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Molecular Detection of *Anaplasma ovis* in Apparently Healthy Sheep and Goats in Tamil Nadu

M. Aravind*, S. Gowrishankar, C. Soundararajan and Bhaskaran Ravi Latha

Department of Veterinary Parasitology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

**Corresponding author*

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A B S T R A C T

Anaplasma species are obligate intracellular rickettsial pathogens transmitted by ticks with an impact on animal health. *Anaplasma ovis* infects sheep and goats and it can be diagnosed by different methods like Giemsa staining, PCR or competitive ELISA. In this study, a PCR method based on major surface protein 4 (MSP4) gene, was utilized for the detection of *Anaplasma* infection. A total of 780 field blood samples were collected of which 360 from sheep and 420 from goat. Out of 780 whole blood samples collected from sheep and goats, 81.02% (632/780) were found positive for *Anaplasma ovis* of which 82.50% (297/360) of sheep and 79.76% (335/420) of goats were positive. This indicates high prevalence and thus, *A. ovis* should be considered as an important constraint of livestock production, and further efforts are needed to better understand the epidemiology and to implement suitable control measures.

Introduction

Anaplasmosis is a tick-borne rickettsial disease that impact human and animal health, wide spread in tropical and subtropical areas (Dumler *et al.,* 2001; Kocan *et al.,* 2004). The species such as *A. centrale, A. bovis, A. platys,* and *A. phagocytophilum. A. marginale and A. ovis* infection can result in anaplasmosis, a mild to severe intraerythrocytic disease that produces considerable economic losses in some regions (Kocan *et al.,* 2004). *Anaplasma ovis* is the main causative agent of ovine anaplasmosis, which is an intraerythro-cytic rickettsial pathogen of sheep, goats and wild ruminants. *A. ovis* infection in sheep and goats is usually asymptomatic (de la Fuente *et al.,* 2007; Torina *et al.,* 2012). The clinical signs usually develop in the event of immunosuppression in sheep, which is similar to those seen in *A. marginale* infection of cattle characterized by severe anemia, fever, weight loss, abortion, pallor of mucous membrane and jaundice

(Splitter *et al.,* 1955; Kocan *et al.,* 2003). The disease development is often predisposed by co-infection with other pathogens and stress induced by various factors, such as hot climate and transportation (Friedhoff 1997, Khayyat and Gilder 1947 and Manickam 1987). Therefore, control of *A. ovis* infection is vital for successful sheep and goat farming, and tick control is an integral part of any *A. ovis* control strategy, as tick control prevents *A. ovis* transmission from ticks to ruminants and vice versa.

Major surface proteins (MSPs) play a crucial role in the interaction of *Anaplasma* spp. with host cells, and they are subjected to selective pressures exerted by host immune systems (Brayton *et al.,* 2006; de la Fuente *et al.,* 2005; Dunning Hotopp *et al.,* 2006; Kocan *et al.,* 2004). MSP PCR has been used for the diagnosis of *Anaplasma* infection (de la Fuente *et al.,* 2005; Hornok *et al.,* 2010, 2012; Torina *et al.,* 2008). Therefore, this study investigated the prevalence of Anaplasmosis in sheep and goats with the aim of improving understanding of their distribution towards effective control.

Materials and Methods

Blood sampling and DNA isolation

Blood samples were collected from apparently healthy sheep (n = 360) and goats (n = 420) from several herds of Tamil Nadu. Blood was taken from the jugular vein of each animal and collected into a tube containing anticoagulant (EDTA). DNA was isolated success-fully from 200 µl of blood using Qiagen blood DNA extraction kit (Qiagen, Germany), following the manufacturer's instructions and stored at −20 ◦C until use.

PCR detection of *A.ovis*

Major surface protein 4 (MSP4) gene of *A. ovis* was targeted for its detection and identification. A primer set (Forward: 5' TGA AGGGAGCGGGGTCATGGG 3'/ Reverse: 5' GAGTAATTGCAGCCAGGCA CTCT 3') was used for amplification which was earlier reported by Torina *et al.,* (2012). PCR assay was carried out in Bio-Rad thermocycler. Each 20µl reaction mixture comprised 5 µl of template DNA, 10 µl Red dye PCR Master Mix (2X) (Ampliqon), 1 µl of each primer set at 10 pmol/µl (forward and reverse primer), and 3µl nuclease free water. The PCR conditions includes initial denaturation at 94°C for 3 min(Initial denaturation); followed by 30 cycles of 94°C for 30 s (Denaturation), 62°C for 15s(Primer annealing), and 72°C for 1min (Extension); with a final extension of 72°C for 5 min. 10 µl of amplified PCR product was loaded for electrophoresis in 1.5% agarose gel along with 100 bp DNA ladder. The images were captured and documented using gel documentation system (Bio Rad., USA).

DNA sequencing and analysis

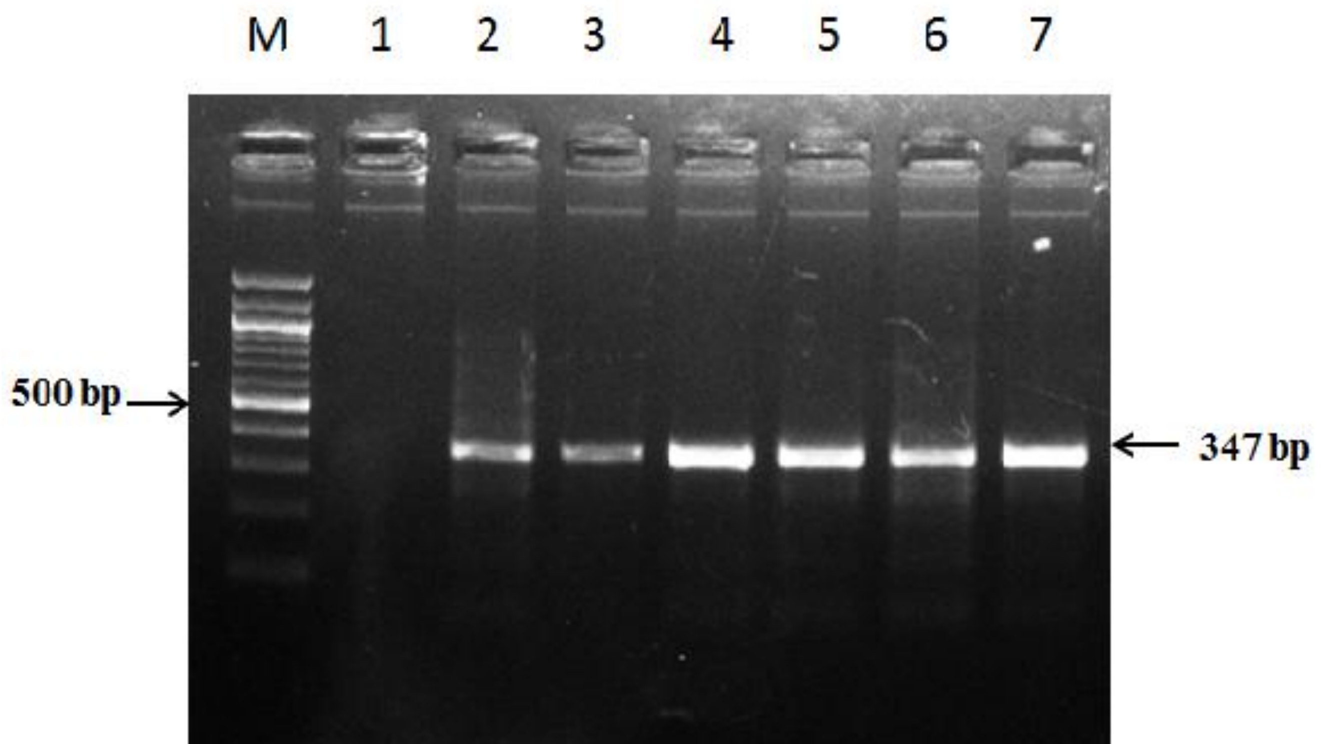
Two Amplicons from each of the positive samples were randomly selected for sequencing. The amplified PCR products were recovered from the agarose gel using QIAquick Gel Extraction Kit (Qiagen, Germany), and the fragments were cloned into a pTZ57R/T vector according to the commercial protocol of the InsTAclone PCR Cloning Kit (Thermo Scientific, USA). After transformation, three recombinant clones were selected for sequencing. The resultant plasmid was subjected to complete sequence analysis with M13 forward and reverse primer by Sanger's dideoxy chain termination method at Xcelris Labs Ltd., Ahmedabad, India. The nucleotide sequence identities and similarities of obtained sequence were analyzed by a BLASTn search in Gen-Bank and the percent identities between nucleotide sequences were calculated using a ClustalV in MegAlign component of the DNA Starprogramme (Version 5.0 DNASTAR, Madision, Wis.).

Results and Discussion

Anaplasma ovis was detected by the *msp4* PCR assay from both sheep and goats showed 81.02% (632/780) were positive. The overall *A. ovis* positive rates were comparable between sheep and goats analysed in this study. Of 360 sheep and 420 goat DNA samples, 297 (82.50%) and 335 (79.76%) (335/420) were positive for *A. ovis* infection. (71.3%) were positive for *A. ovis* infection. This study showed higher prevalence compared to the study by Mo Zhou *et al.,* (2016) and Enkhtaivan *et al.,* (2019) who observed 60 and 70 per cent respectively.

The positive rates were comparable to those determined in several other endemic countries, including Portugal, Sudan, and Iraq (Renneker *et al.,* 2013). The amplicon was resolved as a single band of 347 bp (Fig. 1). It was further purified for ligation in pTZ57R/T cloning vector. The selection of positive colonies was done by colony PCR using the specific primers.

Fig.1 PCR for *Anaplasma ovis*



Lane M : 100 bp ladder (Thermo scientific)
Lane 1: Negative control
Lane 2-6: Field DNA samples
Lane 7: Positive control

The positive clones were selected and custom sequenced for nucleotides. The sequence information revealed 347 bp nucleotides. The nucleotide sequences of MSP4 gene of *A.ovis* obtained in this study was aligned and analysed *in silico* using DNA STAR and MEGA version 7.0 softwares against the same sequence from other isolates of *A.ovis* available in public domain. Sequence analysis indicated that the nucleotide sequences were

of the expected size of 347 bp. The percentage identity of sequences obtained in this study shared 100% homology with the isolates of Kenya (MF 360029), South Africa (MF 945973), China (MN 394791) and 99.7% homology with the isolates of Turkey (MT 344082), Portugal (LC 229602), Kerala (MN 075143) and Tunisia (MT 292904). The infections were confirmed by sequencing the MSP4 gene, which has been proved useful for characterization of *A. ovis* (de la Fuente *et al.,* 2007).

It is seen that *A. ovis* seems to be widely distributed in the investigated geographical regions. Though it causes only mild clinical symptoms (Friedhoff, 1997) its adverse effect is aggravated in infected sheep and goats, when the animals are stressed by other factors such as co-infection, poor health conditions, hot weather, vaccination, deworming or heavy tick infestation (Khayyat and Gilder, 1947; Manickam, 1987). There is an increasing demand for a better understanding of the diseases affecting these animals as they seem to contribute to a decrease in productivity. Therefore, further investigations should allow establishing epidemiologically sound data on *A. ovis* to analyze impact on the health of small ruminants (Khayyat and Gilder, 1947; Myalo, 1957; Göksu, 1965) and to assess the socio-economic impact of ovine anaplasmosis.

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