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Molecular Identification and Prevalence of Tick (*Rhipicephalus sanguineus*) Infestation in Dogs from Guwahati, Assam, India

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ABSTRACT

The experiment was conducted in and around Guwahati, Assam, India for a period of one calendar year from March, 2021 to February, 2022 to determine the prevalence and molecular identification of tick infestation in dogs in and around Guwahati, Assam, India. A total of 582 dogs of different breeds, age groups (below 1 year and above 1 year age group), sex and different categories (stray dogs, pet dogs and working dogs) were examined. Study revealed that overall prevalence of tick was found to be 58.76%. Breed-wise, highest prevalence of tick infestation was observed in mongrels (75%) followed by Germam shepherd (66.66%). Sex-wise, tick infestation was more in male dogs (70.10%) than females (47.42%). According to the age, tick infestation was found more in dogs of below 1 year of age (89.38%) than in dogs of above 1 year of age (39.32%). Category wise, stray dogs (92.77%) showed higher prevalence of tick infestation than working (64.36%) and pet dogs (40.12%). Ticks were observed throughout the year of which highest prevalence was recorded in monsoon season (70.79%) followed by post monsoon (62.22%), pre monsoon (57.35%) and winter (36.92%). Morphological as well as molecular identification based on amplification and sequence analysis of the 16S rRNA gene showed that the isolated ticks belonged to *Rhipicephalus sanguineus* (ON428306.1, ON428307.1 and ON428308.1). Phylogenetic analysis based on a portion of 16S rRNA gene showed divergence at nucleotide level among the tick isolates.

KEYWORDS: *Rhipicephalus sanguineus*, prevalence, dog, 16S rRNA gene

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

Dogs are the most popular and trustworthy companion animal to human being. Because of various fundamental behavioural traits like adoptability, obedience, loyalty and sensitive olfaction etc., dogs have acquired an important place in human life. Dogs are prone to many parasitic diseases viz., helminthic, protozoal and ecto-parasitic and amongst those, ticks were the most common ectoparasite that infests them. Ticks are widely distributed in tropical and subtropical countries including India. They are also considered as one of the important and harmful blood sucking ectoparasites that transmit most of the pathogenic species than any other group of blood feeding arthropods of livestock and humans (Benelli et al., 2016). Several characteristics of ticks viz. wide host range, high reproductive potential, tendency to feed on several hosts during their life cycle and attachment with hosts for relatively longer periods give them ample time to acquire and transmit pathogens which make them outstanding vectors of pathogenic agents (Ghosh and Nagar, 2014). Tick bite leads to intense itching, constant irritation and restlessness which may cause the dog to lick, rub, scratch, chew and bite the affected areas that results into a coarse, roughened hair coat and skin, redness and inflammation (Lema, 2020). Apart from that, biting of the tick releases some neuro-toxins with their saliva that can cause paralysis, systemic illness and hypersensitivity in animals (Sahu et al., 2013). Though, hosts and vegetation both can modulate the dynamics of tick populations but vegetation serves as the major modifier of local climatic conditions, to which ticks must adapt for their development and survival (EstradaPeña, Ayllón and De La Fuente, 2012). Recently, climate change has altered the distribution of different tick species as well as the introduction of some tick species and infectious pathogens into previously unaffected regions (Tirosh-Levy et al., 2018). This expanding nature of different tick population due to climate change has now become a matter of concern (Dumont et al., 2014). Depending on the geographical area, ticks of different genera such as *Rhipicephalus, Haemaphysalis, Dermacentor* and *Hyalomma* act as vectors of various haemoparasitic infection viz., babesiosis, ehrlichiosis, anaplasmosis and hepatozoonosis in dogs. These diseases cause anaemia in the host animal that may lead to death also, if not treated early. *Rhipicephalus sanguineus*, the brown dog tick is the most widespread tick of the world (Dantas-Torres, 2010). Because of the wide range of host adoptability, capacity to survive and proliferate in kennels and residences, *R. sanguineus* are found almost everywhere in the world (Chandra et al., 2020, Barker and Barker, 2023). The increase in the global distribution of ticks increases many emerging and re-emerging tick-borne diseases world-wide (Guglielmone et al., 2013, De la Fuente et al., 2017). Therefore, widespread distribution of ticks and their ability to transmit zoonotic pathogens necessitates the regular screening of dogs for tick infestation.

Identification of ticks has traditionally been based on their morphological features like mean size, genital aperture, sexual dimorphism and capitulum (Walker et al., 2000). However, it is often been found difficult to identify different ticks solely based on morphological descriptions at some of their developmental stages such as larva and nymph. Whereas, molecular tools can serve as a reliable means for accurate identification of tick species by targeting different genetic marker (Ghosh et al., 2020). Previously, prevalence of ticks in dogs has been reported in different states of India by several workers (Sahu et al., 2013, Bhadesiya et al., 2016). In Guwahati, the occurrence of tick borne diseases in dogs was rising day by day. Hence, the present study was designed to explore the prevalence as well as molecular identification of tick infestation in dogs in and around Guwahati, Assam, India.

2. MATERIALS AND METHODS

The present study was conducted in and around Guwahati, the biggest city of the state of Assam which is also the largest urban centre of North-Eastern India. The study was conducted for one calendar year starting from March, 2021 to February, 2022. The laboratory works were conducted at the Department of Parasitology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India.

2.1. Animals, tick collection and identification

A total of 582 dogs of different breeds, age groups (below 1 year and above 1 year age group), sex and different categories (stray dogs, pet dogs and working dogs) were examined for the presence of ticks. The study was conducted covering four main seasons viz., pre-monsoon, monsoon, post monsoon and winter. The samples were collected from the dogs presented in the Veterinary Clinical Complex of the College of Veterinary Sciences, Assam Agricultural University, Khanapara, Guwahati as well as from the privet clinics, NGO's in and around the Guwahati city. The external body coat of dogs mainly both the inner and outer surface of ear, inter-digital spaces, dorsal and ventral surface of neck and the tail were examined thoroughly for the presence of ticks. After collection, the ticks they were gently preserved into clean glass vials containing 70% alcohol for morphological identification and in phosphate buffer saline (PBS) solution for molecular identification. Permanent slides of ticks were prepared as per the procedures specified in Soulsby (1982). The ticks were identified based on the morphology under a stereoscopic binocular microscope and compound microscope following the guidelines described by Sen and Fletcher (1962) and Soulsby (1982).

2.2. DNA extraction from ticks

For extraction of DNA from ticks, 8–10 numbers of adult ticks collected from same animal were crushed with the help of pestle and mortar & DNA was isolated from ticks using DNeasy Blood and Tissue kit (Qiagen® Kit,) following the manufacturer's recommendations with minor modifications. The isolated DNA samples were stored at -20°C until further use. Four such isolated DNA samples were used for molecular detection by PCR amplification and were numbered as L1, L2, L3 and L4.

2.3. PCR amplification of 16S rRNA gene

The PCR amplification of the portion of 16S rRNA gene was carried out from tick DNA samples with the following primer pairs: Forward 5'- CCG GTC TGA ACT CAG ATC AAG T -3' Reverse 5'- GCT CAA TGA TTT TTT AAA TTG CTG -3' (Norris et al., 1996). PCR reaction was performed following the reaction composition and reaction condition specified by Norris et al., 1996. with some minor modifications for amplification of an expected 450 bp product. Briefly, the PCR reaction was carried out in a 50µl reaction volume containing 5µl of extracted tick DNA, 25µl Dream Taq master mix (2x) (Thermo scientific Dream Taq green PCR MM), 2.5 µl of each forward and Reverse primer and 15µl of Nuclease Free Water. PCR was performed with Initial denaturation at 94°C for 3 minutes, followed by 32 cycles, each cycle consisting 1 minute of denaturation at 94°C, annealing of 1 minute at 50.5°C and an extension of 1 minute at 74°C. Final extension was done at 72°C for 15 minutes. PCR products were resolved on 1.5% Agarose gels and compared with 100 bp DNA ladder (Thermo scientific gene rular 100 bp).

2.4. DNA sequencing and construction of phylogenetic tree

The amplified products from three DNA samples (L1, L2 & L3) were sent to 1st Base Sequencing Service (Apical Scientific Sdn. Bhd., Malasia) for column purification and then bidirectional commercial DNA sequencing by the Sanger's di-deoxy nucleotide chain termination method using both forward and reverse primers on at least two independently generated PCR products. The generated sequences were compared using BLASTn algorithm with the sequences available in the National Centre for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov) to identify the tick species. The accession number for the sequences was obtained from the NCBI Gen Bank database. Further, the sequences were used to construct a specific phylogenetic tree based on sequence homology with the published sequences originated from different countries using the UPGMA method in Mega 7.0.21 software.

2.5. Statistical analysis

Prevalence data was expressed as the percentage. To test the significant association between the difference groups, Fisher's exact or Chi square test was performed. The statistical analysis was done at 1% probability level using the Statistical Package for the Social Sciences (SPSS), Version 25 software (SPSS Inc., Chicago, IL, USA).

3. RESULTS AND DISCUSSION

3.1. Prevalence of tick infestation

Out of 582 dog examined, 342 were found to be infested with ticks, with overall prevalence of 58.76% (95% CI: 54.6–62.8). Previously, Bhadesiya et al. (2016) also recorded a higher prevalence of 58.11 tick infestation in dogs from India. However, the present prevalence percentage was found more in comparison to the findings of Krishna Murthy et al. (2016). Variation in the overall prevalence of the ticks may be due to the difference in study period, sample size, geographical area, change in the temperature and the humidity. A total of 780 numbers of ticks of different stages and sex (nymphal stage and adult male and female) were collected during the present study. (Figure 1). All the ticks were examined under microscope and identified as *Rhipicephalus sanguineus,* by their characteristics such as hexagonal basis capitulum, adenal plate and comma shaped spiracle (Figure 2). Earlier, Vijayakumar et al. (2013) in Bareilly, Uttar Pradesh, Hadi et al. (2016) in Indonesia also found *R. sanguineus* as the sole tick species in their study. However, Sahu et al. (2013), Bhadesiya et al. (2016) also reported simultaneous presence other species of ticks also

Figure 1: Ticks at various stages of development collected from the body of dogs

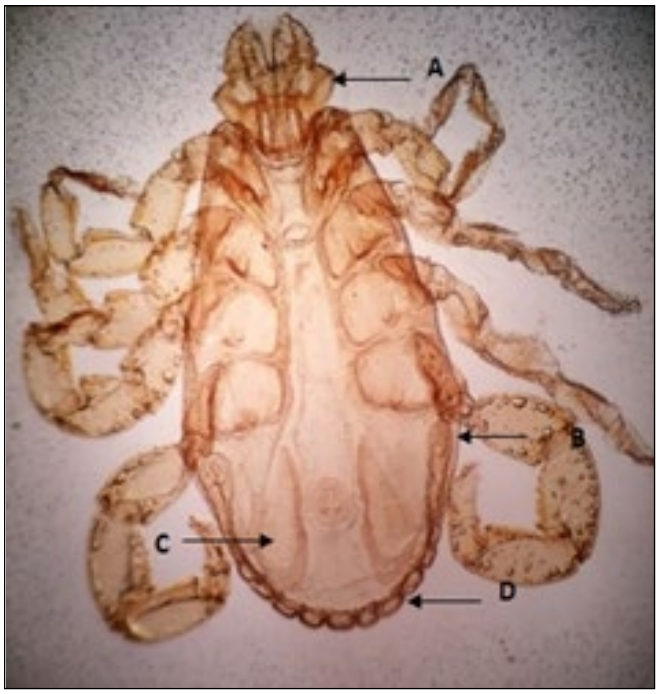

Figure 2: Ventral side of a *Rhipicephalus sanguineus* showing various parts of the body, A: hexagonal basis capituli, B: comma shaped spiracle, C: adenal plate, D: festoon

in their study. This difference in the distribution of the ticks might be due to change in geographical regions as well as different environmental conditions. *Rhipicephalus sanguineus* is highly adoptable in urban conditions and is highly efficient in multiplication in the urban environment therefore it is also called urban dog tick (Roopesh, 2017). As the samples for the present study were collected from the urban areas mostly in and around the Guwahati city, the detection of *Rhipicephalus sanguineus* as only tick species is aptly justified.

Breed wise, highest prevalence of tick was recorded in Mongrel (75.00%) followed by German shepherd (66.66%) (Table 1). Statistically, the prevalence of tick infestation on different breeds of dogs was found significant (p<0.01). Highest prevalence of tick in mongrels was also reported by Raut et al. (2006) in Maharashtra. However, highest prevalence of tick in German shepherd was reported by Hadi et al. (2016) and Kebbi et al. (2019) in Algeria. The highest prevalence in mongrels in the present study could be due to the fact that they were mainly ownerless, free roaming and stray which enables them higher chances of gating and spreading tick infestation from one animal to another. The higher in German shepherd might be due to the fact that these dogs were mostly used by the police and military forces for different out-door activities like bomb detection, forensic and crime detection and therefore are more susceptible for acquiring external parasites.

Table 1: Prevalence of tick infestation in dogs from Guwahati, Assam, India according to season, category and breed

Variables	No. of dogs screened	No. of dogs infected	Prevalence (%)	chi-square statistic	p value
Season					
Pre monsoon	136	78	57.35	39.65	<0.01*
Monsoon	226	160	70.79		
Post monsoon	90	56	62.22		
Winter	130	48	36.92		
Category of dogs					
Pet dogs	329	132	40.12	134.19	<0.01*
Stray dogs	166	154	92.77		
Working dogs	87	56	64.36		
Breed					
Mongrel	252	189	75.00	78.91	<0.01*
German shepherd	69	46	66.66		
Labrador retriever	110	41	37.27		
Pug	45	29	64.44		
Spitz	48	15	31.25		
Golden retriever	18	09	50.00		
Doberman pinscher	16	06	37.50		
Cocker spaniel	10	04	40.00		
Pomeranian	08	02	25.00		
Bull mastiff	06	01	16.66		

*Denotes statistically significant p value (p<0.01), Chi square statistics

Tick infestation was recorded throughout the year. However, highest prevalence was observed in monsoon (70.79%) and least in winter (36.92%) season (Table 1). The influence of seasons in the prevalence of ticks was found statistically significant (p<0.01). Higher tick prevalence in monsoon season was also reported by Sahu et al. (2013) and Vijayakumar et al. (2013). The warm and humid climate in the monsoon might have favoured the growth and

multiplication of Ixodid ticks in monsoon (Soulsby, 1982). Importantly, the present findings show that temperature and humidity plays an essential role in the growth and development of tick. During monsoon and post-monsoon season, the temperature remained high and relative humidity was also ideal for the rapid multiplication which in terms resulted in high population of ticks. Whereas, during the winter season, low temperature and dry environment slowed down the multiplication of ticks which resulted in low prevalence rate observed in the present study.

Category-wise, the prevalence of tick infestation was found to be significantly (p<0.01) higher in stray dogs (92.77%) followed by working dogs (64.36%) and pet dogs (40.12%) (Table. 1). The higher prevalence of ticks in stray dogs was also reported by Ayodhya (2014). Papazahariadou et al. (2003) also found higher tick infestation in dogs living outdoors than the dogs living indoor. The much higher prevalence of ticks in stray dogs might be on account of stress due to poor management, care and unhygienic living conditions (Totton et al., 2011). On the other hand, low prevalence of tick in pet dogs might be due to their better hygiene, good shelter and proper care taken by their owner.

Sex-wise, the prevalence of tick infestation was found significantly (p<0.01) higher in males (70.10%) than in female (47.42%) (Table 2). Our finding correlates with that of Sahu et al. (2013), Vijayakumar et al. (2013) and Hadi et al. (2016) who recorded higher prevalence of tick infestation in male dogs. The higher prevalence of tick infestation in male dogs might be due to the scavenging and wandering habit of male dogs during breeding season which enables them to acquire and spreading of ticks. It was also observed that certain hormonal factors are responsible for predisposing male dogs to tick infestation (Sahu et al., 2013).

Age-wise prevalence of tick infestation was highest in young group (below 1 year) with 89.38% than in adult group (above 1 year) 39.32% (Table. 2). Statistically, the influence of age on tick infestation was found significant (p<0.01). Vijayakumar et al. (2013), Ayodhya (2014) and Kebbi et al. (2019) also reported higher prevalence of tick infestation in young dogs than the older ones. Higher prevalence in young dogs might be due to of their underdeveloped immunity. Further, the constant exposure of the young pups to their carrier mother might also have contributed for higher prevalence of tick in the young group. Moreover, the lower prevalence in adult group might be because of development of resistance against tick and also due to more scratching activity of the adults (Sahu et al., 2013).

Table 2: Prevalence of tick infestation in dogs from Guwahati, Assam, India according to various epidemiological factors

Epidemiological factor	No. of dogs screened	No. of dogs infected	Prevalence (%)	Relative risk	Odd Ratio	95% CI	p value
Sex							
Male	291	204	70.10	0.005	2.6	1.85–3.65	<0.01*
Female	291	138	47.42				
Age group							
Young (<1 year)	226	202	89.38	0.004	12.99	8.09–20.85	<0.01*
Adult (>1 year)	356	140	39.32				

CI: Confidence interval; *Denotes statistically significant p value (p<0.01), Fishers' exact test

3.2. Molecular identification of tick

The PCR amplicons of all four extracted DNA samples from ticks showed single distinct band of 450 bp when compared with the DNA ladder in agarose gel (1.5%) (Figure 3). On BLAST analysis of the sequences, the three samples (L1, L2 and L3) showed maximum 99.53%, 99.76% and 100% similarity at nucleotide level with the sequences of *Rhipicephalus sanguineus* available in the NCBI GenBank database which further confirmed the specificity. Phylogenetic analysis of 16S rRNA gene sequence of the Assam isolates (ON428306.1, ON428307.1, ON428308.1) of *R. sanguineus* was carried out with additional 7 sequences of the same gene available in GenBank derived from tick isolates of different countries (China OL757514.1, Mexico MT322611.1, Thailand KC170744.1, Cuba KP830114.1, USA OM985246.1, Taiwan AY883865.1) as well as a Kerala state of India (India MG066692) (Figure 4). The generated phylogenetic tree depicted that the L1 (ON428306.1) and L2 (ON428307.1) were in two different clade whereas, the L3 (ON428308.1) was grouped in another clade with the USA isolate.

On investigation it was found that the L3 was isolated from a dog that came from Shillong, Meghalaya, India and was presented in the Teaching Veterinary Complex, College of Veterinary Sciences, Khanapara, Guwahati, Assam. The dog had some travelling history with his owner few weeks back

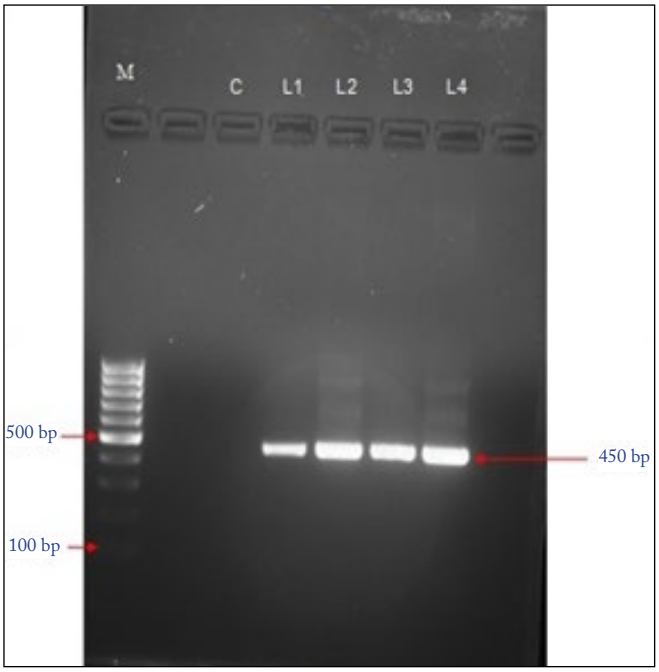

Figure 3: PCR amplification of 16S rRNA gene fragment (450 bp) of *Rhipicephalus sanguineus* isolated from dog from Guwahati, Assam. Lane L_1, L_2, L_3 and L_4: Tick DNA samples, Lane C- Non template control, Lane M:100 bp DNA ladder

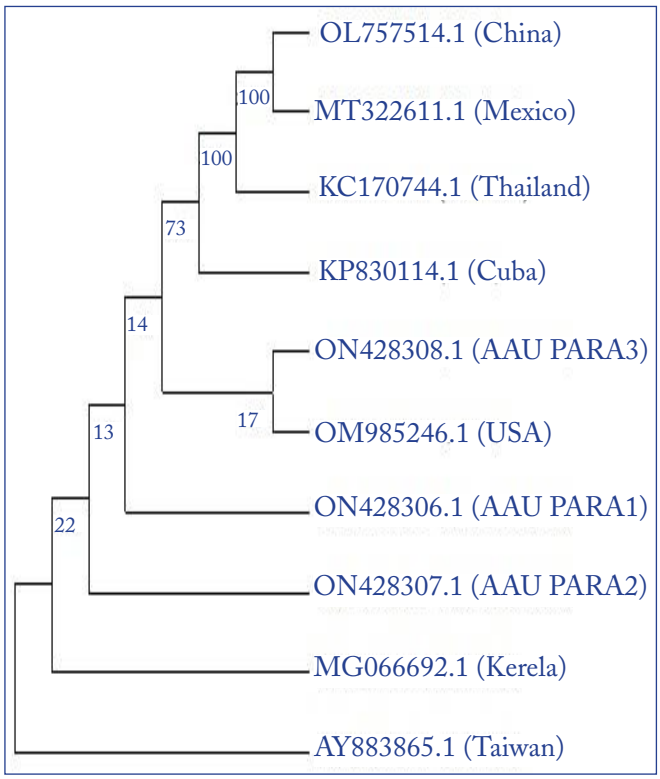

Figure 4: Phylogenetic tree constructed for *Rhipicephalus sanguineus* (ON428306.1, ON428307.1, ON428308.1) from the sequence of 16S rRNA gene fagment using UPGMA method Mega 7.0.21

from abroad. Therefore, there is every possibility that the dog might have carried some immature tick in its body from there and it was noticed only a few days before collection. As because dogs now a day's travel in different parts of the world with their owners, the chance of transboundary tick infestation has increased. Further, the divergence in nucleotide sequence among the tick DNA samples indicated that variability exists among the *R. sanguineus* ticks prevalent in the study area. However, the impact (if any) of this divergence in DNA sequence of 16S rRNA gene on the vector biology and/ or transmission of haemoparasitic disease is required to be explored.

4. CONCLUSION

Overall prevalence of tick in dogs in and around Guwahati, Assam, India was 58.76%. The breed, sex, age, season and category wise study showed the highest prevalence of tick infestation in mongrels, in males, in dogs of below 1 year of age, in monsoon season and in stray dogs. Morphological as well as phylogenetic analysis depicted only 1 species of tick i.e *Rhipicephalus sanguineus* could be recorded in the present study.

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6. REFERENCES

Ayodhya, S., 2014. Management of tick infestation in dogs. Journal of Advanced Veterinary and Animal Research 1(3), 145–7.

Bhadesiya, C.M., Raval, S.K., Hasnani, J.J., Neha, R., 2016. Epidemiological aspects of tick infestation by *Otobius magnini* and *Dermacentor variabilis* in dogs of Anand, Gujarat. Environment and Ecology 34(2A), 636–639.

Barker, S.C., Barker, D., 2023. Ticks of Australasia: 125 species of ticks in and around Australia. Zootaxa 5253(1), 1–670.

Benelli, G., Pavela, R., Canale, A., Mehlhorn, H., 2016. Tick repellents and acaricides of botanical origin: a green roadmap to control tick-borne diseases? Parasitology Research 115, 2545–2560.

Chandra, S., Ma, G.C., Burleigh, A., Brown, G., Norris, J.M., Ward, M.P., Emery, D., Šlapeta, J., 2020. The brown dog tick *Rhipicephalus sanguineus sensu* Roberts, 1965 across Australia: Morphological and molecular identification of *R. sanguineus sl* tropical lineage. Ticks and Tick-Borne Diseases 11(1), 101305.

Dantas-Torres, F., 2010. Biology and ecology of the brown dog tick, *Rhipicephalus sanguineus*. Parasites & Vectors 3, 26.

De la Fuente, J., Antunes, S., Bonnet, S., Cabezas-Cruz, A., Domingos, A.G., Estrada-Pena, A., Johnson, N., Kocan, K.M., Mansfield, K.L., Nijhof, A.M., Papa, A., Rudenko, N., Villar, M., Alberdi, P., Torina, A., Ayllon, N., Vancova, M., Golovchenko, M., Grubhoffer, L., Caracappa, S., Fooks, A.R., Gortazar, C., Rego, R.O.M., 2017. Tick pathogen interactions and vector competence: identification of molecular drivers for tick-borne diseases. Frontiers in Cellular and Infection Microbiology 7, 114.

Dumont, P., Blair, J., Fourie, J.J., Chester, T.S., Larsen, D.L., 2014. Evaluation of the efficacy of afoxolaner against two European dog tick species: *Dermacentor reticulatus* and *Ixodes ricinus*. Veterinary Parasitology 201(3–4), 216–219.

Estrada-Peña, A., Ayllón, N., De La Fuente, J., 2012. Impact of climate trends on tick-borne pathogen transmission. Frontiers in Physiology 3, 64.

Ghosh, P., Tikar, S.N., Gupta, M.K., Sukumaran, D., 2020. Molecular characterization using 16S rRNA and COI gene sequence in hard ticks of Gwalior, India. Defence Life Science Journal 5(3), 173–179.

Ghosh, S., Nagar, G., 2014. Problem of ticks and tick-borne diseases in India with special emphasis on progress in tick control research: a review. Journal of Vector Borne Diseases 51(4), 259–270.

Guglielmone, A.A., Robbins, R.G., Apanaskevich, D.A., Petney, T.N., Estrada-Peña, A., Horak, I.G., 2013. The Hard Ticks of the World (Acari: Ixodida: Ixodidae). Springer Dordrecht 10, 978–994.

Hadi, U.K., Soviana, S., Pratomo, I.R.C., 2016. Prevalence of tick and tick-borne diseases in Indonesian Dogs. Journal of Veterinary Science and Technology 7, 330.

Kebbi, R., Nait-Mouloud, M., Hassissen, L., Ayad, A., 2019. Seasonal activity of ticks infesting domestic dogs in Bejaia province, Northern Algeria. Onderstepoort Journal of Veterinary Research 86(1), 1–6.

Krishna Murthy, C.M., Ananda, K.J., Adeppa, J., 2016. Prevalence of ectoparasites in dogs of Shimoga, Karnataka. Journal of Parasitic Diseases 41(1), 167–170.

Lema, S.Y., Haruna, A., Ibrahim, J., Suleiman, J., 2020. The Prevalence of Ticks Infestation of Dogs in Runjin Sambo Area of Sokoto, Sokoto State, Nigeria. Asian Journal of Research Zoology 3(3), 15–20. http://geographical.openuniversityarchive.com/id/eprint/594.

Norris, D.E., Klompen, J.S.H., Keirans, J.E., Black, W.C., 1996. Population genetics of *Ixodes scapularis* (Acari: Ixodidae) based on mitochondrial 16S and 12S genes. Journal of Medical Entomology 33(1), 78–89.

Papazahariadou, M.G., Saridomichelakis, M.N., Koutinas, A.F., Papadopoulos, E.G., Leontides, L., 2003. Tick infestation of dogs in Thessaloniki, northern Greece. Medical and Veterinary Entomology 17, 110–113.

Raut, P.A., Maske, D.K., Jayraw, A.K., Sonkusale, V.G., 2006. Ectoparasitism in dogs from the eastern zone of Maharashtra state. Journal of Parasitic Diseases 30(2), 138–141.

Roopesh, M.P., 2017. Epidemiological studies on Babesiosis in dogs in Bengaluru district. M.V.Sc thesis submitted to Department of Veterinary Parasitology, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar.

Sahu, A., Mohanty, B., Panda, M.R., Sardar, K.K., Dehuri, M., 2013. Prevalence of tick infestation in dogs in and around Bhubaneswar. Veterinary World 6(12), 982.

Sen, S.K., Fletcher, T.B., 1962. Veterinary Entomology and Acarology for India. (1st edn.) ICAR, New Delhi.

Soulsby, E.J.L., 1982. Helminths, Arthropods and Protozoa of Domesticated Animals, 7th Edn., English Language Book Society, Bailliere, Tindall, London.

Tirosh-Levy, S., Gottlieb, Y., Apanaskevich, D.A., Mumcuoglu, K.Y., Steinman, A., 2018. Species distribution and seasonal dynamics of equine tick infestation in two Mediterranean climate niches in Israel. Parasites & Vectors 11, 1–10.

Totton, S.C., Wandeler, A.I., Ribble, C.S., Rosatte, R.C., McEwen, S.A., 2011. Stray dog population health in Jodhpur, India in the wake of an animal birth control (ABC) program. Preventive Veterinary Medicine 98, 215–220.

Vijayakumar, H., Latchumikanthan, A., Gurav, A., Mahapatra, R.R., Mondal, D.B., 2013. Ticks and Tick-borne Diseases in Canines-A Prevalence study. Intas Polivet 14(2), 264–266.

Walker, J.B., Keirans, J.E., Horak, I.G., 2000. The genus *Rhipicephalus* (Acari, Ixodidae): A guide to the brown ticks of the world. Cambridge University Press, 655.