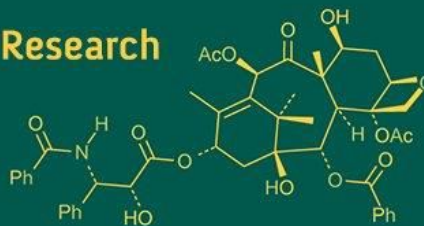
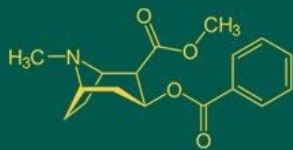


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2025; SP-9(5): 366-371
www.biochemjournal.com
Received: 21-02-2025
Accepted: 26-03-2025

Kolangath SM
Wildlife Research & Training
Centre, Maharashtra Animal &
Fishery Sciences University,
Nagpur, Maharashtra, India

SV Upadhye
Wildlife Research & Training
Centre, Maharashtra Animal &
Fishery Sciences University,
Nagpur, Maharashtra, India

MD Pawshe
Wildlife Research & Training
Centre, Maharashtra Animal &
Fishery Sciences University,
Nagpur, Maharashtra, India

AP Gawande
Wildlife Research & Training
Centre, Maharashtra Animal &
Fishery Sciences University,
Nagpur, Maharashtra, India

VM Dhoot
Wildlife Research & Training
Centre, Maharashtra Animal &
Fishery Sciences University,
Nagpur, Maharashtra, India

Shalini AS
Wildlife Research & Training
Centre, Maharashtra Animal &
Fishery Sciences University,
Nagpur, Maharashtra, India

Priyanka Mulmule
Wildlife Research & Training
Centre, Maharashtra Animal &
Fishery Sciences University,
Nagpur, Maharashtra, India

Shruti Patwa
Wildlife Research & Training
Centre, Maharashtra Animal &
Fishery Sciences University,
Nagpur, Maharashtra, India

Kolangath RM
Department of Biotechnology &
Biochemistry, Saint Francis
DeSales College, Seminary Hills,
Nagpur, Maharashtra, India

Corresponding Author:
SV Upadhye
Wildlife Research & Training
Centre, Maharashtra Animal &
Fishery Sciences University,
Nagpur, Maharashtra, India

Molecular evidence of *Anaplasma bovis* in spotted deer (*Axis axis*): A case report

Kolangath SM, SV Upadhye, MD Pawshe, AP Gawande, VM Dhoot, Shalini AS, Priyanka Mulmule, Shruti Patwa and Kolangath RM

DOI: <https://www.doi.org/10.33545/26174693.2025.v9.i5Se.4441>

Abstract

Anaplasmosis is a tick-borne disease caused by bacteria of the genus *Anaplasma* (Theiler, 1910). The disease is characterized by fever, thrombocytopenia, anemia, and a reduction in total erythrocyte count (TEC). In wildlife, anaplasmosis often goes undiagnosed due to the challenges associated with sampling free-ranging animals. Ungulates are particularly susceptible to infection due to high tick infestations and the spatial overlap of their habitats with domestic animals, which serve as reservoirs for the pathogen. This case report describes the clinical presentation and molecular diagnosis of anaplasmosis in a free-ranging spotted deer (*Axis axis*). The animal was found recumbent in the wild and subsequently rescued. During the tranquilization attempt, the deer succumbed. Hematological analysis revealed neutrophilia, severe anemia (low hemoglobin concentration), reduced TEC, and thrombocytopenia. Molecular screening for haemoparasites confirmed the presence of *Anaplasma bovis*, which was further validated through sequencing. The obtained sequence was submitted to the NCBI GenBank database, and an accession number was assigned. Phylogenetic analysis demonstrated a close genetic relationship with *A. bovis* strains previously reported in deer from Japan. This report underscores the need for enhanced surveillance and molecular diagnostics for tick-borne pathogens in wildlife populations.

Keywords: Anaplasmosis, spotted deer, *Axis axis*, *Anaplasma bovis*, wildlife

Introduction

Anaplasmosis is an important tick-borne disease caused by bacteria from the genus *Anaplasma*, affecting a wide range of domestic and wild animals, and occasionally humans [1]. The disease is caused primarily by species like *Anaplasma marginale*, *Anaplasma phagocytophilum*, and *Anaplasma bovis*. The transmission is majorly through the tick bite and involves *Ixodes* ticks [2]. Clinical manifestations of anaplasmosis vary depending on the specific *Anaplasma* species and the host involved. The disease in ruminants is sometimes acute and characterised by severe anaemia, hemoglobinuria, and icterus due to hemolysis [2]. In humans, *A. phagocytophilum* is reported to cause Human Granulocytic Anaplasmosis (HGA) [3]. *Anaplasma bovis* has been extensively documented in Asia, the Middle East, and Africa. Japan and South Korea have reported the disease from several species. It has been reported in a variety of domestic animals including dogs [4], cattle [5], Horses [6], Sheep [7]. While in the wild animals, it has been documented in a few species like *Apodemus agrarius*, *Bandicota indica*, *Crocidura suaveolens*, *Mus caroli*, *Mus formosanus*, *Rattus losea*, and *Suncus murinus* [8], Cottontail Rabbits (*Sylvilagus* spp) [9], Wild Deer (*Cervus nippon*) [10], Raccoons (*Procyon lotor*) [11], Hokkaido Brown Bears (*Ursus arctos yesoensis*) [12], Wildcats (*Felis silvestris*) [13]. In the free-range wild population, especially cervids, anaplasmosis can compromise the overall fitness of the population by affecting reproductive success, increasing susceptibility to predation and predisposal to opportunistic infection. Both *A. phagocytophilum* and *A. bovis* have caused significant deer population declines in endemic areas across the globe [14, 15]. India has several deer species that populate the forests of the country. In recent years, studies from India have targeted anaplasmosis in wild ungulates with feeble success [16]. Recently, the north-eastern hill region of the country reported *A. marginale* in Mithun (*Bos frontalis*) [17]. Apart from these reports, there are no investigations on anaplasmosis even in the most abundant ungulates of the country, the

spotted deers (*Axis axis*).

The infection can impact herd-dwelling species of cervids and can exhibit seasonal surges as the tick population spikes. In the free range, infection is seldom detected and treated as the animals grow weak and are either predated or succumb to the infection. Treatment typically includes antibiotics like tetracyclines to contain the bacteria along with supportive therapy including antistressors, haematinics, liver tonics, minerals and vitamins [2]. Though a few countries like Japan and China have documented the infection extensively, only a few reports of wildlife have emerged from India. Need for continuous monitoring of wildlife population for the disease in many protected areas of the country will help in the generation of the baseline data on the disease. Newer species of the pathogen and host could be identified by intensifying efforts in surveillance. Continued research is essential to develop effective control measures and mitigate the disease's impact on wildlife. The current case report deals with the clinical presentation and diagnosis of anaplasmosis in a wild spotted deer in free range.

Case report

An adult spotted deer (*Axis axis*), weighing 45 Kg, female was found to be recumbent in the Gorewada Reserve Forest hence a rescue was initiated at the request of the Divisional Manager, Gorewada Project, Nagpur. The animal was tranquilised using Xylazine-Ketamine anaesthesia due to its timid nature and struggle at the sight of the rescue team. The animal was managed using the Airway-Breathing-Circulation principle [18]. The animal showed signs of respiratory distress and hyperthermia which was managed rationally using injection Analgin® and cold water spray. The animal showed an uneven pattern of respiration and finally collapsed after 45 minutes of darting. Blood and serum were collected during the management of the anaesthesia and peripheral blood smears were made. The Leishman stain was used to stain the slides. The Complete Blood Check revealed a haemoglobin concentration of 3.2 gm/dl, neutrophilia and a fall in Total Erythrocyte Count (TEC). The deer showed pale mucous membranes and severe tick infestation (Table 1) [19, 20, 21].

To molecularly characterize the infection, DNA was isolated from the blood following the manufacturer's protocol using the QIAamp® Fast DNA Blood and Tissue Kit (Qiagen Inc., MD, USA). Polymerase chain reaction (PCR) for the common prevalent haemoprotozoans was undertaken as in Table 2. An amplification of approximately 680 bp was obtained after agarose gel electrophoresis (Fig 1.), which was purified using PCR purification Kit (Mfg. Qiagen Inc., MD, USA). The amplicon was sequenced using Genetic Analyser 3500 (Applied Biosystems, CA, USA) using the forward and reverse primers to ensure reliability. The blast analysis revealed 100% similarity with *Anaplasma bovis* reported from LC066136 (Bangladesh), MH255935 & HQ913644 (China) majorly from goat and cattle. The sequence was submitted to the NCBI GenBank database and was assigned the accession number PQ192496. For phylogenetic analysis, sequences of *A. bovis* reported from domestic and wild animals in India and other countries were included in the study (Table 3). The analysis was conducted using MEGA XI software, employing the Maximum Likelihood (ML) method with bootstrap values calculated from 1000 replications to ensure the reliability of the

phylogenetic tree [22] (Fig. 2). The phylogenetic tree showed three major clades each maintaining intra-clade specificity. The query sequence was found in clade I alongside other sequences of *A. bovis* from India, China, Zambia and Kyrgyzstan. The deer isolates from Japan (EU697013) were closely identical to the query sequence. The query sequence was placed alongside other sequences of *A. bovis* from India, indicating the possibility of the common host range.

Discussion

This is the first record of *A. bovis* in a spotted deer (*Axis axis*) from India and with molecular analysis; additional epidemiological data has been generated. *Anaplasma bovis* is a tick-borne rickettsial pathogen primarily infecting domestic and wild ruminants. The pathogen has been reported in various domestic and wild ruminants, predominantly in tropical and subtropical regions, including India, Bangladesh, China, Japan, and Africa [23]. While *A. bovis* primarily infects monocytes, leading to anaplasmosis in cattle and small ruminants, its role in wildlife is still under investigation. The detection of *A. bovis* in a spotted deer suggests potential spillover from domestic livestock to wild ungulates due to shared habitat and vector populations. Studies have indicated that asymptomatic carriers in wildlife may serve as reservoirs for anaplasmosis [24].

In the current case, the deer was found recumbent due to the low haemoglobin concentration and resulting weakness. The complete blood check indicated a low haemoglobin concentration, thrombocytopenia, acute neutrophilia and low Total Erythrocyte Count (TEC). The findings are in complete agreement with the findings of other ruminants like cattle and goats [25]. Xylazine-ketamine anaesthesia has innate complications in cervids, xylazine is a respiratory depressant and can cause hypotension and bradycardia [26]. In animals that have a compromised total erythrocyte count and haemoglobin concentration, the outcomes of the anaesthesia can be unpredictable. During emergency field rescue operations, such complications are encountered and require intense emergency management to save the animal. The clinical presentation characterised by respiratory distress, hyperthermia and eventual mortality highlights the pathogenic potential. Though an isolated case, such infection can compromise overall fitness by inducing severe anaemia and increasing vulnerability to environmental stressors and predation. In endemic areas, domestic animals that graze in the protected areas may serve as a potential reservoir of infection to susceptible wild species. The phylogenetic analysis revealed three major clades each maintaining intra-clade specificity. The sequences in clade I were majorly from cattle reported from India and deer sequences from Japan closely aligned with the query sequence. The phylogenetic analysis indicated the possibility of shared host range and of wild-domestic transmission cycle.

Although *A. bovis* is limited to ruminants and there is no evidence of spillover to humans, the disease can have a great impact on deer populations in the free range. Tick-borne pathogens pose a significant threat to wildlife; particularly in protected areas with significant wildlife domestic animal interaction. The spotted deer are an important prey species supporting a population of leopards and tigers in the landscape. The possibility of transmission of *A. bovis* to large cats is poorly documented, but there is evidence of the infection in cats [27]. The potential

consequences for apex predators, particularly those dependent on cervid prey populations, highlight the need for further research into the pathogen's impact across trophic levels. Thus, extensive surveillance of the wild population will provide clarity on whether the infection can impact the deer population and dependent predator species. The current

findings highlight the need for continued surveillance and research to elucidate the transmission dynamics, genetic diversity, and ecological impact of *A. bovis*. Integrating such knowledge into wildlife conservation and management plans is essential to mitigate the effects of such diseases on biodiversity and ecosystem health.

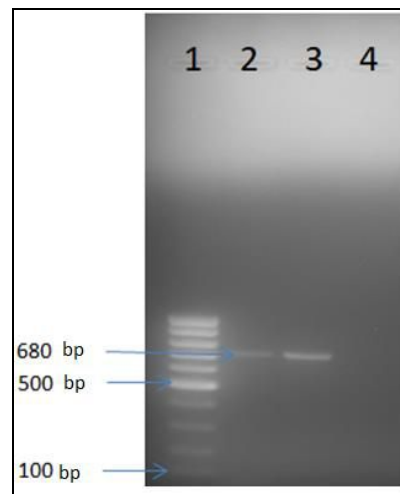


Fig 1: 1% Agarose gel electrophoresis Lane 1: 100 bp Ladder, Lane 2: Sample, Lane 3: Positive Control, Lane 4 Negative Control.

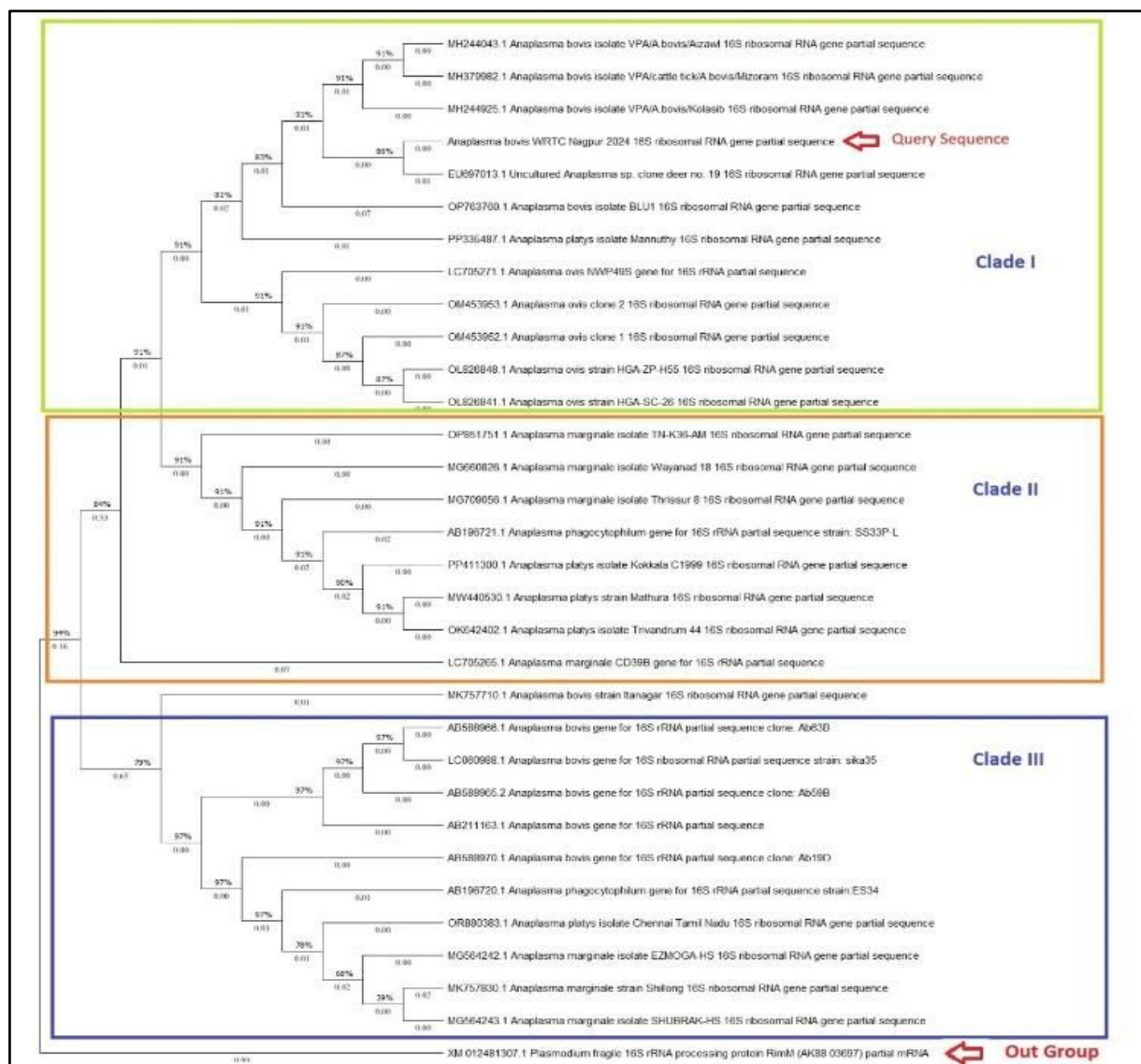


Fig 2: Phylogenetic Analysis by Maximum Likelihood (ML) method with bootstrap values estimated from 1,000 replications to enhance the reliability of the analysis.

Table 1: Haematological parameters of the deer sample conducted on HM5 (Mfg. Abaxis) equipment ^[28]

Sr. No.	Parameter	Value	Reference Value
1	Lymphocytes (%)	1.8	50-70
2	Monocytes (%)	0.6	2-10
3	Neutrophils (%)	97.6	30-48
4	Eosinophils (%)	00	1-8
5	Basophils (%)	00	0-2
6	WBC (10 ³ /μl)	7.41	4-13
7	RBC (Mil/μl)	3.65	8-18
8	Platelets (lakhs/cmm)	0.47	2-9
9	Haemoglobin (g/dL)	3.4	8-12
10	Mean Corpuscular Volume (MCV) (fL)	34	16-25
11	Packed Cell Volume (PCV) (%)	28	22-38
12	Mean Concentration Haemoglobin (MCH) (pg)	9.4	5.2-8.0
13	MCHC (g/dl)	27.9	30-36
14	RDWc (%)	27.7	14-19
15	RDWs (fl)	32.8	20-30

Table 2: Primers used under the study for the detection of haemoprotozoan diseases.

Sr. No.	Primer	Target Disease	Primer Sequence	Amplification (bp)	Target Gene	Reference
1	BTH-1F	Babesiosis, Theileriosis and Hepatozoonosis	5'-CCTGMGARACGGCTACCACATCT-3'	730	18S rRNA	[19]
2	BTH-1R		5'-TTGCGACCATACTCCCCCA-3'			
3	Hep-F	Hepatozoonosis	5'-ATACATGAGCAAAATCTCAAC-3'	666	18S rRNA	[20]
4	Hep-R		5'-CTTATTATTCCATGCTGCAG-3'			
5	PlatysF	Anaplasma Spp.	5'-GATTTTGTGTCGTAGCTTGCTATG-3'	679	16S rRNA	[21]
6	EHR16SR		5'-TAGCACTCATCGTTTACAGC-3'			

Table 3: List of sequences utilised for the Phylogenetic analysis with their attributes.

Sr. No.	Accession No.	Host	Country	Reference
1	MH244043	Cattle	India	Unpublished
2	MH379982	Tick	India	Unpublished
3	MH244925	Cattle	India	[29]
4	PQ192496	Spotted Deer	India	Query Sequence
5	EU697013	Deer	Japan	[30]
6	OP763760	Cattle	India	Unpublished
7	PP335487	Dog	India	Unpublished
8	LC705271	Sable antelope	Zambia	Unpublished
9	OM453953	Sheep	Kyrgyzstan	[31]
10	OM453952	Sheep	Kyrgyzstan	[31]
11	OL826848	<i>Ornithodoros lahorensis</i>	China	Unpublished
12	OL826841	<i>Ornithodoros lahorensis</i>	China	Unpublished
13	OP851751	bovine	India	Unpublished
14	MG660826	Cattle	India	[32]
15	MG709056	Cattle	India	[32]
16	AB196721	Deer	Japan	[33]
17	PP411300	Dog	India	[34]
18	MW440530	Dog	India	[35]
19	OK642402	Dog	India	Unpublished
20	LC705265	Cattle	Zambia	[36]
21	MK757710	<i>Haemaphysalis bispinosa</i>	India	Unpublished
22	AB588966	<i>Sus scrofa</i>	Japan	[37]
23	LC060988	<i>Cervus nippon</i>	Japan	Unpublished
24	AB588965	<i>Sus scrofa</i>	Japan	[37]
25	AB211163	Deer	Japan	[38]
26	AB588970	<i>Cervus nippon</i>	Japan	[37]
27	AB196720	Deer	Japan	[38]
28	OR880383	<i>Canis lupus familiaris</i>	India	Unpublished
29	MG564242	<i>Rhipicephalus annulatus</i>	Egypt	Unpublished
30	MK757830	<i>Rhipicephalus annulatus</i>	Egypt	Unpublished
31	MG564243	<i>Rhipicephalus annulatus</i>	Egypt	Unpublished
32	XM012481307	<i>Plasmodium fragile</i>		Unpublished

Abbreviations

TEC: Total Erythrocyte Count

NCBI: National Center for Biotechnology Information

HGA: Human Granulocytic Anaplasmosis

DNA: Deoxyribonucleic Acid

PCR: Polymerase Chain Reaction

ML: Maximum Likelihood

Declarations

Ethics approval and consent to participate: This study is a clinical case, where the sample was collected for the diagnosis and treatment of an animal presented at the Wildlife Research & Training Centre, Gorewada, Nagpur. Therefore, ethical committee approval was not required. However, in accordance with the Wildlife Protection Act, 1972, permission for the study and publication of scientific findings was obtained from the Principal Chief Conservator of Forests (Wildlife), Maharashtra State. All sample collection procedures were carried out in compliance with the ARRIVE guidelines.

Consent for publication: Not Applicable.

Availability of Data & Materials: The sequence identified in the study is available in the public domain database of NCBI under Accession No. PQ192496 <https://www.ncbi.nlm.nih.gov/nuccore/PQ192496>

Competing interests: The authors declare that they have no competing interests.

Funding: The authors self-funded the study. The Corresponding author is not in receipt of any funding from research agencies in India or abroad.

Authors' Contributions

KSM major contributor and involved in all phases of research, including writing the manuscript, performing the PCR and sequencing. SVU, VMD, APG SAS and MDP collected samples, undertook haematology and clinically managed the case. KRM performed phylogenetic analysis and PM and SP assisted in manuscript preparation and proof reading. All authors have read and approved the final manuscript.

Acknowledgment

The authors express their gratitude to the Principal Chief Conservator of Forests (Wildlife) & Chief Wildlife Warden, Maharashtra State, for granting approval to conduct this research. They also acknowledge the support of the Managing Director, Forest Development Corporation of Maharashtra Ltd., Nagpur; the Chief Executive Officer, FGZ Ltd., Nagpur; and the Office of the Divisional Manager, Gorewada Project, Nagpur, for providing the necessary facilities for the study. Special thanks are extended to the Director, Wildlife Research & Training Centre, for facilitating the research work at WRTC, Gorewada, Nagpur.

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