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Occurrence of *Trypanosoma evansi* in bovines in Bareilly district of Uttar Pradesh

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Abstract
Trypanosoma evansi, the causative agent of surra, is a hemoflagellate protozoa which is mechanically transmitted by hematophagous flies. The present study was undertaken to record occurrence of *T. evansi* infection in bovines of Bareilly district, Uttar Pradesh during July, 2023 to December, 2023using conventional and molecular techniques .Blood samples were collected from 132 bovines (94 cattle and 38 buffaloes) of different dairy farms and individual livestock owners of Bareilly. Examination of Giemsa stained thin blood smears revealed that 5 samples (3.78%) were positive for *T. evansi* out of 132animals examined, while *T. evansi* paraflagellar rod 2 (PFR2) gene based PCR revealed that 15samples were positive for *T. evansi* DNA. The overall occurence of *T. evansi* in bovines of Bareilly district was found to be 11.36%, with the 8.51% and 18.42% infection rate in cattle and buffaloes, respectively. The sex wise occurence was reported to be 12.84% (14/109) in females and 4.34% (1/23) in males, whereas age wise occurence of try panosomosis was found as 15.94% in age group of >5 years and 8.51% in age group of 2-5 years while none of the animals below two years of age were positive. These findings suggest that the bovines in Bareilly district are acting as carriers of *T. evansi* infection and there is a need to coduct a large scale epidemiological surveys using highly sensitive and specific molecular techniques to identify such carrier animals, so that effective management of *T. evansi* infection in bovines could be implemented.

Keywords: Giemsa stained, hematophagous flies, protozoa, PCR, *Trypanosoma evansi*

1. Introduction
Trypanosomosis commonly known as “Surra” is caused by a haemoflagelate *i.e. Trypanosoma evansi*, which is mechanically transmitted by haematophagus flies *viz*., *Tabanus* spp. and *Stomoxys* spp. The hot and humid climate has been found to favor the propagation of vectors (flies), and thus the maintenance of this disease could be seen in large geographical areas including Asia, Africa and Central and South America (Konnai *et al*., 2009) [12]. Further, this disease poses a severe impact on the overall health of livestock leading to severe economic loss to the dairy industry. In general, this disease is characterized by intermittent fever, anaemia, anorexia, hypoglycaemia, production losses and widespread tissue damage in different organs (Jaiswal *et al*., 2015) [1]. The clinical signs of surra are not pathognomonic for making the confirmatory diagnosis and thus, demonstration of trypanosomes in the blood smear remains the choice for making a definitive diagnosis. However, detection of trypanosomes in thin smear may not be always feasible because of low level of parasitaemia that is commonly encountered in chronic disease condition and is usually reported in cattle and buffaloes which act as the reservoir hosts of *T. evansi*, and play a major role in the epidemiology of the surra. Molecular techniques like PCR assays have been found to be very specific and highly sensitive (Alanazi, 2018) [2] and thus are being preferred in epidemiological investigations and in herd level screening of surra. The present study was therefore performed with the aim to investigate the occurrence of *Trypanosoma evansi* infection in bovines in Bareilly district of Uttar Pradesh using conventional microscopy and PCR based assay.

Materials and Methods
About animals and sample collection
A total of 132 bovines (94 cattle and 38 buffaloes) belonging to various livestock owners and

dairy farms located in Bareilly District of Uttar Pradesh during six months from July, 2023 to December, 2023, were included in this study. Out of these 132 animals, only 02 animals were showing clinical signs *viz.* fever, anaemia, anorexia, oedema of dependent parts, nervous signs etc. suggestive of trypansosmosis, while rest of the animals were asymptomatic.

Blood sample (1-2 ml) was collected aseptically from jugular vein of all the animals with the help of 18 gauge needle and stored in clean, dry, sterilized glass vials containing EDTA @ 1mg/ml of blood. The blood samples were transported on ice to the Clinical and Wildlife Parasitology Laboratory, Division of Parasitology, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly for further screening for *T. evansi* infection using conventional microscopy (thin blood smear) and molecular (PCR) assays.

Blood smear examination

Freshly collected blood sample of each animal was used for preparing the thin blood smear for microscopic examination. Briefly, a small drop of blood was placed on a clean glass slide and a thin blood film was made. The film was air-dried, fixed in absolute methanol for 2 minutes and allowed to dry. The smears were then stained with Giemsa stain (diluted 1:10 with distilled water) for 30 minutes, washed with tap water and then air dried. Slides were seen under microscope at 100x using immersion oil (Jain, 1986) [10].

Polymerase chain reaction (PCR)

Genomic DNA was isolated from individual blood sample using the QIAamp DNA mini kit (Qiagen, Germany) following the manufacturer instructions and same was stored at -20 °C until further used. PCR assay was performed using the self-designed *T. evansi* specific primer pair i.e. Tevans-F1 (CGGAAAGGAAGTTGAAGGTGTTGTGAG) and Tevans-R1 (CACGCACAGACATCTCAAGACCACAG) to amplify 440 bp region of the paraflagellar rod 2 (PFR2) protein gene. PCR was performed in a thermocycler with initial denaturation at 94 °C for 5 min., followed by 35 cycles of denaturation (94 °C for 30 sec.), annealing(52 °C for 45 sec.) and extension (72 °C for 45 sec.), and a final extension at 72 °C for 10 min. The amplified PCR product was electrophoresed in 1.0% agarose gel and amplicon was documented using Gel documentation system (Syngene).

Results and Discussion

In the present study, blood samples collected from cattle and buffaloes were screened for the detection of *T. evansi.* Out of 132bovineblood smears screened by microscopy, only 5(3.78%) were positive for *T. evansi*. The haemoflagellates detected in the study corresponded well with the morphology of *T. evansi* as described by Soulsby (1982). The trypanosomes were 20 to 31 µm long with sub terminal kinetoplast, distinct undulating membrane and a free flagellum. The nucleus was conspicuous and centrally placed (Figure 1).However, the PCR based screening of all the animals resulted in amplification of the paraflagellar rod 2 gene (440 bp) in 15 animals (11.36%). All the 5 microscopically *T. evansi* positive samples were detected positive by PCR as well. This higher occurrence at may be due to prevailing ecological conditions favorable for vector development in the area under study. In India particularly in bovines, almost similar trend was also reported by Bal *et al.* (2014) [4], Gangwar *et al.* (2019) [8] and Maharana *et al.* (2019) [14].

Incidence of trypanosomiasis in bovines in India was found to be directly proportional to onset of monsoon to post monsoon (Ray *et al*., 1992; Soodan *et al*., 1995; Raina *et al*., 2000; Agrawal *et al*., 2003; Sinha *et al*., 2006) [20, 25, 18, 3, 24]. The disease is mostly asymptomatic, but factors like flooding, intercurrent disease (Gupta *et al*., 2009) [9], vaccination (Singla *et al*., 2010) [22], transport (Kalra *et al*., 1994) [11] and malnutrition (Malik *et al*., 2000) [15] often changes an unapparent infection into clinical disease. Microscopic examination observation of fresh blood can be easily carried out by wet mount of blood, thin blood smear, thick blood smear but have low sensitivity with detects parasites above 10^5 trypanosomes per mille litter of blood. However, more than 50-80% of the infections are cryptic and undetectable by direct microscopy; therefore these methods are not sufficient to know the epidemiology and magnitude of the surra in country. Comparative studies suggests that PCR methods are mostsensitive (Pruvot *et al*., 2013) [17] and this can detect as less as 5-10trypanosomes per milliliter of blood.

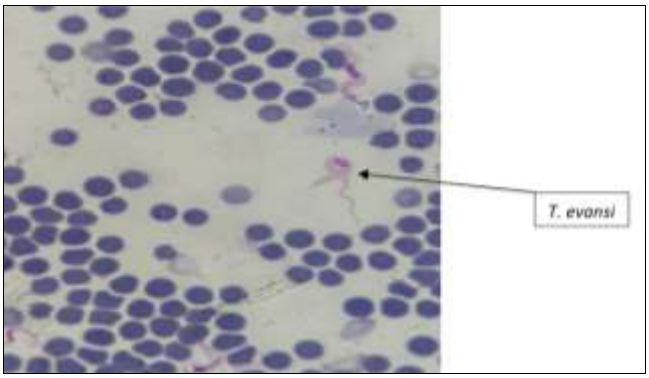
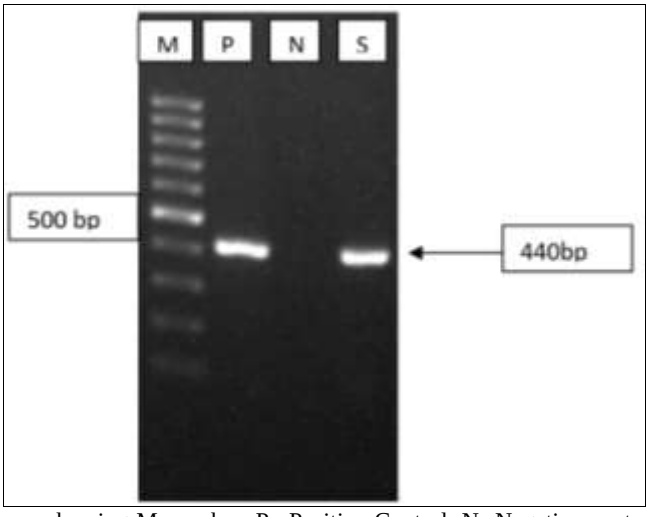


Fig 1: *Trypanosoma evansi* in stained thin blood smear under high power magnification (100x).



Lane showing M- marker, P –Positive Control, N- Negative control and S- Samples

Fig 2: PCR amplification of paraflagellar rod 2 gene of *T. evansi* (440 bp)

During the present study, higher occurrence of *T. evansi* infection was recorded in female animals (12.84%) than in the males (4.34%) as shown in Table 2. Gangwar *et al.* (2019) [8] and Chanie *et al.* (2012) [6] have also reported the higher prevalence of the infection in females than the males. Higher incidence in female population may be due to hormonal disturbances which pretence it to weakened immune system (Maharana *et al*., 2015) [13].

The present study also showed a significant increase in occurence of *T. evansi* infection in bovines with increase in age as 15.94% animals were positive in the age group of >5 years, 8.51% in age group of 2-5 years and none of the animals was positive in below two yearage group (Table 3). The findings of the present study are in agreement with the findings of Muraleedharan *et al.* (2005) [16]; Bhutto *et al.* (2010) [5]; Singla *et al.* (2013) [23] and Rani *et al.* (2015) [19] who have reported the highest prevalence in adult animals greater than 5 years old. It may be due to the predisposing stress factors *viz*. lactation, pregnancy, nutritional and climatic changes etc. These findings are also tallied with the results obtained by Singh *et al.*, (2017) [21] who have also found the highest prevalence in >5 years followed by 2 to 5 years of age and lowest in below 2 years of age group of the buffaloes infected with trypanosome infection. The possible reason of lower prevalence in younger animal is their natural protection to some extent by maternal antibodies.

Table 1: Occurrence of trypanosomosis in bovines of Bareilly

S. No.	Sex	Total no. of bovine examined	No. of positive animals (%)
1	Cattle	94	8 (8.51)
2	Buffaloes	38	7 (18.42)
3.	Total	132	15 (11.36)

Table 2: Sex wise occurrence of trypanosomosis in bovines

S. No.	Sex	Total no. of bovine examined	No. of positive animals (%)
1	Male	23	1 (4.34)
2	Female	109	14 (12.84)
3.	Total	132	15 (11.36)

Table 3: Age wise occurrence of trypanosomosis in bovines

S. No.	Age Group	Total no. of bovine examined	No. of positive animals (%)
1	0-2 yr	16	0
2	2-5 yr	47	4 (8.51)
3	Above 5yr	69	11 (15.94)
4	Total	132	15 (11.36)

Conclusion

The overall occurrence of trypanosomosis in bovines was recorded to be 11.36% in Bareilly district of Uttar Pradesh, with higher occurrence in female animals (12.84%) than in males (4.34%). Further, a higher occurrence of infection was recorded in animals of more than 5 years of age (15.94%) than in animals of 2 to 5 years of age (8.51%). The possible cause for maintenance of infection in area could be the prevailing environmental condition favorable for propagation of vectors particularly during the monsoon and post-monsoon season. Further, it is evidenced that a large number of cattle and buffaloes are acting as carriers of infection as *T. evansi* infection could be detected in them by PCR assay only and such animals act as a source of infection to healthy animals. Thus, it is concluded that PCR assay may be used in conjunction with blood smear examination to increase the diagnostic sensitivity to effectively control the spread of *T. evansi* infection in other host as well as in the new geographical areas.

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