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ORIGINAL PAPER

Endemic status of *Trypanosoma evansi* infection in a horse stable of eastern region of India – a field investigation

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Abstract Diagnosis of *Trypanosoma evansi* infection in a horse stable of Eastern Region of India on the basis of examination of Giemsa stained blood smears have been done. A high percentage (12.74%) of horses of this stable was found suffering from *T. evansi* infection. This high prevalence of *T. evansi* in horses, in this area could be considered as an alarming situation which has never been explored previously in horses of Eastern Region of India. After a period of 2 months and 18 days of treatment with quinapyramine sulphate and quinapyramine chloride, reinfection with *T. evansi* in treated horses of this stable were noticed. Clinical signs of affected horses and possible causes of reinfection have been discussed.

Keywords Horse · Investigation · *Trypanosoma evansi*

Abbreviations

Anon.	anonymous
Hb	haemoglobin
Mm	mucous membrane
PT	post treatment

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Introduction

Trypanosoma evansi is a protozoan parasite of mammalian blood causing a disease popularly known as 'surra' in several different animals such as cattle, buffalo, horse, camel, pig and deer. This infection display typical signs in affected animals such as fever, anaemia, weight loss, oedema, lymphadenomegaly, conjunctivitis, loss of appetite and sudden death (Silva *et al.* 1995; Brun *et al.* 1998; Aquino *et al.* 1999). The disease causes considerable economic losses to the livestock owners (Seidl *et al.* 1998). 'Surra' in India is generally considered as a disease prevalent mostly in animals of Northern India and prevalence of the disease in equines of Northern India have been reported earlier (Chaudhri *et al.* 1985; Singh *et al.* 1995; Soodan *et al.* 1995). As there are scanty reports of *T. evansi* infection in equines from Eastern Region of India, a detailed investigation about this disease in horses of this stable was undertaken and reported.

Materials and methods

In July 2005, mortality in horses maintained in the stable of Bengal Chemicals and Pharmaceuticals Limited (A Govt. of India Enterprise), Kolkata, West Bengal, India, were noticed. It was observed that these horses were suffering from long standing intermittent

fever, edema of legs and ventral abdomen and weakness of hind legs. Splenomegaly, enlargement of lymph nodes and petechial haemorrhages in kidneys were found during post mortem examination of these died horses. Simultaneously, three horses of this stable (Identification Nos. 215, 217 and 094) were found suffering from anaemia and fever. Smears were prepared by taking the blood of these affected horses and stained by Giemsa stain. The dry stained smears were then examined first under high power of microscope (450X) and then under oil immersion lens (1000X) for detection of parasites. Parasitaemia in the Giemsa stained blood smears were denoted as '+'=1 to 4 number of parasites/field (X1000), '++'=5 to 9 number of parasites / field (X1000) and '+++'=more than 9 parasites/field (X1000) as described by Laha *et al.* (2004). After the proper diagnosis of the disease in these three horses , rest 99 horses (total strength of the horse was 102) were screened for detection of 'surra' by examination of Giemsa stained blood smears, as described earlier. At least five blood smears of each animal were examined. The haemoglobin (gm%) value in the blood of each animal and temperature of each animal during collection of blood were recorded. All animals were treated with a mixture of quinapyramine sulphate and chloride at the recommended doses, subcutaneously. After treatment, all horses were kept under strict observation whether any symptoms of 'surra' happens to occur or not.

Results

Among three horses (Identification Nos. 215, 217 and 094) which were examined firstly for the diagnosis, Giemsa stained blood smears of one animal (Identification No. 215) was found positive for the presence of parasites, morphology of which were indistinguishable from *T. evansi*. Among rest 99 horses, 12 were found positive for *T. evansi* infection. Thus out of 102 horses, 13 horses (12.74%) were found positive for *T. evansi* infection as observed by examination of Giemsa stained blood smears. The temperature of affected animals were varied from 102.0°F to 106.0°F. The Hb (gm %) value of affected animals were ranged from 6.4 gm% to 9.0 gm%. The identification numbers of *T. evansi* infected horses, their clinical signs, parasitaemia, condition after treatment and status of reinfection have been presented in Table 1.

Discussion

In case of horses, reproductive organs were found to be affected as observed by swelling of testicles. Besides the involvement of testicles, oedema of legs and lower abdomen were also observed, in addition to increased temperature, anaemia and emaciation. Several factors are responsible for causing anaemia due to *T. evansi* infection. The first one is the production of haemolysin by trypanosomes resulting into haemolysis of RBCs (Bhatia 2000) and extravascular destruction of RBCs may be responsible for development of anaemia. This destruction may be through the erythrophagocytosis or may be immune mediated (Chakrabarti 2003). Depression of erythropoiesis and non-specific factors, which increase red cell fragility, may be responsible for anaemia in *T. evansi* infection. Oedema during T. *evansi* infection may be due to release of kinin from antigen-antibody complexes, which may cause increased endothelial permeability of vessels leading to oedema. Pyrexia may or may not reflect the degree of parasitaemia and it is due to direct contact of monocytes and macrophages with trypanosoma antigen producing pyrogen or antigen-antibody complex, which stimulates for pyrogen release. A decrease in haemoglobin values of affected animals were observed in the present study and in support of the present finding, an average haemoglobin value (gm%) in healthy horses and *T. evansi* infected horses have been estimated as 13.67±0.66 and 7.13±0.59 respectively by earlier workers (Jani and Jani 1993).

A high percentage (12.74%) of horses of this stable was found suffering from *T. evansi* infection. This high prevalence of *T. evansi* in horses, in this area could be considered as an alarming situation which has never been explored previously in horses of Eastern Region of India, except one (Laha *et al.* 2004). The blood smears of horses which were infected and treated, were again re-examined after a period of 2 months and 18 days PT to observe the status of infection within them. All animals were found negative for *T. evansi* infection. After a period of 4 months and 4 days PT, one horse (No. 213) was suspected for 'surra' by

Table 1 Clinical signs, parasitaemia, status after treatment and status of reinfection in horses

Identification Nos. of infected horses	Clinical signs observed	Parasitaemia	Condition after Treatment	Status of Reinfection	Parasitaemia
215	Swelling of scrotum and lower abdomen, severe, emaciation, lacrimation, congestion in eyes, edema of legs, weakness in hind legs	+++			
210	Severe anaemic, wooden appearance, congestion in eyes, lacrimation	+++	Died on day 2 PT		
206	Severe emaciation, lacrimation, congestion in eyes, edema of legs and abdomen, weakness in hind legs, swollen testicles	+++	Died on day 1 PT		
193	Emaciation, anaemic	+		Reinfected	++
123	Severe emaciation, wooden appearance, congestion in eyes	++			
213	Cachectic appearance, paler visible m.m., congestion in eyes, edema of the legs and abdomen	+++		Reinfected	+++
208	Lacrimation, congested eyes	+			
211	Severe anaemic, Lie down condition, wooden appearance, congestion in eyes	+++	Died on day 1 PT		
192	Anorexia	+			
146	Oedema of legs, wooden appearance, paler visible, mucous membrane, congestion in eyes	++			
183	Cachectic appearance, congestion in eyes	++			
209	Emaciation, anaemic, lacrimation	+			
159	Chronic emaciation	+			
201	Emaciation, anaemic			Infected	++

clinical signs exhibited by the animals. Examination of Giemsa stained blood smears of these horses revealed positive for *T. evansi* which indicated possible reinfection in this stable. Again, all the above mentioned (Table 1) previously infected and treated horses, including another one horse (No. 201) which was found negative earlier and was also suspected for *T. evansi* infection, were re-examined by examination of Giemsa stained blood smears and two horses (193 and 201) were found positive for *T. evansi* infection. So, it could be mentioned that between the period of 2 months 18 days and 4 months 4 days of PT, reinfection has occurred. After the detection of higher prevalence of *T. evansi* infection in this stable, all horses (total Nos. 102) were treated with quinapyramine sulphate and chloride at the recommended doses subcutaneously, for therapeutic as well as chemoprophylactic control of the disease, still reinfection observed in horses after 2 months 18 days PT. A mixture of quinapyramine sulphate and chloride reported to give protection against *T. evansi* infection up to 2–3 months (Soulsby 1982). If the situation is critically analysed, it could be opined that as long as prophylactic level of quinapyramine chloride was maintained, the animals were not re-infected or it could be opined that resistance against this drug within these horses might have developed. Because if we go through the history of this stable, then we could find that in this farm *T. evansi* was detected earlier (Anon. 2003, 2004) and subsequently treated with quinapyramine sulphate and chloride. In the year 2002, amongst 20 horses suspected for 'surra', five were found positive for the presence of *T. evansi* as observed by examination of Giemsa stained blood smears. In the year 2003, 73 horses of this stable were screened to observe *T. evansi* infection by examination of Giemsa stained blood smears and 8 (10.95%) were found positive for *T. evansi* infection, which were treated with quinapyramine sulphate and chloride. Recurrence of infection was not observed at that time even after two months and two weeks of PT (Anon. 2004). The horses of this stable are used to prepare antivenom

serum which is likely to be considered as a stress factor for these horses and flare up of infection. This might be the cause of high prevalence of *T. evansi* infection in this stable and subsequent reinfection. Such type of correlated study on *T. evansi* infection in horses has not yet been done earlier in India. If we consider the development of drug resistance is a cause of reinfection, then repeated use of the same medicine might be the cause of development of drug resistance against *T. evansi* infection and development of drug resistance is now a severe and increasing problem in trypanosomosis (Zhou *et al.* 2004; Witola *et al.* 2005).

Relapse of parasitaemia after treatment with quinapyramine sulphate and chloride were observed earlier by Joshi and Singh (2000) who described relapse of parasitaemia after treatment with quinapyramine has been attributed to survival of the trypanosomes in cerebrospinal fluid as the drug could not penetrate the blood brain barrier. Tuntasuvan *et al.* (1997) also isolated the parasite from the cerebrum, cerebellum, pons and spinal cord of affected animals by MIT. Schonefeld (1979) also found trypanosomes in aquous humour and CSF in all cases of death in ponies.

The horses with identification nos. 206 and 211, in spite of treatment with quinapyramine sulphate and chloride, died 24 hours of PT and horse with Identification no. 210 died 48 hours of PT. If we go through the level of parasitaemia of these three animals, we could see that parasitaemia in the Giemsa stained blood smears of these three animals were '+++' which indicates a higher parasitaemia in these affected horses. Though other two horses (Identification nos. 215 and 213), which also survived after treatment in spite of '+++' parasitaemia might be due to individual resistance. The similar observations of death after 12 hours of treatment of a mule, in spite of treatment with quinapyramine sulphate and chloride and death of a mare treated with Diminazene aceturate on third day of treatment have also been reported earlier (Bhadwal *et al.* 1995; Bharkad *et al.* 2005). The reinfected horse of identification no. 213 and others when detected as infected, it was observed '++' to '+++' parasitaemia, i.e., huge number of parasites were circulated in the blood of these infected horses at that moment.

It can be concluded from this study that 'surra' is quite prevalent among horses of Eastern Region of India, which is an alarming situation. There may be a chance of development of drug resistance against *T. evansi* infection in horses of this Region which should be taken into consideration.

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