SHORT COMMUNICATION

Haemato-Biochemical Studies on *Theileria Equi* Infection in Seropositive Horses

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Abstract

This study aimed to know the haemato-biochemical changes associated with *Theileria equi (T. equi)* infection in five horse-populated areas/district of Rajasthan *viz.,* Bikaner, Ajmer, Barmer, Nagaur, and Pali. Total 151 horses, irrespective of age and sex, were screened for *T.equi* infection. Whole blood and sera samples of all these horses were studied for haematology (Hb, PCV, total RBC, and WBC count), serum biochemistry (SGOT, ALP, GGT, and total bilirubin), and serological test like c-ELISA. On the basis of c-ELISA test, the overall seroprevalence of *T. equi* was 49.66%. Haemato-biochemical studies in seropositive horses revealed none significantly low value of haemoglobin, packed cell volume, total erythrocyte count, and none significantly higher GGT, ALP, and total bilirubin when compared with apparently healthy seronegative horses. Haemato-biochemical studies in seropositive horses revealed a significantly lower value of total leucocyte count and a significantly higher value of SGOT than apparently healthy seronegative horses.

Keywords: c-ELISA, Haemato-biochemistry, Horses, Rajasthan, *Theileria equi.*

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Introduction

Equine theileriosis is a tick-borne haemoprotozoan disease caused by *Theileria equi (T. equi)* in horses (Mehlhorn and Schein, 1998). It is an acute, sub-acute, or chronic disease, globally distributed and causes heavy economic losses in the horse rearing industry (Zobba *et al.,* 2008; Radostits *et al*., 2007). The disease is also known as equine piroplasmosis and is endemic in tropical and sub-tropical areas of the world including India (Kumar *et al*., 2013). The occurrence of equine theileriosis is always related to the distribution of tick vectors, *viz., Hyalomma, Dermacentor, Amblyomma* and *Rhipicephalus spp.* (Battur *et al*., 2001; OIE, 2014).

Equine piroplasmosis is considered as a severe problem of paramount economic importance as the affected horses due to loss of working capacity (Radostits *et al*., 2007). The clinical form of the disease is diagnosed by stained blood smear examination in acute stage of infection, but it is not suitable in carrier horses because of low level of parasitemia (Nagore *et al*., 2004). Several serological tests have been developed to increase diagnostic sensitivity, especially in those carrier horses that exhibit no clinical signs (OIE, 2014). *T. equi* infection is widely prevalent in different geographical parts of India, including Rajasthan (Kumar *et al*., 2013). Given the above facts, the objective of the present study was to determine haemato logical and biochemical changes associate with *T. equi* infection and the prevalence of *T.equi* infection using c-ELISA test in five horse populated areas of Rajasthan.

Materials and Methods

One hundred fifty-one horses, irrespective of age and sex, were screened for *T. equi* infection. These animals were screened from the Teaching Veterinary Clinical Complex of the college of veterinary and animal sciences, Bikaner, Ajmer, Barmer, Nagaur, and Pali districts of Rajasthan. The whole blood and sera samples were collected aseptically from each horse by jugular venipuncture in sterile vacutainers with or without anticoagulant. For haematology, 1.0 mL blood was collected into an anticoagulant (EDTA, @ 1 mg/mL) coated vacutainers. For biochemical studies, 10.0 mL of blood was drawn into a clot activator vacutainer without anticoagulant. The samples were transported to the postgraduate research laboratory of the Department of epidemiology and preventive veterinary medicine, Bikaner, at 4°C.

Blood slants were made and incubated for 1hr at 37°C. Blood clots were broken, and tubes were centrifuged at 2,500

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rpm for 30 min. The serum was pipetted out in small Pyrex tubes and was stored in the deep freeze at -20°C till further processing. Values of haemato-biochemical parameters were recorded to compare apparently healthy (seronegative) and seropositive horse groups.

Haemato-Biochemical Examination

Whole blood samples were subjected to haematological examination for haemoglobin concentration (Hb), red blood cell (RBC) count, white blood cell (WBC) count and packed cell volume (PCV) as per methods described by Jain (1986).

Biochemical analysis of serum samples was done to estimate serum glutamic-oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP), total bilirubin, gamma-glutamyl transferase (GGT) on a biochemistry analyzer. SGOT in sera samples were estimated using kits supplied by Spinreact, S.A. - Ctra. Santa Coloma, Spain. Alkaline phosphatase was determined using kits supplied by Span Diagnostics Ltd., Sachin, Surat, India. Total bilirubin was estimated (as per the Diazo method of Pearlman and Lee) using kits supplied by TransasiaBiomedicals Ltd., Malpur, Baddi, Solan (HP), India.

Serodiagnosis of *T. equi*

Serum samples were analyzed using c-ELISA for *T. equi*. A recombinant protein (rEMA) based c-ELISA has been developed by the equine piroplasmosis laboratory of NRCE (Hissar)for the detection of antibodies against *T.equi* in the horse (Kumar *et al*., 2013).

Data Analysis

The data obtained were statistically analyzed and compared as per the standard statistical procedure suggested by Snedecor and Cochran (1994), and the significance of mean difference was tested by 't' test.

Results and Discussion

In the present investigation, the overall seroprevalence of *T. equi* was 49.66% (75 out of 151 horses) in five horse-populated districts of Rajasthan. Seroprevalence was higher in females (54.31 %, 63/116) than males (34.28%, 12/35). Variable seroprevalence of *T. equi* in horses has also been recorded earlier (38.54% to 52.8%) by Kumar *et al*. (2013), Hussain *et al*. (2014), and Moloi(2010). In the present study, higher seroprevalence was recorded because of presence of tick vectors in the areas and faulty management (Hussain *et al.,* 2014; Asgarali *et al.,* 2007). Clinical findings recorded in seropositive horses with *T. equi* were haemoglobinuria, petechial haemorrhage or congestion on a conjunctival mucous membrane, icterus, fetlock swelling, colic, bronchopneumonia, constipation, lameness, anorexia, debility, and diarrhea. Earlier workers (Hussain *et al.,* 2014; Behera *et al*., 2012; Garba *et al*., 2011; Radostits *et al*., 2007) have also documented similar observations.

The piroplasmosis in horses is caused by protozoa *T. equi* or *B. caballi,* and these are responsible for significant losses in the form of morbidity, poor health, anemia, loss of working capacity etc. The *T. equi* transmitted by a tick is a significant risk factor associated with the endemicity of these parasites. Horses suffering from current diseases are more likely to show the signs of disease, and after infections, some horses show severe signs or become a carrier of infection.

The mean (± SE) values of haemoglobin, packed cell volume, and total erythrocyte count of *T. equi* seropositive horses were similar, while total leucocytes count was significantly ($p<0.05$) lower (9.02 ± 0.29 vs 9.83 ± 0.24 thousand/cu mm) than the healthy seronegative horses (Table 1). The lower values of haemoglobin, packed cell volume, total erythrocyte count in seropositive horses recorded by some authors may be due to anemic condition and suppressed erythropoietic activity in bone marrow due to progressive weakness (Hussain *et al.,* 2014; Alsaad, 2014). The finding of significantly ($p<0.05$) lower mean value of total leucocytes count in *T. equi* seropositive horses observed in the present study concurred with Hussain *et al.* (2014) and Rudolph *et al*. (1975), who observed varied leukogram depending on infection stages and severity. Zobba *et al*. (2008) reported weight loss associated with insignificant leukopenia.

The mean value of serum glutamic-oxaloacetic transaminase (SGOT) of seropositive horses was found significantly ($p<0.05$) higher as compared to the value of apparently healthy seronegative horses (368.69 ± 11.58 vs. 333.29 ± 9.10 U/L). However, the values of gamma-glutamyl transferase (GGT) differed non-significantly between two groups. Further, the mean values of serum,gamma-glutamyl transferase (U/L), alkaline phosphatase (U/L), total bilirubin of apparently healthy seronegative and seropositive horses were

Table 1: The mean ± SE values of a haematological parameter of apparently healthy seronegative and seropositive horses

No.	*Haematological parameter*	*Apparently healthy T. equi seronegative*	*T. equi Seropositive*
1	Haemoglobin	11.69 ± 0.27	11.59 ± 0.30
2	Pack cell volume	39.54 ± 0.74	39.03 ± 0.89
3	Total erythrocyte count	8.67 ± 0.29	8.33 ± 0.34
4	Total leucocytes count	9.83 ± 0.24	9.02 ± 0.29*

*p<0.05, between groups

Table 2: The mean ± SE value of biochemical parameter of apparently healthy seronegative and seropositive horses

No.	*Biochemical parameter*	*Apparently healthy T. equi seronegative*	*T. equi Seropositive*
1	SGOT	333.29 ± 9.10*	368.69 ± 11.58
2	GGT	24.52 ± 1.58	26.37±1.62
3	ALP	491.03 ± 26.43	492.33 ± 28.85
4	Total bilirubin	1.50 ± 0.049	1.56 ± 0.044

*p<0.05, between groups.

statistically the same (Table 2). The findings of seropositive horses in the present study confirmed with the observations of Behera *et al*. (2012), Zobba *et al*. (2008). The elevations of liver enzymes are attributed to reduced blood flow to the liver (Frerichs and Holbrook, 1974). Liver enzymes were elevated due to haemolytic anemia, prolonged anorexia, increased amount of unconjugated bilirubin, and destruction of RBCs (Radostits *et al*., 2007).

Conclusion

This study demonstrated wide exposure of *T.equi* infection in horses of Rajasthan. It is a significant disease that affected horses and showed different clinical signs. A significant change in total leucocytes count and serum glutamic-oxaloacetic transaminase values was recorded between infected seropositive and healthy seronegative horses.

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