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First Report of *Haemaphysalis Shimoga* from Cattle in West Bengal, India

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ABSTRACT

Background: Ticks are one of the most common ectoparasites of cattle. Ticks can cause symptoms of infestation, including tick-toxicosis and paralysis and can also transmit some tick-borne diseases. Some tick species are not commonly found in cattle, but can occasionally be transmitted to them from wild reservoirs. A study was conducted to investigate and identify the ticks infesting cattle from the adjacent villages of Gorumara National Park in West Bengal, India.

Methods: Ticks collected from cattle were studied using a stereo zoom microscope. Then, DNA was extracted from them and the mitochondrial 16S rRNA sequence of the tick was amplified using gene-specific primers. The amplified fragments were sequenced and aligned with other sequences using the NCBI BLAST. This further validated the results of morphological analysis.

Result: One of the tick species collected was first identified as *Haemaphysalis shimoga* based on its morphological features. After sequencing the amplified 16S rRNA sequence PCR product, its length was found to be 456 bp long. Phylogenetic analysis of the collected *H. shimoga* revealed 99-100% sequence similarity with the reference sequences of *H. shimoga* from Thailand and Malaysia. This is the first report on the morphological and molecular identification of *H. shimoga* in cattle from West Bengal, India.

Key words: 16s rRNA, Cattle, *Haemaphysalis shimoga,* Phylogenetic analysis.

INTRODUCTION

India has one of the largest cattle populations (192.5 million in 2019) in the world (National Dairy Development Board). With more than 19 million cattle, West Bengal ranks first among all states in India (20th Livestock Census, DADF and Government of India (https://www.nddb.coop/information/stats/pop). A substantial part of the animal husbandry economy in West Bengal is influenced by milk-producing animals, to which cattle contribute a major part. One of the major factors affecting the health of cattle is the presence of ectoparasites on their bodies. The ectoparasites of cattle include ticks, mites, fleas and lice (Mondal *et al.*, 2012). Ticks are hematophagous ectoparasites belonging to the subphylum Chelicerata and class Arachnida (Kabir *et al.*, 2011). It is a causative agent of a broad range of hemo-protozoal, rickettsial, bacterial and viral diseases in livestock, some of which are of zoonotic importance (Singh and Rath, 2013). Ticks can cause anaemia, irritation, pruritis, damage to the hide and can also cause tick toxicosis (Marcondes and Dantas-Torres, 2016). These symptoms not only cause discomfort to the animals but also lead to reduced production and reproduction efficiency. If the hide of the animal gets damaged, it results in a lower selling cost. All this may impart an additional burden on the output of local farmers. Though the common breed of goat (Black Bengal) from West Bengal has one of the best skin qualities among different breeds of goat found in India, still, two tick species have also been reported from the goat population of West Bengal (Amin and Sulabh, 2025). One of the tick species, *Haemaphysalis bispinosa,* found in the case of goats of West Bengal, also causes infestation and transmission of tick-borne pathogens in cattle (Amin and Sulabh, 2025).

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Most of the identification of Ticks in India is based on morpho-taxonomic studies. However, morphological identification is sometimes strenuous for damaged, engorged and identical tick samples or for those that are in sub-adult phases (Li *et al.*, 2018). Therefore, accurate and effective molecular identification techniques using various genetic markers for the authentic identification of tick species are indispensable. Mitochondrial 16S rRNA has been widely used to analyse the phylogeny and systematic evolution of ticks and gives precise results to identify tick species (Black and Piesman, 1994; Beati and Keirans, 2001). Genetic markers are used in domestic animals and other species and help in the accurate identification of specific gene sequences and consequent study of variation that can help in selection and identifying species (Sulabh *et al.*, 2016; Gupta *et al.*, 2016).

Gorumara National Park is located in the Dooars region of northern West Bengal, India, at the foothills of the Eastern Himalayas. This region is a submontane Terai belt characterised by rolling forests and riverine grasslands. Gorumara National Park has a tropical monsoon climate. The area remains optimally humid throughout the year, peaking during the monsoon season, which is favourable for tick population growth (Kumar and Ranjan, 2020). Ruminants from villages present around the park usually go to the outskirts for grazing and browsing.

H. shimoga has been reported in a few southern states of India and is known to infest both wild and domestic animals (Kumar *et al.*, 2018; Rajan *et al.*, 2023). However, no reports from West Bengal are available regarding the infestation of *H. shimoga* in cattle (Sanyal and De, 2001). The present study was conducted to identify the presence of *H. shimoga* in the cattle population from adjacent villages of Gorumara National Park in the Jalpaiguri district of West Bengal. To confirm the identity of the tick species, both morphological parameters and a molecular study involving mitochondrial 16S rRNA amplification and sequencing were undertaken.

MATERIALS AND METHODS

Study area and tick sampling

A survey was conducted from January 2022 to December 2022 to identify the presence of ticks in cattle in nearby villages of Gorumara National Park in the Jalpaiguri district of West Bengal, India (Fig 1). A total of 524 cattle were randomly examined for the presence of ticks. All examined cattle were maintained by the farmers in a semi-intensive system (cattle were permitted to graze during the daytime in field conditions and at night time they were kept in farms under stall feeding). The presence of ticks on the animal's coat was checked manually and if found, the ticks were carefully collected using blunt forceps, ensuring that their mouthparts were not damaged. The attachment sites of the ticks were found to be the ear and dewlap of the cattle. Most of the ticks were collected in glass vials containing 70% ethanol for morphological identification, whereas a few ticks were preserved in 95% ethanol and later stored at -20°C in the Animal Breeding and Genetics Laboratory of the Department of Animal Science, Kazi Nazrul University, Asansol, West Bengal, for molecular analysis.

Morphological identification of ticks

Collected ticks were first treated in 10% KOH and later were followed with 30%, 50%, 70%, 90% and 100% of ethyl alcohol for 20 minutes each for dehydration. The dehydrated samples were transferred to xylene and finally mounted in Canada balsam. Ticks were observed under a stereo zoom microscope (Leica M205 A, Germany) and were identified to species level based on their morphology as described by Sharif (1928), Walker (1994) and Geevarghes and Misra (2011).

Molecular diagnosis

After morphological identification, species identification was performed through PCR amplification of unique sequences of the mitochondrial DNA of the tick.

DNA extraction

Tick samples were washed in different descending grades of ethanol (70%, 50%, 30% and 10%) for 1 h for each concentration and finally in double-distilled water for 1 h. Each tick sample was then frozen and crushed using a mortar and pestle (Chitimia *et al.*, 2010). Subsequently, DNA was extracted using the GSure Insects DNA Isolation kit (G45276B) according to the manufacturer's instructions**.**

DNA amplification

A specific primer set of 16S+1 (5′-CTGCTCAATGATTTTT TAAATTGCTGTGG-3′) and 16S-1 (5′-CCG GTCTGAA CT C AGATCAAGT-3′) was used to target the mitochondrial 16S rRNA gene (Black and Piesman, 1994). Amplification of the

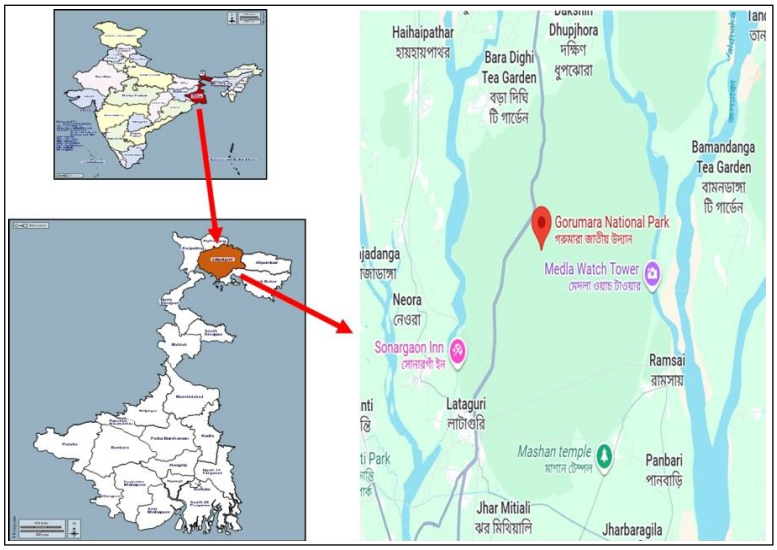


Fig 1: Study area - Nearby villages of Gorumara National Park.

16S rRNA gene was performed using polymerase chain reaction (PCR) with 2X Hi-G9 Taq PCR Master Mix (G4804) 25 µL, forward (10 pM) and reverse primer (10 pM) 1 µL each, template 25ng and nuclease-free water to make up the PCR mixture final volume of 50 µL for yielding a product of 456 bp. All PCRs were performed in a Bio-Rad T100 thermal cycler and amplified for 10 cycles under the following conditions: denaturation at 92°C for 1 min, annealing at 48°C for 1 min and extension at 72°C for 90 s, followed by 32 cycles under the following conditions: denaturation at 92°C for 40 s, annealing at 54°C for 35 s and extension at 72°C for 90 s. The amplified PCR products were separated by electrophoresis on a 2% agarose gel.

Amplified products were purified using the GSure PCR Purification Kit (G4628A) and sequenced using a 3730XL Genetic Analyzer. An accurate sequence of *H. shimoga* was obtained by removing low-quality sequences from both ends and the obtained tick sequences were submitted to the National Centre for Biotechnology Information (NCBI) BankIt portal. The obtained tick sequences were analysed using the NCBI BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Phylogenetic tree construction

The accession number of the submitted sequence of *H. shimoga* in the NCBI database is PX108288. The final sequence was used in NCBI-BLAST to identify closely related sequences of similar and other species of ticks. For phylogenetic analysis, 17 sequences (Table 1) from India and other countries were chosen based on sequence identity for comparison. The selected sequences were then downloaded from the NCBI GenBank database. Multiple sequence alignments were performed using the MUSCLE algorithm in MEGA 11 software (Tamura *et al.*, 2021). The aligned sequences were then used to construct a phylogenetic tree using the neighbor-joining method.

RESULTS AND DISCUSSION

Morphological identification of *Haemaphysalis shimoga* ticks

Male-the scutum was oval and elongated (Fig 2 and 3). Basis capituli (a) was rectangular (Fig 4). Lateral grooves (b) were shallow, whereas cervical grooves (c) were deep and short (Fig 5 and 6). Punctations (d) were small in size and numerous (Fig 7). The 1st coxal spurs (f) were long and triangular, whereas the 2nd coxal spurs (g) were larger than the 3rd coxal spurs (h) (Fig 8 and 9). Two elongated spurs (i) were observed in the coxae 4 (Fig 10). Palpi were short and wide. Palpal segment 2 with a prominent ventrolateral spur (j) that was wide, pointed and posteriorly directed (Fig 11). Palpal segment 3 (k) was dorsally twice as wide as long and its basal margin extended laterally to form a wing, overlapping the apical margin of segment 2 (Fig 12). Hypostomal dentition (l) was 5/5 (Fig 13).

The ticks were morphologically identified as *H. shimoga* males. *H. shimoga* female ticks were not found during the survey; therefore, morphological identification was not possible in the case of *H. shimoga* females.

Phylogenetic analysis of *Haemaphysalis shimoga*

The sequence of the 16s rRNA gene of *H. shimoga* in our study showed 99-100% sequence similarity with the reference sequences of *H. shimoga* from Thailand (KC170730 and MZ330747) and Malaysia (LC602444)

Table 1: Tick sequences used for phylogenetic analysis in this study.

Tick species/strain	Source of tick specimen	Genbank accession number 16S rRNA gene
*Haemaphysalis shimoga*x isolate S3	West Bengal, India	PX108288
Haemaphysalis shimoga	Thailand	KC170730
Haemaphysalis shimoga	Kerala, India	MH044717
Haemaphysalis shimoga	Thailand	MZ330747
Haemaphysalis shimoga	Malaysia	LC602444
Haemaphysalis cornigera	China	OQ725578
Haemaphysalis cornigera	China	PQ681359
Haemaphysalis sp.	Thailand	PP831996
Haemaphysalis montgomeryi	Pakistan	PV687431
Haemaphysalis longicornis	China	PP486230
Haemaphysalis hystricis	Thailand	KC170733
Haemaphysalis punctata	China	MZ617267
Haemaphysalis lagrangei	Thailand	KC170731
Rhipicephalus microplus	China	PQ681640
Haemaphysalis doenitzi	China	JF979402
Haemaphysalis asiatica	Thailand	KC170734
Haemaphysalis humerosa	Australia	OM830395
Haemaphysalis campanulata	China	PV558769

xThe GenBank accession number for the 16S rRNA gene (PX108288) was submitted by the authors of the current study.

(Table 1) (Fig 14). *H. shimoga* collected from cattle showed 98.45% sequence similarity with the reference sequences of *H. shimoga* from Kerala, India (MH044717) (Table 1) (Fig 14). *H. shimoga* of the present study showed 94.79% (PQ681359), 92.79% (PV687431), 92.71% (OM830395), 92.59% (PP486230) and 92.58% (KC170733) sequence similarity to *Haemaphysalis cornigera, Haemaphysalis montgomeryi*, *Haemaphysalis humerosa, Haemaphysalis longicornis* and *Haemaphysalis hystricis* respectively (Table 1) (Fig 14).

In the present study, *H. shimoga* was found in cattle from the surrounding villages of Gorumara National Park in the Jalpaiguri district of West Bengal. To date, no report is available on the infestation of *H. shimoga* in domestic animals of West Bengal. However, similar findings were observed by Li *et al.* (2018), who identified *H. shimoga* and *H. kitaokai* using 16s rRNA from the China-Myanmar border. *H. shimoga*, *Haemaphysalis spinigera*, *Haemaphysalis bispinosa* and *Haemaphysalis indica,* using morpho-taxonomic keys, were identified in wild mammals from Kerala, India (Kumar *et al.*, 2018). In another study, *H. bispinosa*, *H. intermedia*, *H. spinigera*, *H. turturis* and *H. shimoga* were recorded in domestic animals from Kerala, India (Rajan *et al.*, 2023). In Vietnam, *H. cornigera*, *Rhipicephalus linnaei*, *Rhipicephalus haemaphysaloides* and *Amblyomma integrum* have been reported in cattle



Fig 2: *H. shimoga* Male (Dorsal view).



Fig 3: *H. shimoga* Male (Ventral view).



Fig 4: Rectangular basis capituli (a).



Fig 5: Shallow lateral groove (b).



Fig 6: Short cervical groove (c).



Fig 7: Small sized punctations (d).

using COX1 and 16s rRNA as genetic markers (Hornok *et al.*, 2024). The mitochondrial 16S rRNA sequence is conserved and is useful in the identification of tick species. The 16S rRNA gene offers advantages over the COI gene primarily in higher sequencing quality and more reliable PCR amplification success, especially with degraded tick samples or challenging tick species (Lv *et al.*, 2014); (Wells *et al.,* 2012) recorded the infestation of *H. cornigera*, *H. bispinosa*, *Haemaphysalis koenigsbergi* and *Haemaphysalis semermis* in domestic dogs from forest areas of Northern Borneo. They also suggested that the infestation of dogs with *Haemaphysalis* ticks indicates a crucial connection for pathogen transmission between dogs and forest wildlife. Sambar Deer is well well-known host of *H. shimoga*

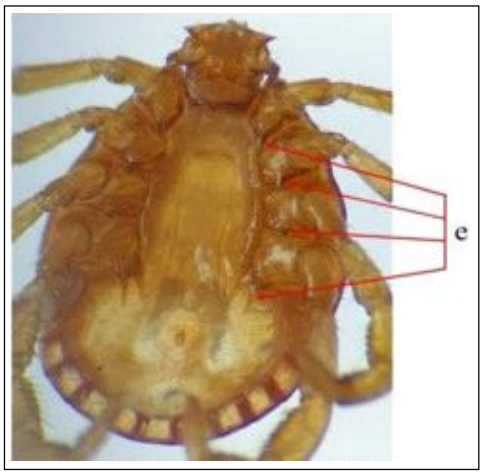


Fig 8: Coxal spurs (e).

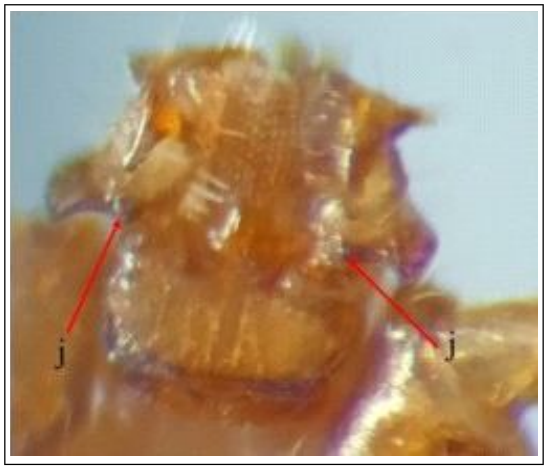


Fig 11: Spurs (j) on palpal segment 2.

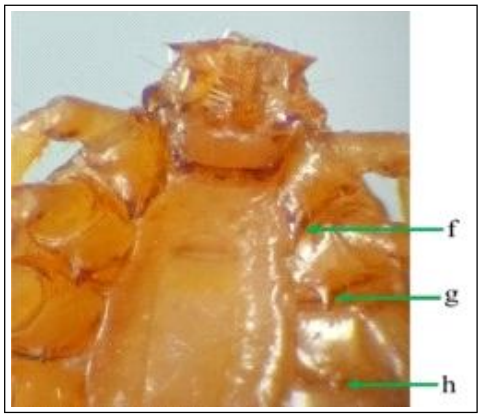


Fig 9: 1st (f), 2nd (g) and 3rd (h) Coxal spurs.

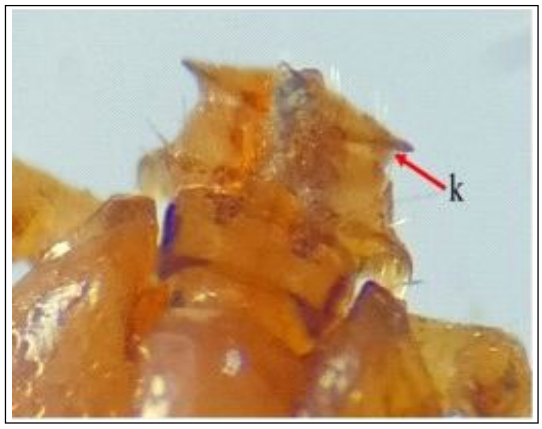


Fig 12: Palpal segment 3 (k).



Fig 10: 4th Coxal spurs (i).

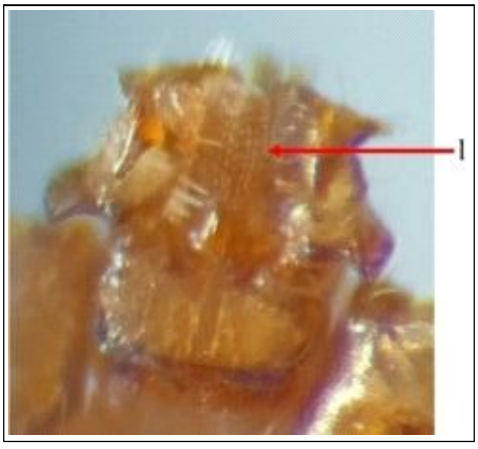
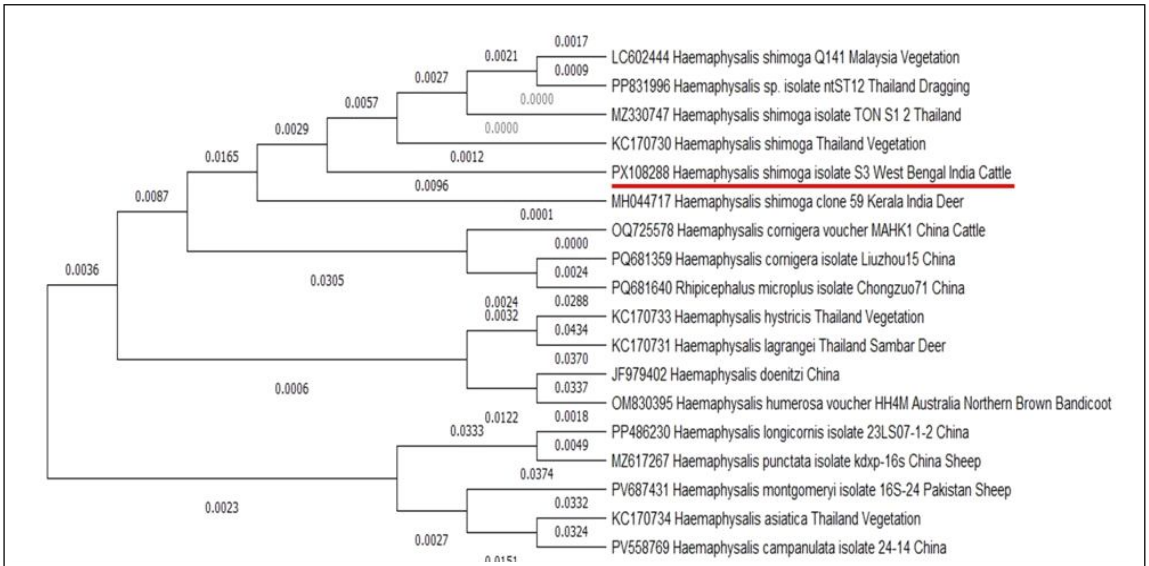


Fig 13: Hypostomal dentition (l).



The evolutionary distances were computed using the Maximum Composite Likelihood method and are expressed in the units of the number of base substitutions per site (Tamura *et al.*, 2004). This analysis involved 18 nucleotide sequences. All ambiguous positions were removed from each sequence pair (pairwise deletion option). The final dataset comprised 471 positions. Evolutionary analyses were conducted in MEGA11 (Tamura *et al.*, 2021).

Fig 14: The evolutionary history of *H. shimoga* was inferred using the neighbour-joining method (Saitou and Nei, 1987).

tick. Infestation of cattle with *H. shimoga* ticks identified an important link between cattle and forest wildlife for potential pathogen transmission to cattle.

The sequence of the 16s rRNA gene of *H. shimoga* in the present study showed 99-100% sequence similarity with *H. shimoga* from Thailand and Malaysia (Fig 14). *H. shimoga* in our study revealed 98.45% sequence similarity with the reference sequences of *H. shimoga* from Kerala, India (MH044717) (Fig 14). *H. shimoga* collected from cattle showed 94.79% (PQ681359), 92.79% (PV687431), 92.71% (OM830395), 92.59% (PP486230) and 92.58% (KC170733) sequence similarity to *Haemaphysalis cornigera, Haemaphysalis montgomeryi*, *Haemaphysalis humerosa, Haemaphysalis longicornis* and *Haemaphysalis hystricis,* respectively (Fig 14). Some of the literature suggests the genetic manipulation of ticks' genomes and formation of transgenic ticks for different studies and specific purposes (Sulabh and Kumar, 2018; Nuss *et al.*, 2021).

H. shimoga houses a wide range of pathogens like *Rickettsia* sp., *Coxeilla* sp., Coxiella-like endosymbiont, Marsupial-associated *Babesia* sp., *Ehrlichia* sp., *Mycobacterium* sp., *Candidatus* Rhabdochlamydia, *Stenotrophomonas* sp. and *Anaplasma* sp. in its body. *Rickettsia* sp. has been confirmed in *H. shimoga* and *H. lagrangei and* a novel Coxeilla-like agent or *Coxeilla* sp. was also detected in *H. shimoga* in the same study from Thailand (Ahantarig *et al.*, 2011). The presence of Coxiella-like endosymbionts has been reported in *Haemaphysalis hystricis*, *H. lagrangei, Haemaphysalis obesa and H. shimoga* ticks from Thailand (Arthan *et al.*, 2015). *H. shimoga* has been reported in the vegetation of Thailand and *Anaplasma* was also detected in it (Malaisri *et al.*, 2015). Marsupial-associated *Babesia* sp. has been detected in male *H. shimoga* from Malaysia (Lau *et al.*, 2022). *Coxiella, Rickettsia, Ehrlichia, Mycobacterium, Candidatus* Rhabdochlamydia and *Stenotrophomonas* have been detected in *H. shimoga* from Malaysia (Lau *et al.*, 2023). The presence of *Coxiella*-like endosymbionts has also been confirmed in *H. hystricis*, *H. semermis*, *H. shimoga* and *A. testudinarium* collected from vegetation in Thailand using16 S rRNA, *groE, L* and *rpoB* genes (Nooma *et al.*, 2025).

An infestation of *H. shimoga* in domestic animals is rare and usually of wildlife origin; it will be very difficult for the people involved in animal health control to precisely predict the pathogens that may be transmitted from the wild animal population to domestic animals like cattle. This may lead to uncertainty in the preparation for the prevention of the spread of an unwanted or novel disease that may be transmitted from this species, as it may carry a wide variety of pathogens.

CONCLUSION

This is the first report of the molecular identification of *H. shimoga* in cattle in West Bengal, India. Ticks collected from cattle in this area were identified based on morphological keys and 16S rRNA sequences. *H. shimoga* is an important tick species that harbours a range of pathogens. In the case of an increase in the infestation of domestic animals in the surrounding area by *H. shimoga,* there are chances of the introduction of new pathogens in the local population of farm animals. The area remains adequately humid throughout the year, peaking during the monsoon season, which is propitious for the growth of the

tick population and thus heightens the chances of transmission of tick-borne diseases in domestic animals. Proper management and a diligent treatment strategy can prevent the occurrence of tick-borne diseases in cattle.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

No experiments were carried out on the animals.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

REFERENCES

Ahantarig, A., Malaisri, P., Hirunkanokpun, S. Chalao Sumrandee, C., Trinachartvanit, W. (2011). Detection of rickettsia and a novel *Haemaphysalis shimoga* Symbiont bacterium in ticks in Thailand. *Current Microbiology*. **62:** 1496-1502. doi: 10.1007/s00284-011-9887-3.

Amin, M.R., Sulabh, S. (2025). Identification of prevalent tick species in the goat population of West Bengal state of India. *Small Ruminant Research.* pp. 107526. https://doi.org/10.1016j.smallrumres.2025.107526.

Arthan, W., Sumrandee, C., Hirunkanokpun, S., Kitthawee, S., Baimai, V., Trinachartvanit, W., Ahantarig, A. (2015). Detection of Coxiella-like endosymbiont in *Haemaphysalis* tick in Thailand. *Ticks and Tick-borne Diseases.* **6(1):** 63-68. doi: 10.1016/j.ttbdis.2014.09.005.

Black, W.C. and Piesman, J. (1994). Evolution Phylogeny of hard- and soft-tick taxa (Acari: Ixodida) based on mitochondrial 16S rDNA sequences. *Proceedings of the National Academy of Sciences of the United States of America.* **91:** 10034-10038.

Beati, L. and Keirans, J.E. (2001). Analysis of the systematic relationships among ticks of the genera Rhipicephalus and Boophilus (Acari: Ixodidae) based on mitochondrial 12S ribosomal DNA gene sequences and morphological characters. *The Journal of Parasitology.* **87:** 32-48.

Chitimia, L., Lin, R., Cosoroaba, I., Wu, X., Song, H., Yuan, Z., Zhu, X. (2010). Genetic characterization of ticks from southwestern Romania by sequences of mitochondrial cox1 and nad5 genes. *Experimental and Applied Acarology.* **52:** 305-311.

Geevarghese, G. and Mishra, A.C. (2011). *Haemaphysalis* ticks of India. First Ed. Elsevier, London. pp. 104-105.

Gupta, J.P., Bhushan, B., Panigrahi, M., Ranjan, S., Asaf, V.N., Kumar, A., Sulabh, S., Kumar, A., Kumar, P., Sharma, D. (2016). Study on genetic variation of short tandem repeats (STR) markers and their association with somatic cell scores (SCS) in crossbred cows. *Indian Journal of Animal Research.* **50(4):** 450-454. doi: 10.18805/ijar.6709.

Hornok, S., Farkas, R., Duong, N.N., Kontschan, J., Takacs, N., Keve, G., Pham, D.N., Dao, T.T.H. (2024). A morpho phylogenetic update on ixodid ticks infesting cattle and buffalos in Vietnam, with three new species to the fauna and a checklist of all species indigenous to the country. *Parasite and Vectors.* **17:** 319. doi: 10.1186/s13071-024-06384-5.

Kabir, M.H.B., Mondal, M.M.H., Eliyas, M., Mannan, M.A., Hashem, M.A., Debnath, N.C., Miazi, O.F., Mohiuddin, C., Kashem, M.A., Islam, M.R., Elahi, M.F. (2011). An epidemiological survey on investigation of tick infestation in cattle at Chittagong District, Bangladesh. *African Journal of Microbiology Research.* **5(4):** 346-352.

Kumar, K.G.A., Ravindran, R., Johns, J., Rajagopal, K., Chandrasekhar, L., George, A.J., Ghosh, S. (2018). Ixodid tick vectors of wild mammals and reptiles of Southern India. *Journal of Arthropod-Borne Diseases.* **12(3):** 276-285.

Kumar, A. and Ranjan, V. (2020). Life Forms and Biological Spectrum of the Flora of Gorumara National Park, West Bengal, India. *International Journal of Advanced Research in Biological Sciences.* **7(11):** 131-134.

Lau, A.C.C., Qiu, Y., Moustafa, M.A.M., Nakao, R., Shimozuru, M., Onuma, M., Nakajima, N., Sashika, M., Mohd-Azlan, J., Tsubota, T. (2022). Detection of a *Babesia* sp. genotype closely related to marsupial-associated *Babesia* spp. in male *Haemaphysalis shimoga* from Sarawak, Malaysian Borneo. *Journal of Veterinary Medical Science.* **84(11):** 1469-1473. doi: 10.1292/jvms.22-0304.

Lau, A.C.C., Mohamed, W.M.A., Nakao, R., Onuma, M., Qiu, Y., Nakajima, N., Shimozuru, M., Mohd-Azlan, J., Moustafa, M.A.M., Tsubota, T. (2023). The dynamics of the microbiome in Ixodidae are shaped by tick ontogeny and pathogens in Sarawak, Malaysian Borneo. *Microbial Genomics.* **9:** 000954. doi: 10.1099/mgen.0.000954.

Li, L., Zhang, Y., Wang, J., Li, X., Yin, S., Zhu, D., Xue, J., Li, S. (2018). High genetic diversity in hard ticks from a China-Myanmar border county. *Parasite and Vectors.* **11:** 469. doi: 10.1186/s13071-018-3048-5.

Lv, J., Wu, S., Zhang, Y., Chen, Y., Feng, C., Yuan, X., Jia, G., Deng, J., Wang, C., Wang, Q., Mei, L., Lin, X. (2014). Assessment of four DNA fragments (COI, 16S rDNA, ITS2, 12S rDNA) for species identification of the Ixodida (Acari: Ixodida). *Parasites Vectors.* **7:** 93. doi: 10.1186/1756-3305-7-93.

Malaisri, P., Hirunkanokpun, S., Baimai, V., Trinachartvanit, W., Ahantarig, A. (2015). Detection of *Rickettsia* and *Anaplasma* from hard ticks in Thailand. *Journal of Vector Ecology.* **40(2):** 262-268.

Marcondes, C.B., Dantas-Torres, F. (2016). Diseases caused by Acari (ticks and mites). In Arthropod Borne Diseases (pp. 537-548). Cham: *Springer International Publishing.* https://link.springer.com/chapter/10.1007/978-3-319-13884-8-34.

Mondal, D.B., Sarma, K.M. and Saravanan, M. (2012). Upcoming of the integrated tick control program of ruminants with special emphasis on livestock farming system in India. *Ticks and Tick Borne Diseases.* **1:** 1-10.

Nooma, W., Kaenkan, W., Trinachartvanit, W., Baimai, V., Ahantarig, A. (2025). Molecular prevalence of *Coxiella* like endosymbionts and the first record of *Coxiella burnetii* in hard ticks from Southern Thailand. *Scientific Reports.* **15:** 10129.

Nuss, A., Sharma, A., Gulia-Nuss, M. (2021). Genetic manipulation of ticks: A paradigm shift in tick and tick-borne diseases research. *Frontiers in cellular and infection microbiology.* **11:** 678037. https://doi.org/10.3389/fcimb.2021.678037.

Rajan, A., Koyyan, A., Reghu, R.K. (2023). First report on diversity and relative abundance of questing and feeding hard ticks (Acari: Ixodidae) of the tribal communities and wildlife in Aralam Wildlife Sanctuary of Western Ghats, Kerala, India. *International Journal of Acarology.* **49(4-6):** 319-324.

Saitou, N. and Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution.* **4:** 406-425.

Sanyal, A.K. and De, S.K. (2001). Diversity in Ticks (Acari) of West Bengal. *Records of the Zoological Survey of India.* **99(1-4):** 65-74. doi: 10.26515/rzsi/v99/i1-4/2001/59633.

Sharif, M. (1928). A revision of the Indian Ixodidae with special reference to the collections in the Indian Museum. *Records of the Indian Museum.* **30:** 237-284.

Singh, N.K. and Rath, S.S. (2013). Epidemiology of ixodid ticks in cattle population of various agro climatic zones of Punjab, India. *Asian Pacific Journal of Tropical Medicine.* **1:** 947-951.

Sulabh, S., Kumar, A. (2018). Application of transgenic animals in animal production and health. *Agricultural Reviews.* **39(2):** 169-174. doi: 10.18805/ag.R-1782.

Sulabh, S., Verma, A., Gupta, I.D., Kumar, S.R. (2016). Detection of polymorphism of Calgranulin A gene in Indian Murrah buffalo. *Indian Journal of Animal Research.* **50(5):** 658-661. doi: 10.18805/ijar.5715.

Tamura, K., Nei, M., Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences (USA).* **101:** 11030-11035.

Tamura, K., Stecher, G. and Kumar, S. (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution.* **25;38(7):** 3022-3027. doi: 10.1093/molbev/msab120.

Walker, A. (1994). The arthropods of human and domestic animals. First ed. Chapman and Hall, Madras. pp. 41-46.

Wells, K., Beaucournu, J., Durden, L.A., Petney, T.N., Lakim, M.B., O'Hara, R.B. (2012). Ectoparasite infestation patterns of domestic dogs in suburban and rural areas in Borneo. *Parasitology Research.* **111:** 909-919. doi: 10.1007/s00436-012-2917-7.