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SHORT COMMUNICATION

Indirect Fluorescent Antibody Technique based Prevalence of Surra in Equines

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ABSTRACT

This project was carried out to find the prevalence of trypanosomiasis in equine in District Gujranwala by using indirect fluorescent antibody technique and thin smear method. Blood samples were collected from a total of 200 horses and donkeys of different ages and either sex. Duplicate thin blood smears were prepared from each sample and remaining blood samples were centrifuged to separate the serum. Smears from each animal were processed for giemsa staining and indirect fluorescent antibody test (IFAT). Giemsa stained smears revealed Trypanosome infection in 4/200 (2.0%) samples and IFAT in 12/200 (6.0%) animals.

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INTRODUCTION

Surra, an infection of *Trypanosoma evansi*, is one of the most commonly distributed arthropod-born protozoan disease affecting domesticated animals. More severe disease occurs in camels, horses and dogs. Cattle and buffaloes show sub clinical infections and act as carrier. The clinical signs of Surra are indicative but are not sufficiently pathognomonic and confirmed diagnosis must be based on laboratory methods (Akinboade and Dipeolu, 1984). Trypanosomiasis has got intense considerable attention due to its disastrous effects on working animals. Identification of parasite in the thin smears from fresh blood or buffy coat after centrifugation is most commonly employed methods for diagnosis (Queiroz *et al.*, 2000) but these conventional techniques are unable to diagnose chronic or latent infections. These problems could be overcome by the use of advanced techniques like polymerase chain reaction, enzyme linked immunosorbent assay or indirect fluorescent antibody technique (IFAT). Indirect fluorescent antibody technique is a highly sensitive, specific and fast tool for the identification of Trypanosomes in blood (Nantulya, 1990).

In Pakistan, capricious prevalence of Surra has been reported by different authors by using conventional methods (Khan, 1986; Waheed *et al.*, 1998). So, present study had carried out to assess the factual prevalence of the Surra by using IFAT and compares the efficacy of conventional staining technique with indirect fluorescent antibody technique.

MATERIALS AND METHODS

Blood samples (5.0 ml from each animal) were collected from a total of 200 animals (horses and donkeys), randomly, from different localities of district Gujranwala and screened for the presence of *T. evansi* through IFAT and conventional thin smear method. Duplicate thin smears were prepared from each sample and rest of the blood was used to separate serum. One of the two blood smears was processed for Giemsa staining as described by Coles (1986) and other for IFAT (Aquino *et al.*, 1999). Briefly, a drop of blood from a heavily parasitemic horse was distributed on slides with defined cavities of 4 mm (Dunn Labortechnik GmbH). Slides were air-dried, fixed in acetone for 15min and stored at -80°C in individual wrappers. The test slides were brought to room temperature, and diluted serum samples (1:10 to 1:160 in PBS) were placed onto the defined areas on the slides. The slides were then incubated at 37°C for 30min, washed twice in PBS (5min each), rinsed in double-distilled water and dried. After drying, the slides were incubated for another 30min at 37°C with Fluorescein, DTAF –conjugated Affinipure rabbit anti-horse IgG (Jackson Immuno Research Lab) at 1:16 dilution containing 25% Evans blue as a counter stain. The slides were finally washed as previously described, dried and mounted with PBS glycerol and tested under the fluorescence microscope. The IFAT results were recorded at different titers. Animals that showed fluorescence at 1:10 were considered positive.

Statistical Analysis

The difference in the prevalence rate as determined by both techniques was analyzed for significance by Pearson's χ^2 test using SPSS-13.0, a statistical package (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Giemsa stained smears (Fig. 1) and IFAT (Fig. 2) revealed the presence of *T. evansi* infection in 4/200 (2.0%) and 12/200 (6.0%) animals, respectively. The prevalence was higher ($P<0.05$) when determined with IFAT compared with a staining technique. Variable prevalence of Surra 3.0% (Butt *et al*., 1996), 5.18 and 9.09% in horses and donkeys in District Faisalabad and 5% in Lahore (Khan, 1986) and 15.29% (Waheed *et al.*, 1998) in Gujranwala has been reported by using Giemsa stained thin blood smear method. Low level of infection detected through conventional staining technique in present study may be attributable to better management practices or prophylactic chemotherapy in the region.

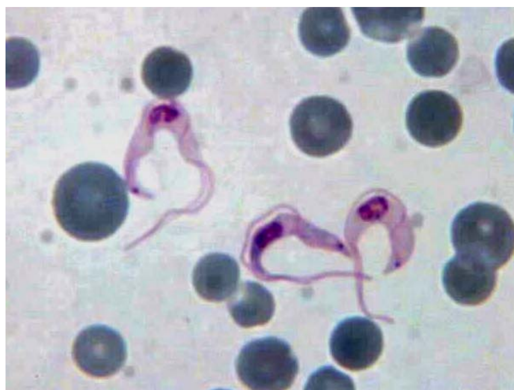

Fig. 1: Photomicrograph of blood smear from a horse with *Trypanosoma evansi* infection. Note elongated trypanosomes.

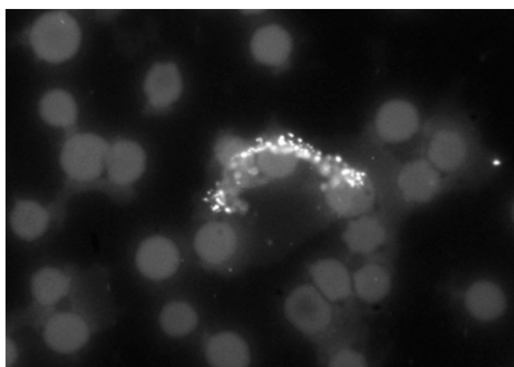

Fig. 2: Photomicrograph showing fluorescent image of *Trypanosoma evansi*

This prevalence of the disease (6%) as evident by IFAT in Pakistan is considerably low as compared to 81.7% (work horses) and 57. 14% (stable horses) reported by Reyna-Bello *et al.* (1998) in Venezuela by using ELISA. This difference in prevalence may be due to disparity in geographical conditions in the two countries. Gutierrez *et al.* (2000) has reported 4.8% *T. evansi* infection in dromedary in Canary Islands by using IFAT. Present study indicated that the IFAT is more sensitive than conventional Giemsa staining method for diagnosis of *T. evansi* infection. Similar findings have been reported by Akinboade and Dipeolu (1984) who described 9 and 55% prevalence by thin blood smear and IFAT, respectively in mules from Nigeria.

From the results of present work, it can be concluded that IFAT is a highly sensitive technique compared with routine blood smear staining method and may be used for the surveillance and diagnosis of Surra which has considerable prevalence in Pakistan. More sensitive techniques will be of great help for disease diagnosis in carrier animals with low parasitaemia or latent infections in cattle and buffalo or chronic infections in camels.

REFERENCES

Akinboade OA and OO Dipeolu, 1984. Comparison of blood smear and indirect fluorescent antibody techniques in detection of hemoparasites infections in mules in Nigeria. Vet Parasitol, 14: 95-104.

Aquino LP, RZ Machado, AC Alessi, LC Marques and Malheiros, 1999. Clinical, parasitological and immunological aspect of experimental infection with *Trypanosoma evansi* in dogs. Mem Inst Oswaldo Cruz, 94: 255-260.

Butt AA, NI Chaudhry, G Muhammad, M Athar and K Iqbal, 1996. Prevalence of haemoparasites among dromedary in and around Faisalabad (Punjab). J Camel Prac Res, 3: 103-106.

Coles EH, 1986. Veterinary Clinical Pathology. 4th Ed., WB Saunders Company London, UK, pp: 46-47.

Gutierrez C, MC Juste, JA Corbera, E Magnus, D Verloo and JA Montoya, 2000. Camel Trypanosomiasis in the Canary Islands: assessment of seroprevalence and infection rates using the indirect fluorescent antibody test and parasite detection tests. Vet Parasitol, 90: 155-159.

Khan MQ, 1986. Prevalence of blood parasites in equines in and around Faisalabad and efficacy and safety of quinapyramine sulphite against trypanosomiasis in naturally infected animals. M Sc (Hons) thesis, Univ Agric, Faisalabad, Pakistan, pp: 53-54.

Nantulya VM, SE Bajyana, and R Hamers, 1989. Detection of circulating trypanosomal antigens in *Trypanosoma evansi* infected animals using a *T. brucei* group-specific monoclonal antibody. Trop Med Parasitol, 40: 263-266.

Queiroz A, P Cabello and A Jansen, 2000. Biological and biochemical characterisation of isolates of *Trypanosoma evansi* from Pantanal of Matogrosso Brazil. Vet Parasitol, 92: 107–118.

Reyna-Bello A, FA. García, M Rivera, B Sansó and PM Aso, 1998. Enzyme-linked immunosorbent assay (ELISA) for detection of anti-*Trypanosoma evansi* equine antibodies. Vet Parasito, 80:149-157.

Waheed MA, IA Khan and MH Khan, 1998. A report on Surra in Gujranwala. Pak Vet J, 18: 170-172.