VETERINARSKI ARHIV 93 (5), 535-548, 2023 DOI: 10.24099/vet.arhiv.1659

Phylogenetic analysis and molecular characterization of field isolates of *Anaplasma* spp. from cattle in India

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KUMAR, A., A. SINGH, A. KUMAR VERMA, P. S. MAURYA, M. R. PRAJAPATI, A. KUMAR, T. K. SARKAR: Phylogenetic analysis and molecular characterization of field isolates of *Anaplasma* spp. from cattle in India. Vet. arhiv 93, 535-548 2023.

ABSTRACT

In India, the economic losses incurred due to ticks and tick-borne diseases are estimated around 498.7 million US dollars. Among these diseases, anaplasmosis causes significant mortality and morbidity in animals, leading to significant production losses. However, only scant information on the molecular characterization, phylogenetic and matrix analysis of *Anaplasma* spp in cattle is available. Therefore, the objective of the present study was to perform phylogenetic and molecular characterization of field isolates of *Anaplasma* spp. infecting dairy animals in Uttar Pradesh, India. Blood samples from 200 cattle showing the clinical signs of bovine anaplasmosis were collected from the western part of Uttar Pradesh, India. To detect *Anaplasma* spp., screening of blood smears and molecular confirmation by PCR was performed. The molecular characterization was done by sequencing the 16S rRNA gene and its bioinformatic analysis using MEGA version X. On the basis of microscopic examination, *Anaplasma* spp. were detected in 106 (53%) samples, while the 16S rRNA gene-based polymerase chain reactions revealed positive results in 176 (88%). The sequencing of the 16S rRNA gene and the bioinformatic analysis of sequences revealed the existence of three different populations of *Anaplasma* spp., viz., *Anaplasma marginale* and two other *Anaplasma* spp. genetically related to *Anaplasma capra* and *Anaplasma ovis,* circulating in the blood of infected cattle. All the field isolates of *A. marginale* and *A. ovis* from Uttar Pradesh, India, were clustered in a single clade with others isolated from Iran, Brazil, Thailand and Israel, while *A. capra* isolates from India, established in same clade of *A. capra,* have been reported from Japan, China and South Korea. To the best of the authors' knowledge, this is the first report of *Anaplasma capra* and *Anaplasma ovis* from bovine in India, and indicates the potential of cattle to serve as reservoirs of these pathogens, leading to the requirement for further studies of these emerging zoonotic pathogens and their possible zoonotic potential.

Key words: bovine anaplasmosis; *Anaplasma capra*; *Anaplasma ovis*; cattle

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ISSN 0372-5480
Printed in Croatia

Introduction

Bovine anaplasmosis is clinically characterized by pyrexia, weight loss, loss of appetite, decreased milk production, abortion and, sometimes, death (AUBRY and GEALE, 2011; FERNANDES et al., 2019). The genus *Anaplasma*, the causative agent of anaplasmosis, comprises Gram negative obligate intracellular parasites that inhabit and replicate within the blood cells, viz., erythrocytes, granulocytes, monocytes and endothelial cells of hosts and are usually transmitted through ticks (DUMLER et al., 2001; RAR and GOLOVLJOVA, 2011; GEORGE et al., 2017; JOUGLIN et al., 2019; PARAMANANDHAM et al., 2019; ZHANG et al., 2020). To date, eight classified species (*A. phagocytophilum, A. bovis, A. centrale, A. marginale, A. ovis, A. platys, A. odocoilei* and *A. capra*) of the genus *Anaplasma* have been found to cause anaplasmosis across the world in a large range of wild and domesticated vertebrates (AUBRY and GEALE, 2011; TATE et al., 2013; LI et al., 2015; KUMAR et al., 2016; BATTILANI et al., 2017; RAR et al., 2021). In India, bovine anaplasmosis was first reported in Odisha state (PATNAIK, 1963) and then two species of *Anaplasma.* i.e. *A. marginale* and *A. bovis* were reported to cause the disease in dairy animals (NAIR et al., 2013; SHARMA et al., 2015; GEORGE et al., 2017). The microscopic examination of Giemsa-stained thin blood smears is the most common method for diagnosis of *Anaplasma* species in cattle. Recently, molecular tests such as PCR (TORIONI et al., 2005; YAN et al., 2020), multiplex PCR (BILGIC et al., 2013; KUNDAVE et al., 2018; VIEIRA et al., 2019; PARODI et al., 2021), PCR-ELISA (GALE et al., 1996), semi-nested PCR (COURTNEY et al., 2004), and real-time PCR (PICOLOTO et al., 2010) are being used for detection of *Anaplasma* spp, with high sensitivity and specificity. Currently, the ribosomal RNA (16S rRNA) gene (PAROLA et al., 2003; STIK et al., 2007; PRADEEP et al., 2019; ZEB et al., 2020; AKTAS and ÇOLAK, 2021), 23S rRNA (DAHMANI et al., 2015; ALANAZI et al., 2021), the major surface protein (msp) (DE LA FUENTE et al., 2002; SHIMADA et al. 2004), heat-shock protein groEL (PARK et al., 2005) and citrate synthase gltA (INOKUMA et al., 2002), msp1α, msp1β, msp4 and groEL with the msp4 gene (QUIROZ-CASTANEDA et al., 2016; RAMOS et al., 2019) and msp5 (WATTHANADIREK et al., 2021) genes are widely used for molecular detection, phylogenetic analysis and inter-species variation of *Anaplasma* spp.

In the last two decades, anaplsmosis has received increasing attention due to the increasingly frequent detection of zoonotic *A. phagocytophilum* and *A. capra,* with first time reports in 1994 and 2015, respectively (LI et al., 2015). *A. phagocytophilum* is the most common pathogen causing human granulocytic anaplasmosis (HGA) infecting the neutrophils of humans and animals around the world (MACQUEEN and CENTELLAS, 2022), while the latter is usually neglected because of its sporadic occurrence (PARAMANANDHAM et al., 2019). *A capra* was first reported in asymptomatic goats in northern China in 2012 (LI et al., 2015) and then isolated from human, sheep and ticks from China (LI et al., 2015; YANG et al., 2016, 2018), cattle and water deer from South Korea (SEO et al., 2018; AMER et al., 2019), and deer from France (JOUGLIN et al., 2019) posing a potential health threat to humans and animals (PENG et al., 2021). *A. ovis* is considered to be the frequent cause of small ruminant anaplasmosis, but it appears to be less pathogenic, leading to subclinical infections with a low grade fever in small ruminants and wild animals (CABEZAS-CRUZ et al., 2019). Although bovine anaplasmosis has a major impact on the dairy sector in India, scant literature is available on the molecular characterization, phylogenetic analysis of *Anaplasma* spp. in cattle from India. Therefore, the present study was conducted for the molecular characterization and phylogenetic analysis of *Anaplasma* spp. from dairy animals in Uttar Pradesh, India.

Materials and methods

*Study area, animals, and sample collection***.** During the present study, about 3 ml of blood from each of the 200 cattle (suspected for bovine anaplasmosis) was collected in aseptic conditions using labeled sterile EDTA vacutainers (BD, USA), in five districts (Baghpat, Hapur, Meerut, Muzaffarnagar, Shamli) of Uttar Pradesh, India. These districts were selected because of the high

numbers of smallholder dairy farmers with good animal husbandry practices. The predominant cattle breeds were Sahiwal and crossbreds of Holstein Friesian and Jersey, while the main buffalo breed was Murrah. The climate of this area is monsoon influenced humid subtropical, characterized by hot summers and cooler winters. The meteorological parameters of the study area are: average annual temperature 24.5°C, varying from 5.2°C to 41.8°C; relative humidity 68.1%, varying from 18.4% to 99.9%; and annual rainfall 845 millimeters/anum (33 inch). Agriculture, along with animal husbandry, are the predominant economic activities in rural areas. A map showing the locations of the different districts within Uttar Pradesh, where blood samples were collected is given in Fig. 1.

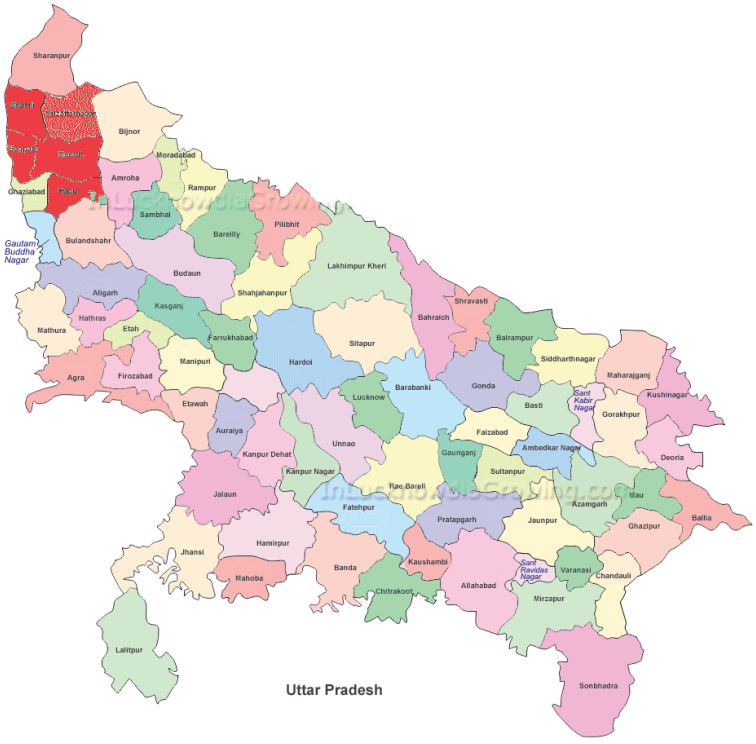

Fig. 1. Administrative map of Uttar Pradesh State showing the study area (Red colour)

Laboratory diagnosis

Thin blood smear preparation and examination. Thin peripheral blood smears were prepared on clean, grease-free glass slides (POTGIETER and STOLTSZ, 2004). The prepared thin smears were fixed in methanol, and then stained using a tenfold dilution of Giemsa's stain (HiMedia, Mumbai) with water for 30-40 min. Stained blood smears were air dried and observed under a microscope first at 40X and then using an oil immersion lens to detect parasitized and abnormal red blood cells (RBCs). The presence of a single parasite in the RBC was considered as a positive sample, while for declaring a sample negative, more than 5000 RBCs were screened per sample.

Polymerase chain reaction. The genomic DNA from the EDTA blood samples (1 ml) were extracted by the phenol chloroform method (SAMBROOK and RUSSEL, 2001) after enzymatic digestion using proteinase K (100 µg/ml) and a 1% (w/v) sodium dodecyl sulfate (SDS). About 50 µL of DNA elution buffer was used for elution of the extracted

DNA and the purity of the extracted genomic DNA was measured by a NanoBio 3.0 Spectrophotometer (Analytica, India) and then stored at −20°C for further analysis. For the positive control of the PCR, the genomic DNA extracted from the blood of *Anaplasma* infected cattle, presented to the Veterinary Clinical Complex, College of Veterinary and Animal Sciences, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, India, was used. The leukocyte DNA from a day old calf from the Livestock Farm Complex, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, India served as the negative control.

A previously described PCR detection assay was used to amplify 270 base pairs (bp) fragment of *Anaplasma spp.* 16S rRNA gene using: Forward (5′-GGC GGT GAT CTG TAG CTG GTC TGA-3′) and Reverse (5′- GCC CAA TAA TTC CGA ACA ACG CTT-3′) primers (KUNDAVE *et al.,* 2018). The PCR reaction was performed using a PCR master mix (Takara, Japan) in an automatic DNA thermocycler (BioRad, USA). A 25 µl reaction volume was used with 200 ng of purified genomic DNA. The PCR conditions consisted of an initial denaturation step of 5 min at 94°C, followed by a second step of 35 cycles of denaturation (45s at 94°C), primer annealing (45 s at 55°C) and extension (45 s at 72°C) along with a final extension step of 10 min at 72°C. A total of 10 µl amplified PCR product was used in 1.0% agarose gel electrophoresis containing 10 µl /ml ethidium bromide.

Sequencing and Phylogenetic analysis. Amplicons of the polymerase chain reaction (16S rRNA gene) were cut from the agarose gel and sent to Biokart India Private Limited, Banglore, India, for automated nucleotide Sanger sequencing. The sequences were submitted to the GenBank database and the sequences of each field isolate of *Anaplasma* were compared to other published sequences available in GenBank using NCBI-BLAST (http://www.ncbi.nlm.nih.gov/BLAST). The molecular evolutionary and phylogenetic analyses were conducted using MEGA version X (KUMAR et al., 2018). Phylogenetic trees were constructed from ClustalW-aligned sequences on MEGA-X (http://www.megasoftware.net/mega.php), using the Maximum-Likelihood method, with 1000 bootstrap replications. The pair-wise sequence identity studies of genomes were performed using Clustal W in the Sequence Demarcation Tool (SDT v1.2) (MUHIRE et al., 2014).

Results

Microscopic examination of thin blood smears stained with Giemsa stain. Out of 200 blood samples of cattle tested, 106 (53.00%) animals were found positive for bovine anaplasmosis by thin smear examination stained with Giemsa stain, and revealed the presence of dense, rounded, intra-erythrocytic bodies situated on or near the margin of the erythrocytes (Fig. 2).

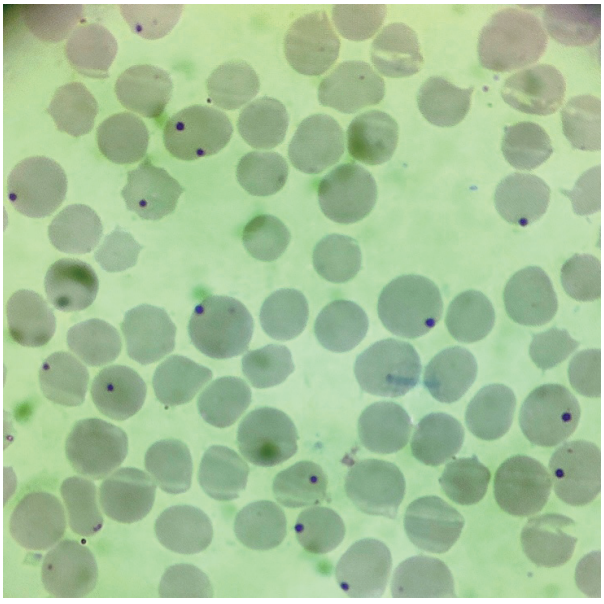

Fig. 2. Giemsa stain revealing the presence of *Anaplasma* spp. in the erythrocytes of blood smears

Molecular characterization of Anaplasma spp. The amplicons of 270bp were amplified in 176 (88.00%) blood samples using 16S rRNA gene based *Anaplasma* specific polymerase chain reaction (Fig. 3). Twenty-four samples were negative in both the tests, i.e. the thin blood smear examination stained with Giemsa stain and 16S rRNA gene based *Anaplasma* specific PCR. PCR detected 70 additional samples as positive that were negative

in the thin blood smear examination stained with Giemsa stain. The PCR based detection showed significantly higher ($P<0.05$) positivity compared to the thin smear method of detection.

*Identification of the Anaplasma species and its evolution and analyses***.** A total of 10 amplicons of *Anaplasma* spp. were submitted for Sanger sequencing by outsourcing. Out of 10, nine samples were sequenced successfully and the results are presented in Table 1. The nucleotide sequences obtained from Sanger sequencing were subjected to BLAST in the NCBI database to identify the species of the *Anaplasma*. It revealed two as *Anaplasma capra*, one as *Anaplasma ovis,* and the rest as *Anaplasma marginale* (Table 1). This seems to be the first report of *Anaplasma capra* and *Anaplasma ovis* in bovine from India. The sequences of all 09 have been submitted to the NCBI database to obtain an accession number.

Table 1. Nucleotide sequence of 16 ribosomal RNA gene of *Anaplasma* spp

S. No.	Accession Number	Organism	16S ribosomal RNA gene nucleotide sequence
1	MW723202	*Anaplasma Capra*	1 ctagttggtg gggtaatggc ctaccaaggc agtgatctgt agctggtctg agaggatgat 61 cagccacact ggaactgaga cacggtccag actcctacgg gaggcagcag tggggaatat 121 tggacaatgg gcgcaagcct gatccagcta tgccgcgtga gtgaggaagg ccttagggtt 181 gtaaaactct ttcagtaggg aagataatga cggtacctac agaagaagtc ccggcaaact 241 ccgtgccagc agccgcggta atacggaggg ggcaagcgtt gttcggaatt attgggcgta 301 aagggcatgt
2	MW990086	*Anaplasma capra*	1 atgatcagcc acactggaac tgagacacgg tccagactcc tacgggaggc agcagtgggg 61 aatattggac aatgggcgca agcctgatcc agctatgccg cgtgagtgag gaaggcctta 121 gggttgtaaa actctttcag taggggaaga taatgacggt acctacagaa gaagtcctcg 181 gcaaacttcc gctgtccagc agccgcggta atacggaggg ggcaagcgtt gttcggaaat 241 tattgggc
3	MW990081	*Anaplasma ovis*	1 ggcggtgatt ctgtagctgg tctgagagga tgatcagcca cactggaact gagacacggt 61 ccagactcct acgggaggca gcagtgggga atattggaca atgggcgcaa gcctgatcca 121 gctatgccgc gtgagtgagg aaggccttag ggttgtaaaa ctctttcagt agggaagata 181 atgacggtac ctacagaaga agtcccggca aactccgtgc ccagcagccg cggtaatacg 241 gagggggcaa gcgttgttcg aatttattgg gca
4	MW990084	*Anaplasma marginale*	1 gctggtctga gaggatgatc agccacactg gaactgagac acggtccaga ctcctacggg 61 aggcagcagt ggggaatatt ggacaatggg cgcaagcctg atccagctat gccgcgtgag 121 tgaggaaggc cttagggttg taaaactctt tcagtaggga agataatgac ggtacctaca 181 gaagaagtcc cggcaaactc cgtgcccagc agccgcggta atacggaggg ggcaagcgtt 241 gttcgaaatt attgggca
5	MW990088	*Anaplasma marginale*	1 ggcggtgatc ctgtagctgg tctgagagga tgatcagcca cactggaact gagacacggt 61 ccagactcct acgggaggca gcagtgggga atattggaca atgggcgcaa gcctgatcca 121 gctatgccgc gtgagtgagg aaggccttag ggttgtaaaa ctctttcagt agggaagata 181 atgacggtac ctacagaaga agtcccggca aactccgtgc ccagcagccg cggtaatacg 241 gagggggcaa gcgttgttcg gaaattattg ggc

Table 1. Nucleotide sequence of 16 ribosomal RNA gene of *Anaplasma* spp (continued)

S. No.	Accession Number	Organism	16S ribosomal RNA gene nucleotide sequence
6	MW990089	*Anaplasma marginale*	1 ggcggtgatt cgtagctggt ctgagaggat gatcagccac actggaactg agatacggtc 61 cagactccta cgggaggcag cagtggggaa tattggacaa tgggcgcaag cctgatccag 121 ctatgccgcg tgagtgagga aggccttagg gttgtaaaac tctttcagtg gggaagataa 181 tgacggtacc tacagaagaa gtcccggcaa actccgtgcc cagcagccgc ggtaatacgg 241 agggggcaag cgttgttcgg aaattattgg g
7	MW990412	*Anaplasma marginale*	1 gtttggcggt gatcctgtag ctggtctgag aggatgatca gccacactgg aactgagaca 61 cggtccagac tcctacggga ggcagcagtg gggaatattg gacaatgggc gcaagcctga 121 tccagctatg ccgcgtgagt gaggaaggcc ttagggttgt aaaactcttt cagtagggaa 181 gataatgacg gtacctacag aagaagtccc ggcaaactcc gtgccagcag ccgcggtaat 241 acggaggggg caagcgttgt tcggaattat tgggcaa
8	MW995952	*Anaplasma marginale*	1 gcggtgattc cgagctggtc tgagaggatg atcagccaca ctggaactga gacacggtcc 61 agactcctac gggaggcagc agtggggaat attggacaat gggcgcaagc ctgatccagc 121 tatgccgcgt gagtgaggaa ggccttaggg ttgtaaaact ctttcagtag ggaagataat 181 gacggtacct acagaagaag tcccggcaaa ctccgtgcca gcagccgcgg taatacggag 241 ggggcaagcg ttgttcggaa ttattgggca a
9	MW995967	*Anaplasma marginale*	1 ggcggtgatc ctgtagctgg tctgagagga tgatcagcca cactgggaac tgagatacgg 61 tccagactcc tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc 121 agctatgccg cgtgagtgag gaaggcctta gggttgtaaa actctttcag tggggaagat 181 aatgacggta cctacagaag aagtcccggc aaactccgtg cccacgcagc cgcggtaata 241 cggagggggc aagcgttgtt cggaattatt ggg

To establish the evolutionary pattern of all these *Anaplasma*, phylogenetic and matrix analysis of the sequences obtained from Sanger sequencing were performed. The analysis revealed different geographical regions of origin and linkage (Fig. 4, Table 2). Phylogenetic analysis of the 16S rRNA gene sequences revealed that the sequences of the present *Anaplasma* spp. clustered into four different clades. One isolate was in clade 1 with 99 - 100% identity to *A. capra* (GenBank AB509223, MT798599, LC432126), one isolate was in clade 2 with 99% identity to *A. capra* (GenBank KP06296451), six isolates were in clade 3 with 98 - 100% identity to *A. marginale* (GenBank MK310488, CP023731, MG018439, KT26418) and one isolate was in clade 4 with 99 – 100% identity to *A. ovis* (Fig. 4). BLASTn searches of the NCBI databases revealed that *A. capra* India shared 99.68% - 100% nucleotide sequence identities with other *A. capra* isolates reported from South Korea (LC432126 and MT798599), Japan (AB509223 and AF283007) and China MG869594, MW577114 and KP062964). The amplified sequence of *A. ovis* (MW990081) in the present study was in a different clade of *A. ovis* from that identified in other countries, such as Iran (MF979844), China (EF587237) and Mongolia (LC194134).

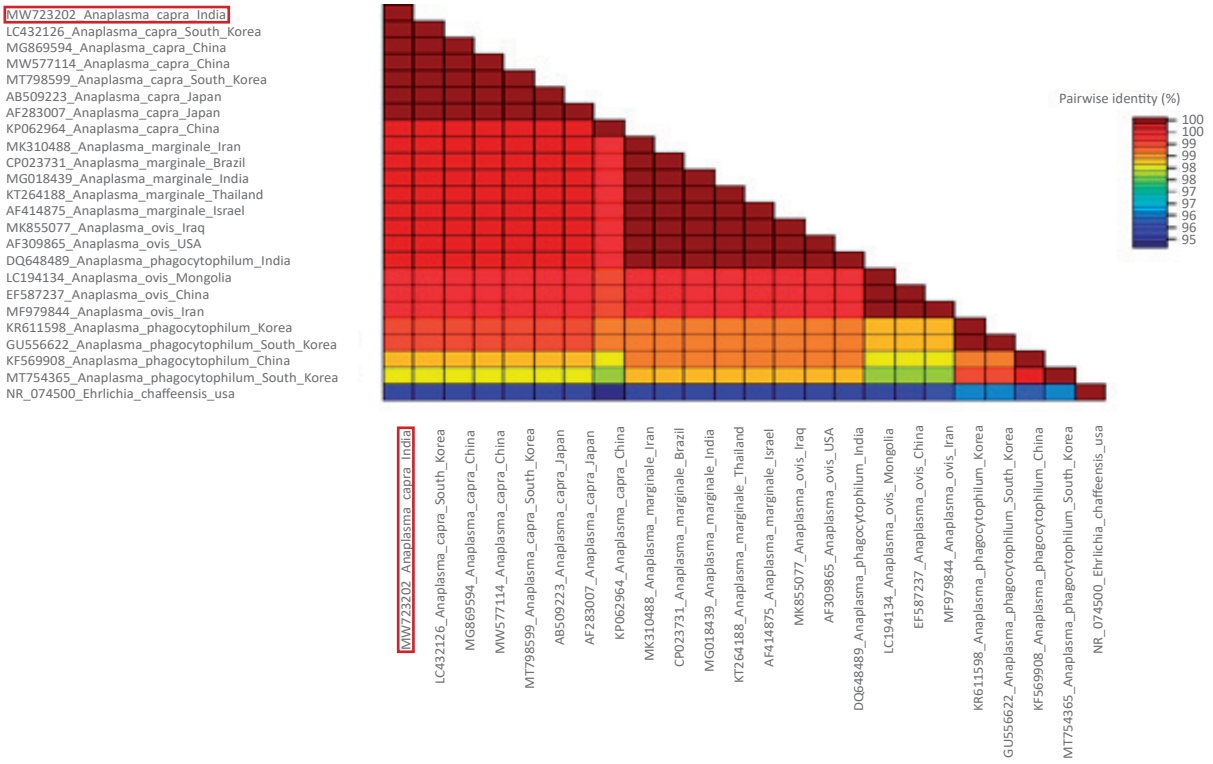

Table 2. Identity matrix of *Anaplasma* spp. sequences with other sequences

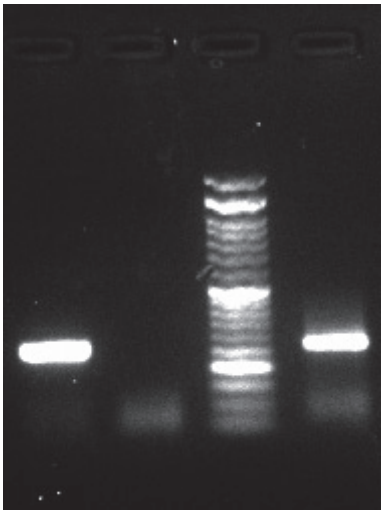

Fig. 3. Amplicication of the 16S rRNA gene of *Anaplasma sp.*

Lane M: 50 bp DNA ladder
Lane NC: Negative Control
Lane 1: Positive control (*Anaplasma spp*)
Lane 2: Sample (Blood)

Discussion

Bovine anaplasmosis is considered to be a major problem for the dairy sector in tropical and subtropical regions (JONGEJAN and UILENBERG, 2004). For diagnosis of bovine anaplasmosis in animals various laboratory tests are used, viz., direct microscopic examination (ME) of blood smear stained with Giemsa stain, serological tests such as ELISA and molecular tests such as PCR. Among these tests, *Anaplasma* infection is routinely diagnosed by direct microscopic examination of Giemsa stained blood smears for detection of dense, rounded, intra-erythrocytic bodies situated on or near the margin of the erythrocytes. Out of 200 blood samples, 106 were positive by thin blood smear examination stained with Giemesa stain, and 176 revealed the expected amplicons, 270 bp in length by polymerase chain reaction.

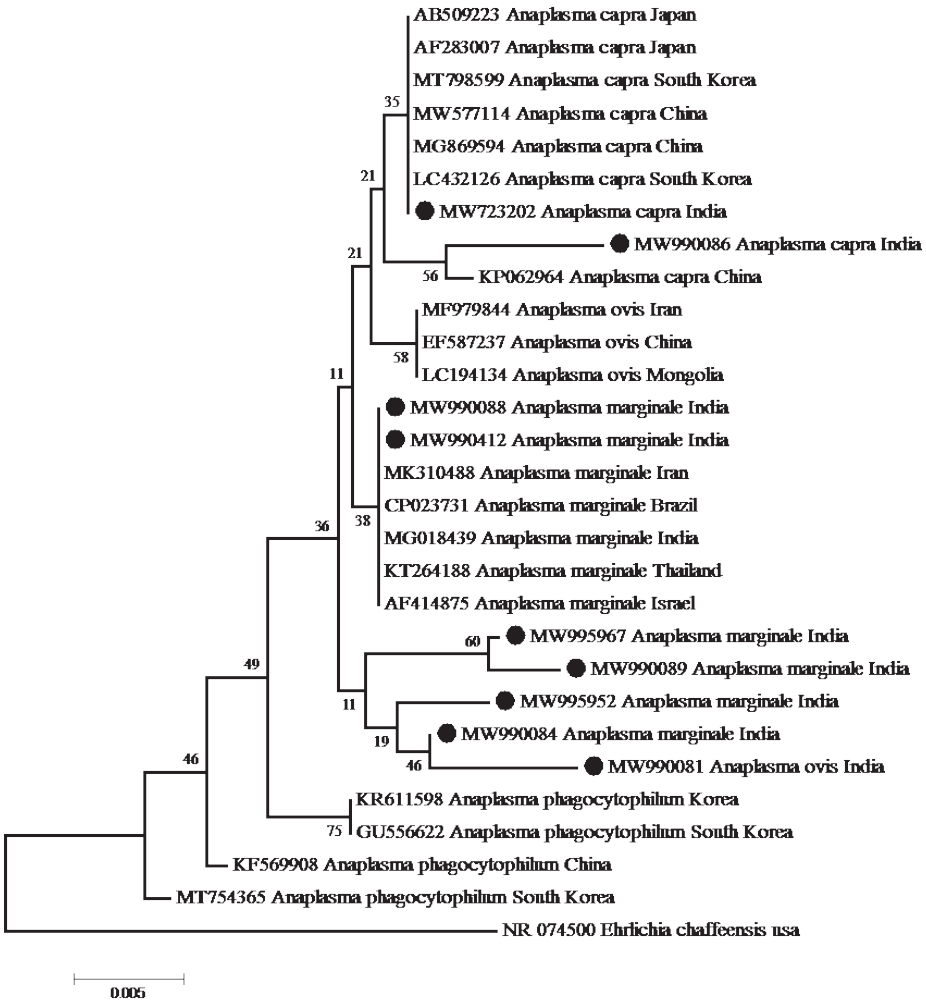

Fig. 4. Phylogenetic tree showing the evolutionary relationship of the *Anaplasma* species (marked with •) with other species

In the present study, only 60.22% of the PCR-positive animals were smear positive, which was in agreement with earlier studies (NAZIFI et al., 2008; NOAMAN and SHAYAN, 2010; SHABANA et al., 2018). This might be due to the early stage of *Anaplasma* infection, when there was a low level of infection beneath the detection limit of microscopic examination (NOAMAN and SHAYAN, 2010; SHABANA et al., 2018). The conventional microscopic examination of blood smears was suitable for diagnosis of acute cases of bovine anaplasmosis, but was not able to detect the lower number of *Anaplasma* infected erythrocytes in the circulation of carrier animals (GE et al., 1995; LIU et al., 2005; CARELLI et al., 2007; NOAMAN and SHAYAN, 2010). Similar to these findings, earlier studies also suggested that Polymerase Chain Reaction has several advantages compared to the conventional microscopic smear method (DUMLER and BROUQUI, 2004) in terms of sensitivity, reliability, and its ability to discriminate and detect co-infection of *Anaplasma* spp. (BAKKEN et al., 2002; TORINA et al., 2012; NOAMAN and SHAYAN, 2010; SHABANA et al., 2018). However, the microscopic smear method for diagnosis of bovine anaplasmosis is economical,

but requires a high level of bacteremia, good quality blood smears and staining, and above all a qualified and well-trained technician. In day to day diagnosis of bovine anaplasmosis the microscopic examination remains a convenient technique, but for early detection of carrier animals for proper control and management of infection, and to reduce the overuse of antimicrobials, PCR may provide a more reliable diagnosis of anaplasmosis (BAKKEN and DUMLER, 2006; CHAPMAN et al., 2006; NOAMAN and SHAYAN, 2010; SHABANA et al., 2018).

Bovine anaplasmosis is caused by various spp of *Anaplasma spp.* (*A. marginale, A. centrale, A. bovis* and zoonotic *A. phagocytophilum*) and has been reported to infect the erythrocytes of the host (GUO et al., 2019; HENKER et al., 2020). The sequencing of the 16S rRNA gene and the bioinformatic analysis of sequences revealed the existence of three different populations of *Anaplasma* spp., viz., *Anaplasma marginale* and two other *Anaplasma* spp. genetically related to *Anaplasma capra* and *Anaplasma ovis,* circulating in the blood of infected cattle. All the field isolates of *A. marginale* from Uttar Pradesh, India, were clustered in a single clade with others isolated from Iran, Brazil, Thailand and Israel, while *A. capra* isolates from India, were in same clade of *A. capra* reported from Japan, China and South Korea. There have been no previous reports on *Anaplasma capra* and *A. ovis* infecting bovines in India, while *A. marginale* was previously reported in India, based on both microscopy and molecular techniques (NAIR et al. 2013; PRADEEP et al., 2019). Similar results were obtained from the Maximum Likelihood based phylogenetic tree of the 16S rRNA gene sequence of *A. capra* and other 16S rRNA gene sequences of *A. capra* from different regions of the world using MEGA X (KUMAR et al., 2018). The phylogenetic tree revealed that *A. capra* India isolates (Accession no. MW723202) were grouped in the same clade with other *A. capra* reported from other countries, while the *A. ovis* India isolate (Acession no. MW990081) was grouped in a different clade from *A. ovis* reported from other countries (Fig. 4). The pair-wise sequence identities (%) of the *A. capra* 16S rRNA gene sequences of MW723202 shared 95%-100% identities with *Anaplasma spp* sequences. *A. capra* is considered to be an emerging zoonotic pathogen, and humans infected with *A. capra* are reported to have fever, headache, malaise, dizziness, chills, nausea, vomiting, diarrhea, and laboratory abnormalities, e.g., high hepatic amino transferase concentrations, leucopenia, and thrombocytopenia (LI et al., 2015). *A. capra* has been reported in animals and hard ticks, and may be transmitted through different species of hard ticks. Therefore, *A. capra* is a potential threat to humans as well as domestic animals, and requires attention to prevent its spread.

Conclusions

The present study confirmed the existence of three different populations of *Anaplasma* spp., viz., *Anaplasma marginale* and two other *Anaplasma* spp. genetically related to *Anaplasma capra* and *Anaplasma ovis,* circulating in the blood of infected cattle. The isolates of *A. marginale* from bovines of Uttar Pradesh, India, were clustered in a single clade with others isolated from Iran, Brazil, Thailand and Israel. To the best of the authors' knowledge, this study is the first report of *Anaplasma capra* and *A. ovis* from cattle in India.

Accession numbers of nucleotide sequences. The sequences obtained in this study have been submitted and deposited in the GenBank database with accession numbers (Table 1).

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgement

The authors thank Hon'ble Vice Chancellor, Sardar vallabhbhai Patel University of Agriculture and Technology, Meerut, India, for providing the funds and necessary support, under which this research work was carried out.

Ethical Approval

All animal studies were approved by the Committee on the Ethics of Animal Experiments of the College of Veterinary and Animal Sciences, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India.

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Received: 9 July 2021
Accepted: 29 June 2023

KUMAR, A., A. SINGH, A. KUMAR VERMA, P. S. MAURYA, M. R. PRAJAPATI, A. KUMAR, T. K. SARKAR: Filogenetska analiza i molekularna karakterizacija terenskih izolata bakterije *Anaplasma* spp. izoliranih iz goveda u Indiji. Vet. arhiv 93, 535-548 2023.

SAŽETAK

Ekonomski gubici koje u Indiji uzrokuju krpelji i bolesti koje se prenose krpeljima procjenjuju se na oko 498,7 milijuna američkih dolara. Među tim je bolestima i anaplazmoza, koja uzrokuje znatnu smrtnost i pobol među životinjama, što vodi do velikih gubitaka u proizvodnji. S obzirom na oskudne podatke o molekularnoj karakterizaciji, filogenetskoj i matriksnoj analizi bakterije *Anaplasma* spp. u goveda, cilj je ovog rada bio dobiti znanstvene informacije iz navedenih područja istraživanja na terenskim izolatima *Anaplasma* spp. koja inficira mliječna goveda u zapadnom dijelu savezne države Uttar Pradesh u Indiji. Prikupljeni su uzorci krvi od 200 goveda koja su pokazivala kliničke znakove anaplozmoze. Za detekciju bakterije učinjena je analiza razmaza krvi i molekularna potvrda PCR-om. Karakterizacija izolata na molekularnoj razini provedena je sekvenciranjem gena 16S rRNA i njegovom bioinformatičkom analizom upotrebom MEGA verzije X. Mikroskopskim je pregledom *Anaplasma* spp. otkrivena u 106 uzoraka (53%), dok je PCR analizom gena 16S rRNA pozitivno bilo 176 uzoraka (88%). Sekvenciranje gena 16S rRNA i bioinformatička analiza slijedova otkrili su postojanje triju različitih populacija bakterije *Anaplasma* spp., i to *Anaplasma marginale* i dviju drugih anaplazmi genetski povezanih s vrstama *Anaplasma capra* i *Anaplasma ovis* koje cirkuliraju u krvi zaraženih goveda. Svi su terenski izolati bakterija *A. marginale* i *A. ovis* iz države Uttar Pradesh svrstani u jednu filogenetsku kladu zajedno s izolatima iz Irana, Brazila, Tajlanda i Izraela, dok su izolati *A. capra* iz Indije svrstani u kladu s *A. capra* iz Japana, Kine i Južne Koreje. Prema autorovim je saznanjima ovo prvo izvješće o vrsti *Anaplasma capra* i *Anaplasma ovis* u goveda u Indiji što upućuje na potencijal goveda da budu rezervoari ovih patogena. To pak upućuje na potrebu za daljnjim istraživanjima bakterije *Anaplasma* spp. s obzirom na njezinu emergenciju i zoonotski potencijal.

Ključne riječi: anaplazmoza bovida; *Anaplasma capra*; *Anaplasma ovis*; goveda