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Microscopic detection of *Trypanosoma evansi* in canines

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Abstract
Trypanosomosis is a widely prevalent haemoprotozoan disease caused by a haemoflagellate, *Trypanosoma evansi.* The present study aimed to detect *Trypanosoma evansi* in dogs using microscopic methods like wet blood film examination and conventional staining techniques i.e., Giemsa staining, Field's staining, Acridine orange staining at the field level. In the current study, 314 dog blood samples were collected and examined from different places in and around Hyderabad, Telangana, India. Out of 314 dogs, 5 (1.59%) dogs were found positive by Giemsa, Field's, Acridine orange staining techniques and only 3 (0.95%) dogs were found positive by peripheral wet blood film examination for *Trypanosoma evansi*.

Keywords: *Trypanosoma evansi*, dogs, wet blood film, staining techniques

1. Introduction
Trypanosoma evansi is the most widespread and pathogenic trypanosome, affecting a wide range of mammalian hosts like equines, bovines, canines, felines, camels etc., in tropical and subtropical countries, and causes a significant disease called "surra" (Desquesnes *et al.*, 2013) [1]. It is mechanically transmitted by several genera of hematophagous flies like *Tabanus*, *Stomoxys* and *Haematobia* (Soulsby, 2007; Radwanska *et al.*, 2018) [2, 3] and also orally transmitted by ingestion of fresh infected meat in carnivores (Sinha *et al.*, 1971; Raina *et al.*, 1985) [4, 5]. Most of the outbreaks were reported in rainy and post-rainy seasons due to the build-up of vector population and increased *T. evansi* infection in domestic animals like cattle and buffalo (Losos, 1980; Singh *et al.*, 1993) [6, 7].
Canine trypanosomosis is usually an acute infection and is characterized by intermittent pyrexia (39℃ - 41℃), inappetance, cachexia, edema of the head and throat, hoarse voice, anaemia, paresis of hindquarters, staggering gait, convulsions, conjunctivitis, lachrymation, and corneal opacity (Vershney *et al.*, 1998) [8]. *T. evansi* infection can lead to severe immunosuppression and increased susceptibility to opportunistic infections. The clinical signs of canine trypanosomosis are not pathognomonic, so a confirmed diagnosis is required for proper treatment and management. In the present study, Parasitological techniques like microscopic examination of fresh blood by wet blood film and staining methods were employed to demonstrate trypanosomes in the canine blood, as these techniques are simple, inexpensive and rapid diagnostic methods that can be easily practiced at the field level.

2. Materials and Methods
2.1 Sampling and collection of blood
A total of 314 dogs of either sex varying in ages from 0 to >8 yr old were examined for trypanosomosis from veterinary clinics, animal birth control centers in different parts of Hyderabad, Telangana, India. Blood samples (1 ml) were aseptically collected by a disposable syringe from the cephalic vein into sodium heparin-coated vacutainer tubes from each suspected dog showing clinical signs of pyrexia, anorexia, hindlimb weakness, bilateral corneal opacity, edema of throat and abdomen, and blood smears were prepared.

2.2 Microscopic examination
2.2.1 Wet blood film examination
A drop of blood was aseptically collected by disposable syringe from the marginal ear vein of a dog onto a clean grease-free microscopic slide from 314 dogs, coverslips were placed over blood drops and slides were examined immediately for live and motile trypanosomes under a 10X, 45X objective of a bright field microscope.
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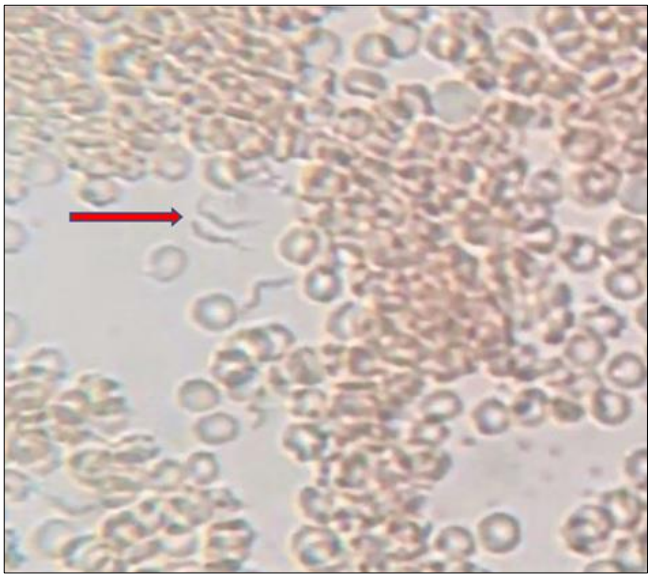

Fig 1: Photomicrograph showing live & motile *Trypanosoma evansi* in wet blood film (45X) of dog blood

2.2.2 Giemsa staining technique

The blood smears were air dried, fixed in methanol for 2 to 3 mins and stained with Giemsa stain of 1:10 dilution (1 part commercial Giemsa stain diluted in 9 parts of distilled water) for 30 mins. These stained smears were rinsed in running tap water to remove excess stain and air dried. A drop of cedar wood oil was placed over the stained smears and slides were observed under the oil immersion lens (100X) of the microscope for the presence of *Trypanosoma evansi*.

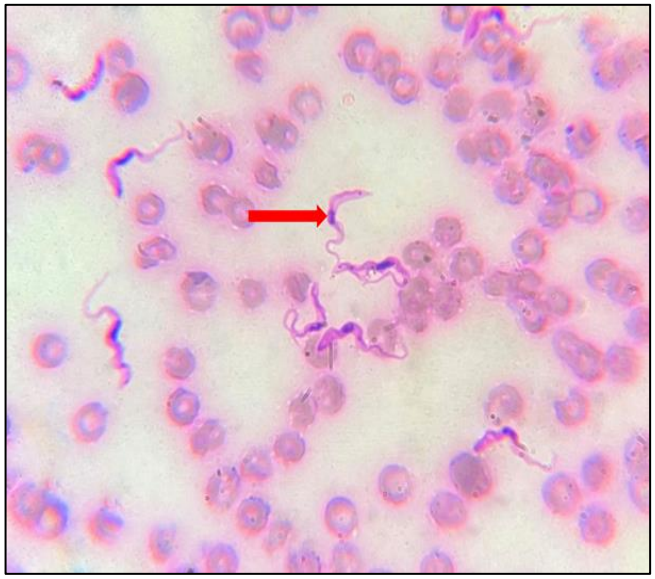

Fig 2: Photomicrograph showing *Trypanosoma evansi* in dog blood smear stained with Giemsa stain (100X)

2.2.3 Field's staining technique

The blood smears were fixed in ethanol for one minute and air dried. The smears were stained with Field Stain B (Eosin Stain) for 5 to 6 seconds and rinsed gently with distilled water. The smears were again stained with Field Stain A (Methylene Blue Stain) for 30 seconds, rinsed and air dried. A drop of cedar wood oil was placed on the stained smears and were observed under oil immersion (100X) lens of a bright field microscope.

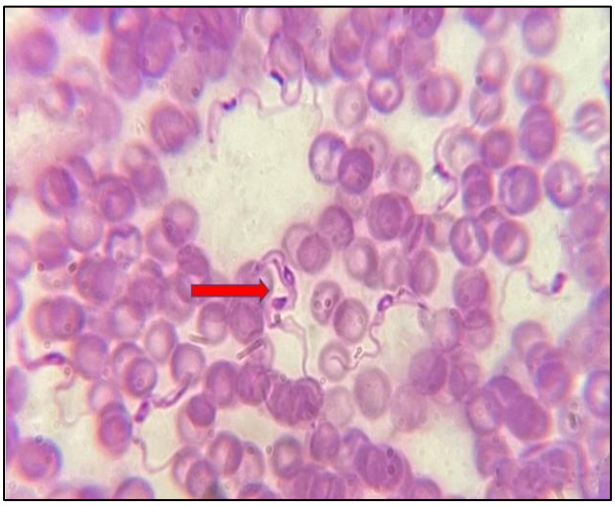

Fig 3: Photomicrograph showing *Trypanosoma evansi* in dog blood smear stained with Field's stain (100X)

2.2.4 Acridine orange staining technique

Blood smears of dogs were fixed in methanol, flooded with a freshly prepared working solution (0.1 mg/ml) of Acridine orange stain and were allowed to stain for 3 minutes. Stained blood smears were rinsed carefully with PBS (pH 7.4), air-dried and examined under 60X magnification of a Fluorescent microscope.

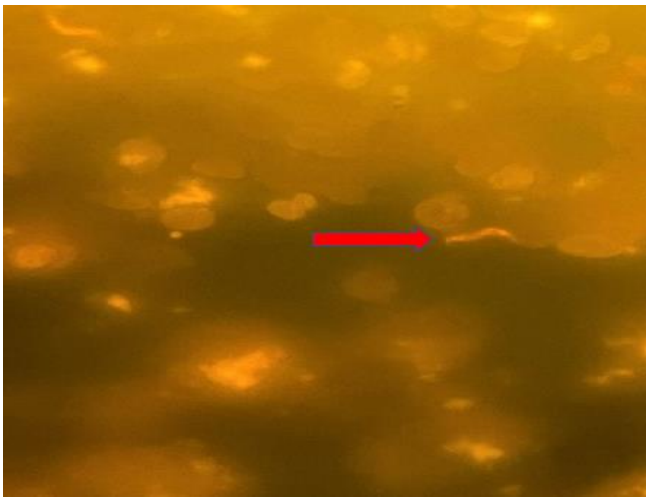

Fig 4: Photomicrograph showing *Trypanosoma evansi* in dog blood smear stained with Acridine orange staining (60X)

3. Results and Discussion

5 out of 314 (1.59%) dogs examined were found positive by Giemsa, Field's and Acridine orange staining techniques. Similar observations were made by Chowdhury *et al.* (2005) [9] who reported a prevalence of 1.72% in dogs in Kolkata, Prasad *et al.* (2015) [10] who reported 1.17% in dogs in Andhra Pradesh, Asif *et al.* (2020) [11] who reported 3.75% in dogs, Alli *et al.* (2020) [12] who reported 0.66% in dogs using staining techniques.

Wet blood film examination of the peripheral blood detected *T. evansi* in only 3 out of 314 (0.95%) dog blood samples screened for the diagnosis of canine trypanosomosis. The results are in agreement with Chowdhury *et al.* (2005) [9] who reported 1.72% in dogs using WBF in Kolkata, Ali *et al.* (2011) [13] who reported 1.7% by WBF in camels in Sudan, Prasad *et al.* (2015) [10] who reported prevalence of 1.60% in dogs by WBF, Alli *et al.* (2020) [12] who reported 0.33%

prevalence in dogs by WBF. The lower incidence recorded by the microscopic examination methods might be due to the collection of blood during stages of low parasitaemia, inherent low sensitivity. The present study concluded that the staining techniques have more diagnostic efficacy over wet blood film examination for the detection of *T. evansi*. Although different techniques have been developed, microscopy remains as the golden standard test and could be used for the demonstration of *T. evansi* in the dog's blood during clinical stages of infections at the field level.

4. Acknowledgement
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