South Gujarat, India. Species specific PCR was used to detect 576 bp of msp5 gene of *A. marginale* in the oviposited ticks.

Result: Crossbred cattle (73.91%) had higher tick prevalence than indigenous ones (55.80%), with *R. (B.) microplus* being the dominant species. Highest tick infestation was noted in cattle >3 years (74.43%), followed by <1 year (70.23%) and 1-3 years old (55.60%). Ticks collected from crossbred exhibited higher prevalence of *A. marginale* compared to indigenous cattle. Significantly highest ($p > 0.05$) ticks infestation rate was noted in cattle with pale conjunctiva, poor body condition and black coat colour. Infection rate of both the parasites was highest during summer, followed by monsoon and winter. Kachcha house and earthen floor favoured the survival of the pathogen. Owners in surveyed regions combat tick infestations using mechanical (7.69%), chemical (83.51%) and herbal/no treatments (10.07%). These findings emphasize the intricate link between tick infestations and *A. marginale* infection, offering crucial insights for cattle health management.

Key words: *Anaplasma marginale*, Cattle, *Rhipicephalus (Boophilus) microplus*, Ticks.

INTRODUCTION

Cattle farming in South Gujarat faces a significant challenge posed by the infestation of the hard tick of species *Rhipicephalus (Boophilus) microplus* (Patel *et al.*, 2019). Ticks harm cattle, spread diseases and lead to significant global economic losses, including reduced production and increased management costs. In India, managing tick-related issues in the livestock costs 787.63 million USD annually (Singh *et al.*, 2022). Ticks' prevalence varies by animal breed, age, conjunctiva color, body condition, coat color, season and housing. Animals in better body condition generally host fewer ticks, while poor housing conditions increase infestation rates, with tick numbers peaking in warmer seasons (Patel *et al.*, 2019).

Among the plethora of pathogens transmitted by ticks, *Anaplasma* species, particularly *A. marginale*, emerge as major limiting factors for the bovine industry (Kumar *et al.*, 2022). The biological transmission of *A. marginale* involves *R. (B.) microplus* ixodid ticks. Additionally, mechanical transmission becomes significant in the absence of biological vectors, occurring through various means such as blood-contaminated fomites and blood-sucking arthropods. Its life cycle depends on tick feeding, with binary fission in ticks' cells, leading to dense colonies that infect various tick tissues including salivary gland and are transmitted to animals during feeding. In bovine, during

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prepatent stage the *A. marginale* invade erythrocytes in a geometric progression. Both cattle and ticks serve as reservoirs for *A. marginale*, potentially maintaining persistent infections (Kocan *et al.*, 2010).

Understanding the complex interactions between *R. (B.) microplus* infestation and *A. marginale* infection is crucial for devising effective strategies to mitigate the impact on cattle health and productivity. This research seeks to

assess the risk factors associated with *R. (B.) microplus* infestation in cattle in South Gujarat and elucidate the implications of such infestations in the context of *A. marginale* infection.

MATERIALS AND METHODS

Study areas, biological samples and risk factors

The study was conducted on 2305 cattle (1495 crossbred and 810 indigenous) of Navsari, Tapi, Surat and Valsad districts of South Gujarat and categorized into three age groups: <1 year (527 animals), 1-3 years (723 animals) and >3 years (1058 animals). Ticks on cattle were examined visually at owners' doorsteps, collected using forceps and placed in sealed glass tubes to transport to the laboratory. Identification was based on morphological characteristics. Concurrently, to address the biotic (animal's breed, age, body condition, conjunctiva and coat colour) and abiotic (housing condition and season) risk factors influencing ticks and *A. marginale* prevalence, a methodical approach was employed to extract the crucial insights from cattle owners and performing examinations of the animals. The body condition score (BCS) was calculated to assess body fat by palpating the vertical (spine) and horizontal (short ribs) processes of the animals (Cornelius *et al.*, 2015). The scoring ranged from 1 (very skinny and emaciated) to 5 (overfat). Genomic DNA was extracted from oviposited *R. (B.) microplus* to detect *A. marginale* in its tissue, using the DNeasy Blood and Tissue DNA Extraction Kit (Qiagen, Germany), following the manufacturer's protocol.

PCR assay for *Anaplasma* DNA detection in *R. (B.) microplus*

To detect *Anaplasma* DNA in *R. (B.) microplus* (from 823 crossbred and 284 indigenous cattle), PCR was performed using a thermal cycler under using the primer pair of 5'-GTGTTCTGGGGTACTCCTATGTG-3' and 5'-AAGCATGTGACCGCTGACAAAC-3' to amplify a 576 bp segment of

the *msp5* gene of *A. marginale* (Kumar *et al.*, 2019). Pooled DNA samples from *R. (B.) microplus* were initially screened for *A. marginale* and individual PCR was performed in cases of positive amplification. For each PCR reaction, a 25 µL mixture was prepared by combining 1 µL (25-50 ng) of DNA template, 1 µL (10-20 pM) of each primer, 12.5 µL of 2x Taq PCR master mix (containing 2x buffer, 0.4 mM deoxynucleotide and 4 mM MgCl₂) from Qiagen, Germany and 9.5 µL of nuclease-free water in a sterile PCR tube. The amplification condition was initial denaturation at 94°C for 5 min followed by 35 cycles of 3 steps consisting of denaturation at 94°C for 1 min, annealing temperature 56°C for 1 min, elongation at 72°C for 1 min, final elongation at 72°C for 10 min and storage at 4°C. Following PCR, the resulting products were visualized under UV light by performing 1% agarose gel electrophoresis and ethidium bromide staining.

Statistical analysis

The data was analyzed using OPSTAT software (Sheoran *et al.*, 1998), comparing means using one-way ANOVA with Duncan's multiple range test. The p value <0.05 was considered significant.

RESULTS AND DISCUSSION

Animal breed and ticks genus-wise ticks prevalence

A combined count of 1105/1495 (73.91%) crossbred cattle and 452/810 (55.80%) indigenous cattle exhibited infestations of ticks. Similar higher prevalence of ticks in crossbred cattle as compared to indigenous has been reported earlier by many workers (Khajuria *et al.*, 2015; Patel *et al.*, 2019; Shirsikar *et al.*, 2023). Valsad had the highest tick incidence, but there was no significant difference across all four districts, possibly due to similar agro-climatic conditions and animal rearing practices. The highest incidence rate was associated with *R. (B.) microplus* ticks, followed by *Haemaphysalis* sp., *Hyalomma* sp. and mixture

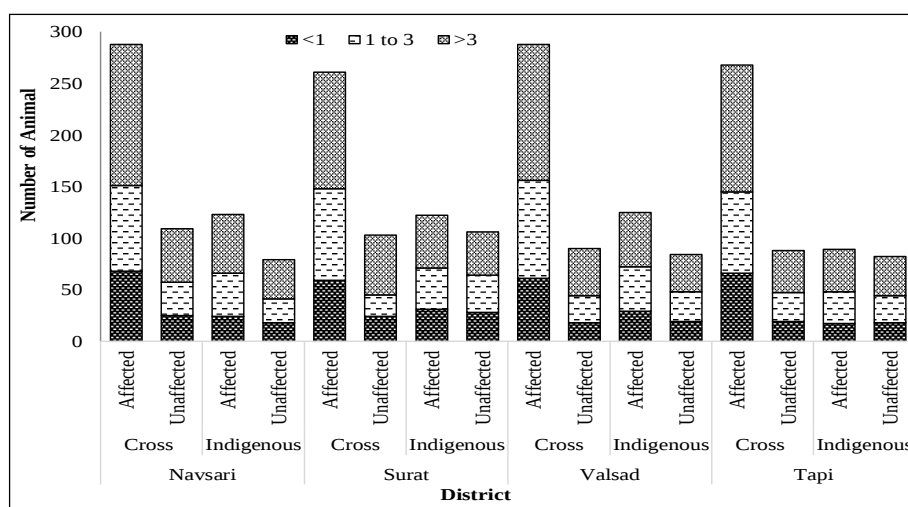


Fig 1: Ticks distribution based on animal age categories.

of ticks. Similarly, Mandloi *et al.* (2016) reported significantly higher prevalence of *R. (B.) microplus* (42.89%) compared to *H. a. anatolicum* (11.82%) and mixed infestations (4.16%). Additionally, Dehuri *et al.* (2017) and Patel *et al.* (2019) had also highlighted *R. (B.) microplus* as the predominant tick species across India.

Animal age-wise ticks prevalence

This study observed ticks' infestation rate of 70.23% (368/524) in cattle aged less than 1 year, 55.60% (402/723) in cattle aged 1-3 years and a rate of 74.43% (786/1056) in those older than 3 years. Notably, both crossbred and indigenous cattle aged over 3 years exhibited a significantly higher overall prevalence ($p < 0.05$), followed by those less than 1 year old and those aged 1-3 years (Fig 1) across all four districts. Similarly, Ghosh *et al.* (2019) and Shirsikar *et al.* (2023) observed higher prevalence of ticks in >3 years age of cattle than <1 year of age. Conversely, Patel *et al.* (2013), Khajuria *et al.* (2015) and Dehuri *et al.* (2017) noted the highest prevalence in animals under 1 year of age, followed by those between 1-3 years of age and lower prevalence in those over 3 years old. In contrast, Mandloi *et al.* (2016) found that tick infestation was significantly higher in cattle aged 1-3 years (78.63%) compared to those over 3 years (56.79%) and below 1 year (52.92%).

Animal conjunctiva colour-wise ticks prevalence

The colour of the conjunctiva exhibited a direct correlation with anaemia in animals. Among 253 surveyed cattle, 51.78% exhibited tick infestation and red-colored conjunctiva, while within the 1557 animals with tick infestations, 40.21% displayed pink-colored conjunctiva and among the 927 animals with pale-colored conjunctiva, 86.29% had tick infestations (Fig 2). The colour of the conjunctiva can be a significant diagnostic tool for tick-induced anaemia, as over 80% of large animals with pale conjunctiva were infested with ticks (O'Kelly and Seifert, 1970).

Animal body condition-wise ticks prevalence

The influence of bovine health on tick infestation was evident in this study. Animals with poor body condition displayed a significantly higher prevalence ($p = 0.00001$) of tick infestation (88.21%, 703/797) followed by those in good body condition (73.98%, 765/1034) and excellent body condition (18.78%, 89/474), confirming the impact of overall health on tick occurrence. This finding aligns with the observations made by Dehuri *et al.* (2017) and Patel *et al.* (2019), who noted elevated tick prevalence in animals with poor body condition.

Animal coat colour-wise ticks prevalence

The infestation rate of ticks differed significantly across cattle with varying body coat colors. The highest infestation rate was observed in cattle with black body coat (73.31%, 533/727), followed by those with mixed body coat (71.75%, 442/616), red/brown body coat (72.13%, 414/674) and white body coat (57.29%, 165/288). Breed-wise ticks prevalence was 75.88% (475/626) in black crossbred, 57.43% (58/101) in black indigenous, 65.22% (240/368) in red/brown crossbred, 56.86% (174/306) in red/brown indigenous, 35.71% (5/14) in white crossbred, 58.39% (160/274) in white indigenous, 79.05% (385/487) in mixed crossbred and 44.19% (57/129) in mixed indigenous. These findings resonate with Jawale *et al.* (2012), who similarly highlighted the significant influence of cattle body coat color on tick incidence, black cattle having the highest infestation rates, followed by mixed, white and brown cattle.

Season-wise ticks prevalence

The study revealed varying tick incidence rates across seasons, with the highest occurrence during summer (87.00%, 857/985), followed by monsoon (67.34%, 431/640) and winter (39.56%, 269/680). Summer and monsoon seasons collectively showed significantly greater infestations compared to winter in cattle. Among crossbred cattle, tick infestation rates were 70.62% (298/422) in summer, 91.27%

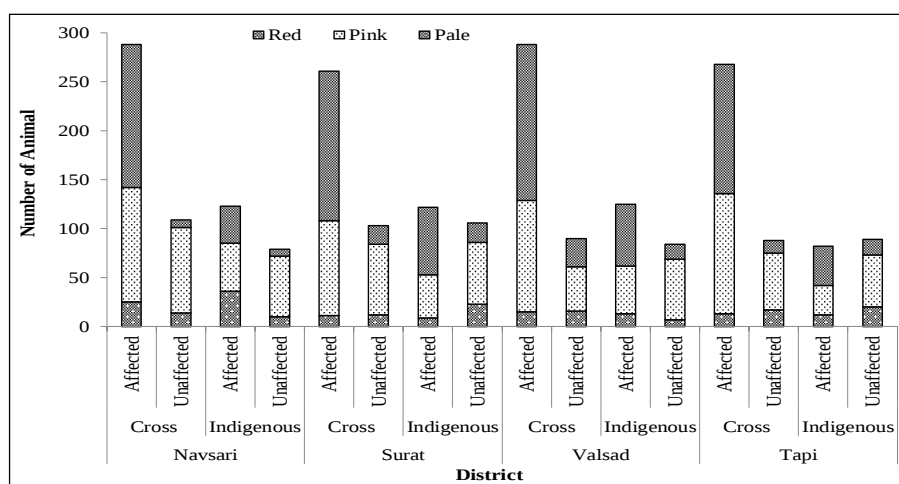


Fig 2: Ticks distribution based on animal conjunctiva colour.

(617/676) in monsoon and 47.86% (190/397) in winter. Similarly, among indigenous cattle, tick infestation rates were 61.01% (133/218) in summer, 77.67% (240/309) in monsoon and 27.92% (79/283) in winter (Fig 3). Similar patterns were noted by Kumar *et al.* (2022), who observed higher tick prevalence during the summer season, followed by the monsoon and winter seasons. Conversely, Khajuria *et al.* (2015); Ghosh *et al.* (2019); Negi and Arunachalam (2020); Jayalakshmi *et al.* (2024) reported higher tick prevalence in the monsoon season, followed by summer and then winter. These variations may arise from differences in geographical locations, topography, soil composition, temperature, humidity and rainfall.

Animal house-wise ticks prevalence

Among 2305 animals, 1069 (46.24%) were housed in wooden structures (kachcha) in which 790 (73.90%) with tick ($p=0.00001$) and 279 (26.10%) without tick, while 1236 (64.00%) were in pucca (permanent) houses in which 767 (62.06%) were with tick and 469 (37.94%) without tick. Ticks were most prevalent among animals housed on earthen floors (78.01%, 667/855), followed by stone-paved floors (63.35%, 261/412) and cement concrete floors (60.60%, 629/1038). Animals in earthen/wooden houses exhibited significantly more ticks ($p=0.00001$) than those in pucca houses. A similar trend was observed by Farooqi *et al.* (2017), who found significantly higher tick incidence among animals housed in traditional wooden structures compared to those in concrete houses. This association is likely due to the presence of cracks and crevices in wooden houses that provide hiding spaces for ticks.

Ticks control measure adopted by the animal owner

The efficacy of traditional and conventional tick control methods has been compromised in recent times due to the

changing climatic conditions, which have facilitated a rapid and widespread proliferation of ticks including resistant population (Patel *et al.*, 2020). To combat tick infestations, the animal owners in the surveyed regions employed mechanical measures in 7.69% of cases, chemical methods in 83.51% of cases and no or herbal therapy in 10.07% of cases (Table 1). The implementation of guidance from clinicians and para-clinicians in the management of animal health was observed in 18.18% and 47.81% of the cases, respectively and a notable proportion of 34.04% of animals received treatment without their direct involvement (Table 1). The frequency of drug administrations to cattle revealed a balanced distribution, with 58.18% receiving treatments at regular intervals, while 41.82% were subjected to irregular treatment schedules. Furthermore, the assessment of treatment and control techniques employed on cattle demonstrated varying degrees of effectiveness, with 62.56% exhibiting positive outcomes and 37.44% indicating less successful results. These findings highlight the diverse perspectives on the efficacy of pest control methods, indicating potential differences in outcomes across regions.

Risk factor-wise prevalence of *A. marginale* in *R. (B.) microplus*

The set of primer pairs specific to the *A. marginale* resulted in the amplification of a 576 bp fragment of msp5 gene. Animals of <1 year age group, crossbred cattle exhibited lower infection rates of *R. (B.) microplus* (9.16%, 137/1495) and *A. marginale* (1.82%, 15/823) in this tick compared to indigenous animals (9.75%, 79/810 and 1.06%, 3/284, respectively). Similarly, in the 1-3 year age group, crossbred cattle continue to demonstrate lower infection rates for both *R. (B.) microplus* (9.97%) and *A. marginale* (3.04%) than indigenous animals (10.86% and 3.87%, respectively). In

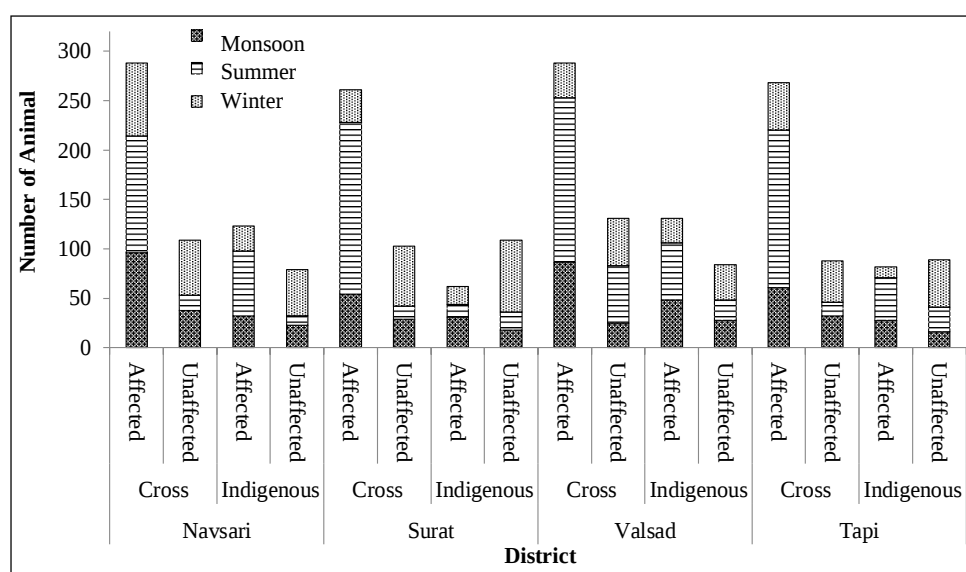


Fig 3: Ticks distribution based on season.

Table 1: Animal owner response related to tick control measures.

Question/information asked	No. (%)										p value
	Navsari		Tapi		Surat		Valsad		Overall		
	No.	%	No.	%	No.	%	No.	%	No.	%	
Method of control	Chemical										
	Mechanical	24	4.01	31	5.88	52	8.78	41	6.98	148	7.69
	Deltamethrin	226	42.72	132	30.41	211	43.51	221	46.33	790	41.04
	Flumethrin	112	21.17	134	30.88	108	22.27	115	24.11	469	24.36
	Ivermectin	182	34.40	163	37.56	151	31.13	129	27.04	625	32.47
	Others	9	1.70	5	1.15	15	3.10	12	2.52	41	2.13
	Sub-total	529	88.31	434	82.35	485	81.93	477	81.26	1925	83.51
	Herbal/ no treatment	46	7.68	62	11.76	55	9.29	69	11.75	232	10.07
	Total	599		527		592		587		2305	0.0001
	Clinician	123	20.53	82	15.56	116	19.59	98	16.70	419	0.35
Drug/ control measure advised by Frequency of drugs/ control measure Effect after drug / control	Para-clinician	279	46.58	231	43.83	299	50.51	313	53.32	1122	0.37
	Self	197	32.89	214	40.61	177	29.90	176	29.98	764	0.05
	Regular	315	52.59	298	56.55	343	57.94	385	65.59	1341	0.23
	Occasional	284	47.41	229	43.45	249	42.06	202	34.41	964	0.06
	Effective	334	55.76	362	68.69	389	65.71	357	60.82	1442	0.24
	Non- effective	265	44.24	165	31.31	203	34.29	230	39.18	863	0.03

contrast, within the >3 years age group, crossbred animals have higher infection rates for both *R. (B.) microplus* (35.92%) ($p=0.0296$) and *A. marginale* (15.07%) ($p=0.116$) compared to indigenous cattle (14.44% and 11.27%, respectively). Cattle in the poor body condition, displayed highest infection rates for both *R. (B.) microplus* (27.63% for crossbred, 13.21% for indigenous) ($p=0.016$) and *A. marginale* (12.52% for crossbred, 9.15% for indigenous) ($p=0.069$) compared to other categories. In contrast, animals in excellent body condition exhibited the lowest infection rates for both *R. (B.) microplus* (4.55% for crossbred, 5.93% for indigenous) and *A. marginale* (0.97% for crossbred, 0.70% for indigenous). When observing seasonal trends, crossbred cattle in the summer season exhibited a higher infection rate of *R. (B.) microplus* (36.52%) and a higher percentage of *A. marginale* infection within ticks (8.63%) compared to indigenous animals (19.38% and 7.39%, respectively) followed by monsoon season [in crossbred *R. (B.) microplus* of 13.31% and *A. marginale* of 9.96%; in indigenous 1.36% and 7.75%, respectively]. Likewise, animals housed in kachcha conditions presented higher infection rates of both *R. (B.) microplus* (29.16% for crossbred, 19.14% for indigenous) and *A. marginale* (7.65% for crossbred, 5.28% for indigenous), in contrast to those housed in pucca facilities with *R. (B.) microplus* infection rates of 25.892% for crossbred and 15.93% for indigenous, along with *A. marginale* infection rates of 12.27% for crossbred and 10.92% for indigenous). Further more, cattle kept on earthen floors show highest infection rates of *R. (B.) microplus* (20.87% for crossbred, 13.70% for indigenous) ($p=0.002$) and *A. marginale* (10.33% for crossbred, 8.45% for indigenous) ($p=0.021$) compared to other floor types. In contrast, animals housed on cement floors exhibited lower infection rates for both *R. (B.) microplus* (14.45% for crossbred, 10.12% for indigenous) and *A. marginale* (3.77% for crossbred, 3.17% for indigenous). Risk factor-wise prevalence of *A. marginale* in the invertebrate host like *R. (B.) microplus* ticks remains unreported, even though some research has been conducted in the vertebrate host. One such study, conducted by Khan *et al.* (2019) on cattle in southern Khyber Pakhtunkhwa, Pakistan, unveiled an overall anaplasmosis prevalence of 19.66% among diseased cattle. Notably higher prevalence was evident in young cattle ($d^{TM}5$ years, 24.85%) compared to adults ($e^{TM}5$ years, 13.13%, $p<0.001$). Cross HF cattle exhibited the highest prevalence (28.10%, $p<0.0000$) among breeds, as opposed to indigenous purebred cattle (6.08%). Prevalence exhibited variations across seasons (summer: 36%, winter: 7%, $p<0.0000$).

CONCLUSION

The in-depth analysis of tick infestations in South Gujarat's cattle reveals diverse risk factors, with crossbred cattle exhibiting higher tick prevalence. *R. (B.) microplus* is

dominant and infestation rates vary with age, conjunctiva color, body condition, coat colors and seasons. The study identifies *A. marginale* in *R. (B.) microplus* infection in PCR, with varying infection rates in ticks from crossbred cattle across age groups, seasons and health conditions, emphasizing the intricate relationship between tick infestations and *A. marginale* infection for comprehensive cattle health management in the region.

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Conflict of interest

The authors declare no competing interests.

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