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Pooja Sree Alli
Department of Veterinary Parasitology, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India

M Udaya Kumar
Department of Veterinary Parasitology, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India

GS Sreenivasamurthy
Department of Veterinary Parasitology, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India

P Kalyani
Department of Veterinary Parasitology, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India

Sameena
Department of Veterinary Parasitology, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India

Corresponding Author:
Pooja Sree Alli
Department of Veterinary Parasitology, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India

Diagnosis of canine trypanosomosis by wet blood film examination and staining techniques

Pooja Sree Alli, M Udaya Kumar, GS Sreenivasamurthy, P Kalyani and Sameena

Abstract
In our present study different traditional parasitological techniques i.e., Wet Blood Film Examination and conventional staining techniques like Giemsa stain, Leishman stain, Field's stain and Acridine orange staining technique were used for the diagnosis of canine trypanosomosis in the field. Out of 302 samples examined wet blood film examination revealed only one dog (0.33%) to be positive for trypanosomosis. Only two blood smears collected from 2 different dogs were found positive by all conventional staining techniques viz., Giemsa staining 0.66% (2/302), Leishman staining 0.66% (2/302), Field's staining 0.66% (2/302) and Acridine orange staining 0.66% (2/302) techniques. The main aim was to study the prevalence of canine trypanosomosis because most of the cases go unnoticed without proper diagnosis. The control of these cases is essential, as these act as carriers to other animals and in rare cases to humans.

Keywords: Canine trypanosomosis, wet blood film examination, staining techniques

Introduction
Trypanosoma evansi, causes 'Surra' (Rawashdeh *et al*., 2000) [1] in wide range of domestic and wild animals. Some rare cases of zoonotic infections of surra were reported in people with frameshift mutations in Apo L-1 allele (Vanhollebeke *et al.*, 2006) [11] indicating near future zoonotic threat. *T. evansi* is transmitted mechanically by hematophagous flies such as *Tabanus* (Soulsby, 2007) [10], *Stomoxys* and *Haematobia* sp. Oral transmission by fresh, infected meat or carcasses (Sinha *et al*., 1971) [9] and iatrogenic transmission by contaminated needles during mass vaccination campaigns (Singh *et al*., 1993) [8] were also reported.
Dogs suffer from most severe *i.e.,* acute and fatal form of surra (Curasson, 1943) and often exhibit strong clinical signs like intermittent pyrexia, oedema of the head, including larynx (to be differentiated from rabies), dysphagia, hoarse voice, oedema of the abdominal wall and legs, anaemia, generalized weakness, lack of appetite leading to emaciation and sometimes, paresis of the hindquarters, staggering gait, convulsions, bilateral corneal opacity, conjunctivitis, keratitis along with alterations in haematological and bio-chemical profiles (Chowdhury *et al.*, 2005) [3] and myocarditis in addition to sexual excitement.
Traditional parasitological techniques widely practised at field level can detect clinical stages of infections. Hence, we made an attempt to standardize Wet Blood Film Examination and conventional staining techniques like Giemsa stain, Leishman stain, Field's stain and Acridine orange staining technique to detect prevalence of canine trypanosomosis in dogs in the field and its efficacy was compared in the study.

Materials and Methods
A total of 302 whole blood and blood smears of dogs showing symptoms like pyrexia, anaemia, generalized weakness, dysphagia, paresis of the hindquarters, convulsions, bilateral corneal opacity etc., were collected from clinics in and around Hyderabad and transported to the laboratory within 4 hr of collection on ice at 4 °C.

Wet Blood Film Examination
A drop of blood was taken from marginal ear vein on to a clean grease free microscopic slide and placed a cover slip for examining motile trypanosomes under 10 X and 45 X objective of bright field microscope. Most of the examinations were done in clinics were facilities were available. In some cases, the blood was transported at 4 °C and examined within 4 hours of collection.

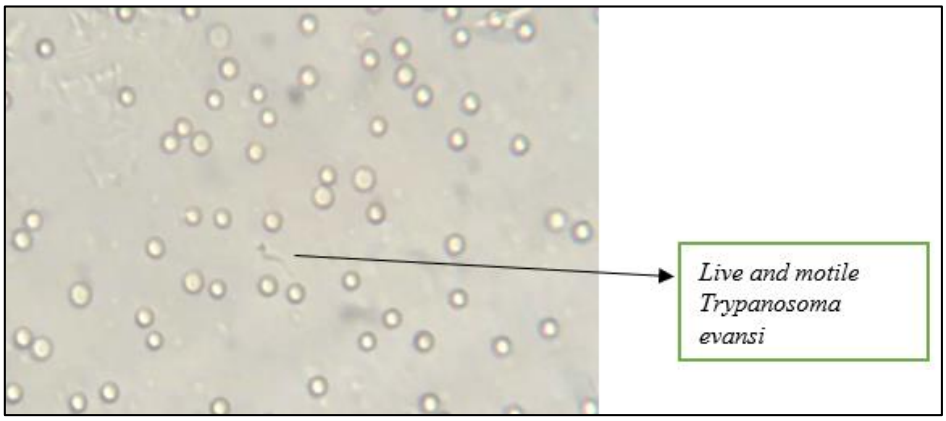

Fig 1: Photomicrograph showing live & motile *T. evansi* in wet blood film examination (45 X)

Giemsa Staining Technique

Blood smears prepared were fixed in methyl alcohol for 2 to 3 minutes followed by staining in Giemsa stain (1 part of stock solution plus 9 parts of distilled water) for 30 min and stained smears were rinsed in running tap water, air dried and observed under 100 x objective of microscope.

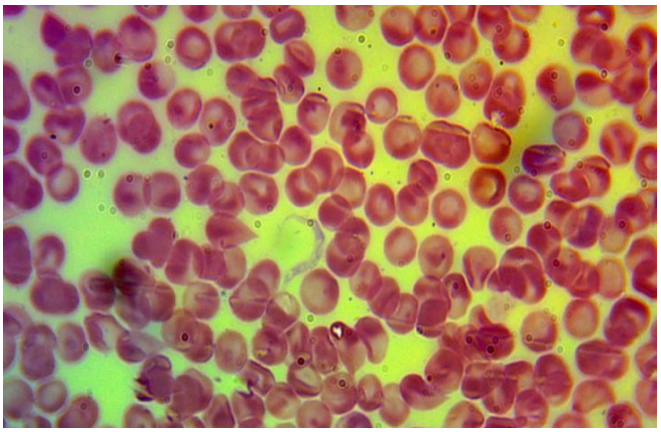

Fig 2: Photomicrograph showing *T. evansi* in dog blood smear stained with Giemsa stain (100 X)

Leishman Staining Technique

Blood smears prepared was flooded with Leishman stain for 3 min. Then added double the quantity of water to the stain and mixed well by blowing air and was allowed to act for 12 min. After rinsing in running tap water and air drying, the slide was observed under oil immersion (100 X) lens of bright field microscope.

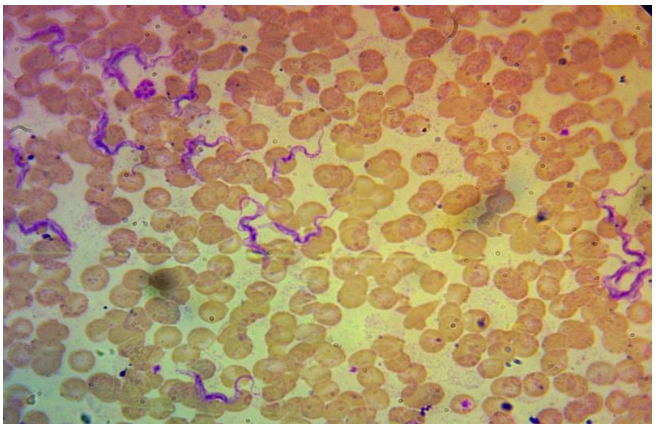

Fig 3: Photomicrograph showing *T. evansi* in dog blood smear stained with Leishman stain (100 X)

Field's Staining Technique

Blood smear prepared was fixed in ethanol for one minute and air dried. The slide was dipped in the Field Stain B (Eosin) for 5 to 6 seconds to fix the smear followed by washing or rinsing the slide in distilled water. The smear was again dipped in Field Stain A (Methylene Blue) for 30 seconds followed by washing in running tap water and air drying. The stained slides were observed under oil immersion (100 X) lens of bright field microscope.

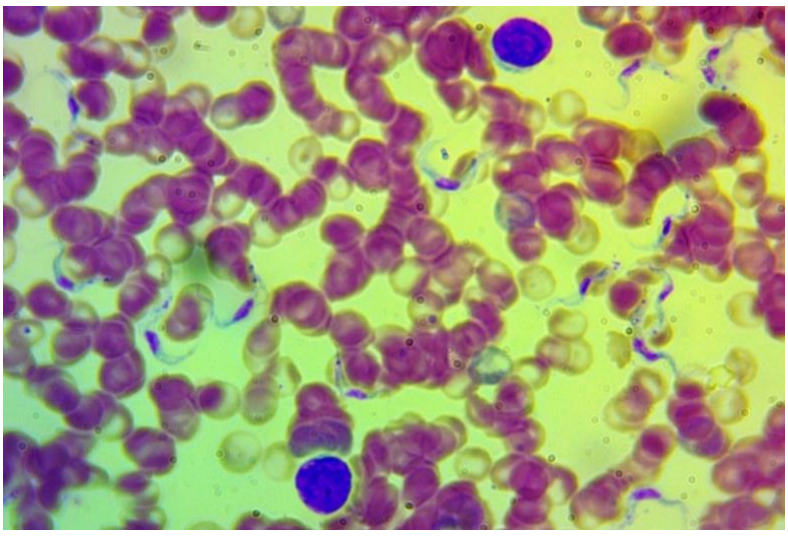

Fig 4: Photomicrograph showing *T. evansi* in dog blood smear stained with Field's stain (100 X)

Acridine Orange Staining Technique

Stock solution of acridine orange (AO) was prepared at 1 mg/ml in distilled water. A working solution of AO was prepared by diluting the stock solution at 1:10 ratio in phosphate buffered saline (PBS) pH 7.4. Methanol fixed blood smears were flooded with freshly prepared AO working solution, and allowed them to stain for 3 minutes. The slides were rinsed carefully with PBS and air dried. The slides were examined at least for 10 min under 60 X magnification of a Fluorescent microscope.

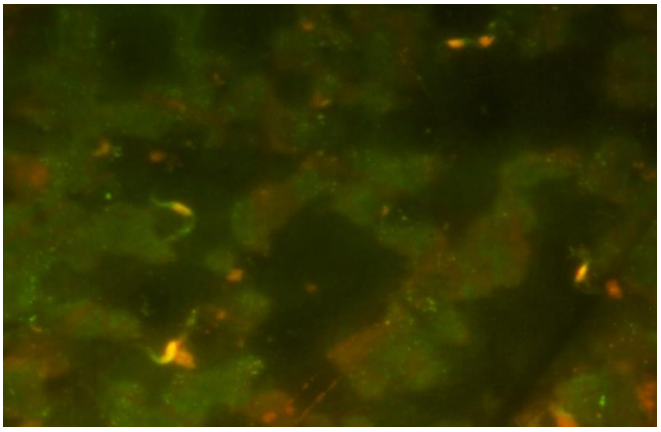

Fig 5: Photomicrograph showing *T. evansi* in dog blood smear stained with Acridine Orange stain (60 X)

Results and Discussion

Wet blood film examination detected only 1 (0.33%) out of 302 blood samples screened for *T. evansi* infection in dogs. The results are in agreement with Malakondaiah (2008) who recorded 0% prevalence in cattle and buffalo by PWBF test in Andhra Pradesh and Karnataka states**.** Slightly higher prevalence was reported using wet blood film examination by Singh *et al*. (1993) [8] in 3.12% (2/64) dogs in Ludhiana, Chowdhury *et al*. (2005) [3] reported 1.72% (5/290) dogs in Kolkata and Lakshmi prasad *et al.* (2013) who recorded 1.60% (15/937) in dogs in Andhra Pradesh. Apart from inherent low sensitivity, the stage of infection while examining the blood could be the reasons for observed low sensitivity in the test.

Out of 302 samples screened, only 2 (0.66%) were found positive for *T. evansi* infection by each of the above conventional staining techniques. Almost similar observations were made by other workers who reported 0.4% (2/4190) in dogs in Chennai by Senthil Kumar *et al.* (2007) [5], 0.13% (2/145) in water buffaloes by Baticados *et al*. (2011) [2], 1.72% (5/290) in dogs in Kolkata by Chowdhury *et al*. (2005) [3], 1.17% (11/937) in dogs in Andhra Pradesh by Lakshmi prasad *et al.* (2013). The lower incidence reported in the present study might be due to inherent low sensitivity of staining techniques and latent stage of infection during collection of blood.

The present study confirmed that, the conventional staining techniques has higher sensitivity over wet blood film examination to diagnose canine trypanosomosis and could be used to screen infections in the field for the effective control of the infection.

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