B-4484
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RESEARCH ARTICLE Indian Journal of Animal Research

# First Molecular Study of Caprine *Trypanosoma evansi* Infection in Central India

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10.18805/IJAR.B-4484

## ABSTRACT

**Background:** Diagnosis of *Trypanosoma evansi* among goat in field is still challenging due to scarce information on clinical manifestation and non availability of rapid reliable diagnostics. Molecular test such as PCR employed to diagnose the *T. evansi* with accuracy in both blood and tissues. Limited reports are available from different parts of the country on natural infection of *T. evansi* in goat. Mainly subclinical form of disease occurred in goats owing to low level of parasitaemia of *T. evansi*.

**Methods:** A Jamunapari goat aged 1.5 years and weighing ~25 Kg was suffering from circling movement, pale conjunctiva and anorexia and was found positive for *T. evansi* by buffy coat examination and polymerase chain reaction. Diminazene aceturate was given intramuscularly as 7% water solution at a dose of 3.5 mg/kg b.wt. After 3 days, blood smear and buffy coat were negative for *T. evansi* and no band was found after running the product on 1.7% agarose gel stained with ethidium bromide.

**Result:** The present study reveals first time report on caprine trypanosomosis from central India by conventional and PCR technique and placed on record. Hence, the goat should also be taken into consideration for control of trypanosomosis as goats are an important source of infection to other animals due to their reservoir nature for *T. evansi.*

**Key words:** Circling movement, Diminazene aceturate, Goat, Polymerase chain reaction, *Trypanosoma evansi.*

## INTRODUCTION

Trypanosomosis or Surra caused by *Trypanosoma evansi* is an economically important arthropod-borne disease and is widely distributed among the animal trypanosomes affecting all domestic livestock in Asia, Africa, Central and South America and imposes a big challenge for successful livestock production (Desquesnes *et al*., 2013). Goat most affectionately labelled as 'poor man's not only provides the nutritional security but also a source of supplementary income to poor farmers. Among the four fundamental pillars of goat farming (breeding, feeding, housing and disease management), prevention of disease is the prerequisite to acquire the maximum profit. The goat population in India is 148.88 million in 2019 which is growing at the rate of 10.1% over the previous census. (Anonymous 20$^{th}$ Livestock Census 2019). Doubling the income of farmers by 2022 could only be achieved by rearing the goat because 76 per cent of marginal farmers rear goat (PTI, 2018). Limited reports are available from different parts of country on natural trypanosomiosis infection in goats. Earlier studies reported 2.15% prevalence of trypanosomosis in goat from different parts of Karnataka state (Krishnappa *et al*., 2002).

The total annual economic loss due to surra among goat was estimated to be INR 340.88 million. Major losses were due to reproductive problems (73.98%) followed by reduced milk (22.87%) and meat yield (3.16%) (Kumar, 2017). Principal host species varies from one geographical area to another geographical area, although buffalo, cattle, camel and horses are mainly susceptible to trypanosomiosis. Acute, subacute, chronic, or subclinical forms of surra have been reported in goat however the cases are very few. Due to low level of parasitaemia, it is considered that in goat, mainly subclinical form of disease occurred. Hence, the goat should also be taken into consideration for control of trypanosomosis as goats are an important source of infection to other animals due to their reservoir nature for *T. evansi*. Diagnosis of *T. evansi* in goat in field condition is still challenging due to scanty information on clinical manifestation and non-availability of rapid reliable diagnostic

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**How to cite this article:** Agrawal, V., Das, G., Maharana, B.R., Jayraw, A.K., Shakya, M., Jatav, G.P. and Jamara, N. (2021). First Molecular Study of Caprine *Trypanosoma evansi* Infection in Central India. Indian Journal of Animal Research. DOI: 10.18805/IJAR.B-4484.

**Submitted:** 13-04-2021 **Accepted:** 23-08-2021 **Online:** 22-09-2021

tests. Molecular test such as PCR used to diagnose the *T. evansi* with accuracy in both blood and tissues (Desquesnes *et al*., 2001). The present study first time report on caprine trypanosomosis from central India by conventional and PCR technique and placed on record.

## MATERIALS AND METHODS

A Jamunapari goat aged 1.5 years and weighing ~25 Kg was suffering from circling movement, pale conjunctiva and anorexia in Panagar village of Jabalpur in the year 2016. (Fig 1A, 1B and 1C). After taking careful consideration of goat habitat, it was reported that goat was fed in free grazing form together with cattle and buffaloes. Goats in this village were kept in open housing system due to which they were exposed to extreme weather conditions and vectors. The village is well-known dairy hub of Jabalpur and a big pond is located nearby that village with the presence of vector like *Tabanus* and unhygienic management in the animal shed. Aseptically, 5 ml blood sample was collected from juglar vein of suffering goat and 3 ml was transferred in vial containing EDTA anticoagulants in order to estimate routine blood parameters as per procedure reported by Sharma and Singh (2000). Peripheral thin blood smear was prepared at the site of collection and immediately fixed with methanol. Blood sample was brought to Department of Veterinary Parasitology, College of Veterinary Science and A.H, Jabalpur. The blood smear stained by Giemsa stain was examined under oil immersion of compound microscope. Method of Murray *et al*. (1977) was followed for detection of trypanosoma infection by buffy coat technique (BCT). Remaining 2 ml of blood sample was transferred for serum separation. Serum samples obtained by centrifugation were used to determine total protein, albumin, alanine transaminase (ALT), aspertate amino transferase (AST), total protein and creatinine using semi auto biochemistry analyzer (Span diagnostic Ltd.) with standard kits of span diagnostic Ltd., Surat, India.

Genomic DNA was isolated from the collected blood sample using QIAamp® DNA blood mini kit following the manufacturer's recommendations. In brief, approximately 200 µl of the blood sample was mixed with 20 µl of proteinase-K and 200 µl of lysis buffer in a 2.0 ml microcentrifuge tube. The homogenous suspension was thoroughly vortexed and incubated at 56°C for 10 min. Subsequently, 200 µl of ethanol was added to the lysate and again vortexed. The mixture was then applied to QIAamp spin column and centrifuged at 8000 rpm for 1 min. Thereafter, 2 washings were given with wash buffers and DNA was eluted in 200 µl of elution buffer and stored at -20°C till further use. Genomic DNA of *T. evansi* was isolated from infected goat blood showing parasitaemia in buffy coat examination and utilized as positive control. Genomic DNA was also isolated from the whole blood of infection-free, 3-day-old goat kid (both microscopically and PCR negative) and used as a negative control.

### PCR assay

Molecular diagnosis of *T. evansi* was done by PCR as per the procedure of Wuyts *et al*. (1995) by using following primers.

TR3: 5´-GCG CGG ATT CTT TGC AGA CGA- 3´ and
TR4: 5´-TGC AGA CAC TGG AAT GTT ACT-3´ specifically targeting repetitive nucleotide sequence of variable surface glycoproteins of *T. evansi*.

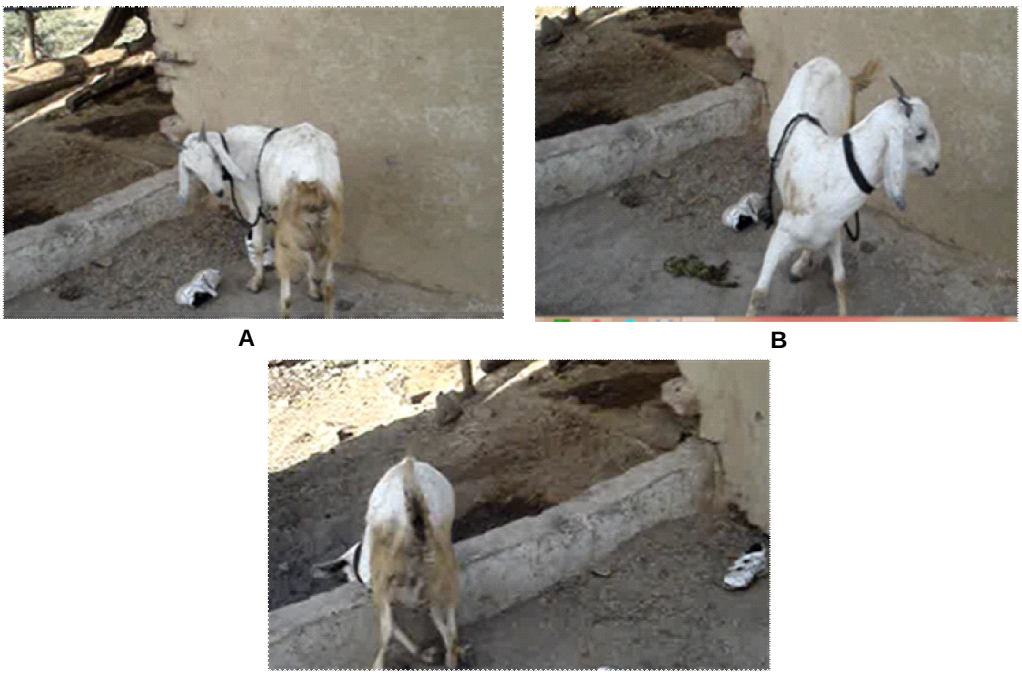

**Fig 1A, 1B and 1C:** Showing circling and staggering movement in *T. evansi* infected goat.

For the PCR assay, following components were used; master mix consisted of 2.5 µL of 10× PCR buffer, 0.5 µL of 10 mM dNTP mix, 2.0µL of 25 mM $MgCl_2$, 1.0 U of recombinant TaqDNA polymerase, 1 µL each (20 pmol) of TR3 Forward and TR4 Reverse primers and 5 µL of template DNA isolated from field and the volume was made up to 25µL with nuclease-free water. The cycling conditions were: initial denaturation at 95°C for 5 min, 30 cycles of denaturation at 95°C for 2 min, annealing at 60°C for 2 min, and elongation at 72°C for 2.5 min and the final elongation was done at 72°C for 10 min. The PCR product was checked for amplification by electrophoresis on a 1.7% agarose gel and visualized using gel documentation system (Syngene, UK).

## RESULTS AND DISCUSSION

Blood sample was positive for *T. evansi* by buffy coat method (Fig 2) and negative by Giemsa stained blood smear. After running the PCR amplified product on 1.7% agarose gel stained with ethidium bromide, an amplicon of 227 bp further confirmed the *T.evansi* infection (Fig 3). This may be attributed to the fact that goat reared with cattle and buffaloes which could be the source of *T. evansi* infection. Detection of *T. evansi* in blood smear of goat is very difficult task due to low parasitaemia. During advanced stage of trypanosomiosis or in febrile conditions, *T. evansi* can be seen in blood smear examination (Ngeranwa *et al.,* 1993). But in present study, *T. evansi* was detected in buffy coat technique. But due to earlier report of goat as reservoir for *T. brucei* and *T. rhodesiense*, it needs critical reappraisal for role of goat in epidemiology of *T. evansi* in other ruminants (Gutierrez *et al*., 2006). Otherwise generally it is believed that goats are highly resistant to trypanosomosis and have little economic importance (Griffin 1978). Susceptibility of goats to trypanosomosis depends upon breed of goat, strain virulence and quantity of infective dose (Otieno and Gacanja, 1976; Ngeranwa *et al*., 1993). Goats are trypanotolerant showing subclinical trypanosomosis with low parasitaemia (Musinguzi *et al*., 2016). In present study Jamunapari goat was reported positive for *T. evansi*. Subclinical (Otieno and Gachanja, 1976), moderate and sever form of infection (Ngeranwa *et al.,* 1993) have been reported in small ruminants by various workers. Ngeranwa *et al.,* 1993 described erratic parasitemia, weight loss and significant drop in PCV in an experimental inoculation using a *T. evansi* strain isolated in Kenya and inoculating in small East African bucks.

Diminazene aceturate was given intramuscularly as 7% water solution at a dose of 3.5 mg/kg b.wt. After 3 days blood smear and buffy coat were negative for *T. evansi* and same was found negative in PCR assay.

The haematological values of goat before and after treatment of *T. evansi* were as follows: RBC (9 $\times 10^6$ $\mu L^{-1}$ Vs 13.0 $\times 10^6$ $\mu L^{-1}$), Hb (7 $gdL^{-1}$ Vs 8.5 $gdL^{-1}$), WBC (11$\times 10^3$ $\mu L^{-1}$ Vs 12 $\times 10^3$ $\mu L^{-1}$), Lymphoctes (55% Vs 50%); Neutrophils (38%Vs 43%); Eosinophils (4% Vs 4%) and Monocytes (3% Vs 3%). Biochemical parameters of naturally infected goat with *T. evansi* were as follows: AST=67.30 $iuL^{-1}$; ALT=26 $iuL^{-1}$; Creatinine=0.75 mg $dL^{-1}$ and total protein=7.74 $gL^{-1}$ whereas for goat treated from trypanosomes were as follows: AST-60.20 $iuL^{-1}$; ALT=21 $iuL^{-1}$; Creatinine=0.60 mg $dL^{-1}$; total protein=4.60 $gL^{-1}$.

Decreased value of RBC and Hb in trypanosoma infected goat might be due to intravascular haemolysis of erythrocytes, haemodilution, erythrophagocytosis of immune-altered erythrocytes or dyshaemopoiesis (Radostitis *et al*., 2000; Dargantes *et al*., 2005). The leucopenia was characterized by neutropenia, eosinopenia and lymphocytosis. Neutropenia in infected goat might be due to increased susceptibility of goat to other infections. There was no changes in value of eosinophils and monocytes in infected animals. Due to trypanosomal antigen coating of leucocytes results in leucophagocytosis and

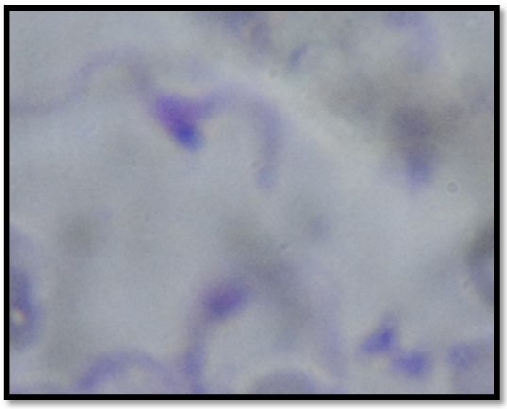

**Fig 2:** Buffy coat showing *T. evansi.*

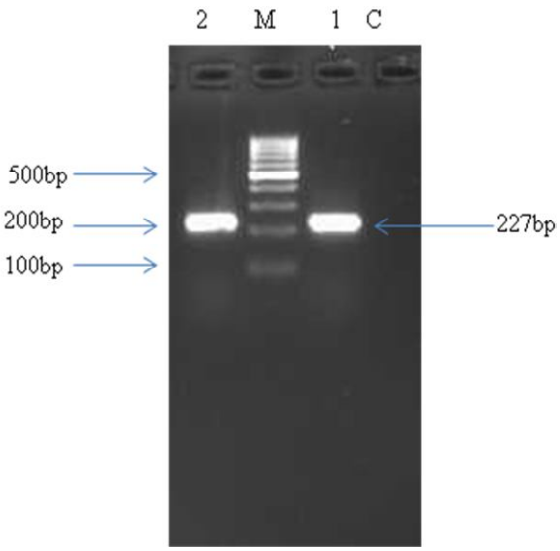


**Fig 3:** Agarose gel electrophoresis (1.7%) showing the band of 227 bp fragment from genomic DNA of *Trypanosoma evansi.* Lane M: 100bp DNA ladder, Lane 1: Amplification of *T. evansi* genomic DNA from the blood of goat positive for infection (positive control) Lane C: Negative control (No temple), Lane 2 Positive processed field samples.

depression of leucocyte production. These are the factors responsible for leucopenia (Sadique *et al*., 2001).

Value of AST, ALT and total protein were high in infected goat owing to breakdown of tissue. High level of AST and ALT indicate the involvement of liver and kidney. High level of total protein indicates immune response due to the infection (Osman *et al*., 2011). Serum creatinine levels were significantly increased in goats infected with trypanosome parasites. Elevation in creatinine level might have resulted from kidney dysfunction, catabolism of muscle.

From the above study, it is clearly inferred that for the control of trypanosomosis, goat should also be taken in to consideration as goats are important source of infection to other animals due to their reservoir nature for *T.evansi*. This is the maiden attempt and first molecular report of caprine trypanosomosis from Cental India and placed on record.

## CONCLUSION

The present study reveals first time report on caprine trypanosomosis from central India by conventional and PCR technique and placed on record. Hence, the goat should also be taken into consideration for control of trypanosomosis as goats are an important source of infection to other animals due to their reservoir nature for *T. evansi.*

## ACKNOWLEDGEMENT

This work was supported by Dean, College of Veterinary Science and Animal Husbandry, Jabalpur, India.

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