SHORT COMMUNICATION

FREEDOM STATUS OF DOURINE *(Trypanosoma equiperdum)* IN MALAYSIA

CHIN S. W.[1], UPPAL P. K.[2], PREMAALATHA B.[1], CHANDRAWATHANI P.[1], NORAZURA A.H.[1] AND RAMLAN M[1]

1.Veterinary Research Institute, 31400 Ipoh, Malaysia

2.770, Sector 17-A, Gurgaon – 122 001 (Haryana), India (E-mail : profpkuppal@gmail.com)

* Corresponding author: tsooichin@gmail.com

ABSTRACT. The objective of this study was to determine the seroprevalence of dourine from imported horses using Complement Fixation Test. Dourine is classified as a OIE listed B disease (OIE Terrestrial Manual 2008) where equines are mainly susceptible to it and it had occurred in many countries such as Africa, the Middle East, South America and South-eastern Europe. Malaysia regularly imports Australian horses for the equestrian sector which encompasses activities such as racing and endurance as well as other recreational or leisure activities in Malaysia. As such, awareness towards this disease is important during importation of horses to avoid dourine embark into Malaysia. A total of 288 horse sera samples obtained from animal quarantine stations and private stables were examined for dourine by complement fixation test. Results showed serologically negative results for dourine with all the samples tested.

Keywords: dourine, complement fixation test

INTRODUCTION

Dourine is a specific, chronic diseases of equines caused by *Trypanosoma equiperdum,* transmitted chiefly by sexual contact, characterised clinically by inflammation of genitalia, followed by edema of subcutaneous tissues (silver dollar plaques) and paralyses. The disease was originally noticed in Asia and North Africa. In USA, diseases were traced with the importation of stallion from France in 1882 by Williams (1886). During the same period 1885 to 1886, the disease was described by Pease (1905) from India. In the past, dourine was widely distributed in Europe, Asia and Africa. The disease was eradicated in the USA, Canada, European Union and India by testing and slaughter policy. In India, the disease was prevented by the adaptation of Dourine Act of 1910 of the Indian Penal Code. There has been no case of dourine in India recorded after 1920-21 (Uppal 2001). The test used to detect infection was complement fixation test which is also presently recommended by the World Animal Health Organisation

(OIE). With the advent of globalisation movement of horses for import and export of horses for breeding, competition, racing and sports events has increased. Therefore, the freedom from equine diseases in horses through standardised quality assured diagnostic system, sustained surveillance, monitoring, reporting of diseases and quarantine has become necessary to demonstrate transparency on the occurrence of diseases, if any. It is with this view the status of dourine in Malaysia by sero diagnostic method is examined so that the equine industry in Malaysia stands to gain. The serological test available for dourine testing is complement fixation test (CFT) and Elisa. However, complement fixation test described by Watson (1915) is the only validated diagnostic test at this moment accepted for the international trade of equines (OIE Z00 - Sanitary code). Dourine is a disease of great economic importance and well documented as a trade barrier for the movement of horses. It is with this objective, the present study has been undertaken to know the serological status of dourine amongst Malaysian horses. This seems to be the first study in this country to know the freedom status of dourine in Malaysia.

MATERIALS AND METHODS

Complement Fixation test on the lines of Uppal and Nilakantan (1966) was used.

Sheep erythrocytes: Throughout the study sheep blood which was not fragile was used. Blood was collected in Alsever's solution 1:1 volume and stored in refrigerator. Blood was centrifuged washed thrice at 1500 RPM for 10 minutes and cells suspended in veronal buffer (VB).

Haemolysin: It was prepared in rabbits using stroma of erythrocytes. It was preserved in equal volume of glycerine. Haemolysin was titrated and suitable dilution was used.

Indicator system: Sheep cells were sensitised with four MHD of haemolysin.

Complement: Pooled guinea pig complement with good lytic activity was obtained from male guinea pigs, housed in the Institute's animal house. The complement was titrated and diluted for test system.

Antigen: German Strain of *Trypanosoma equiperdum* was used. Animal and plant health inspection service of USA National Veterinary services laboratories (NVSL) is also using the same strain. This German strain was originally obtained by the bureau of animal industry in Beltsville, Maryland in 1923 via infected dog and was later given to NVSL. Subsequently, this strain was designated as the American strain. Present antigen used was produced by Uppal in (1999) in bulk at Bundes Institut Fur Gesundheitlichen Verbraucheschutz Und Veterinar Medizin (BGVV) Berlin, Germany in the laboratory of Dr C. Staak. Antigen was prepared from the blood of experimentally infected rats. Pooled blood was filtered and concentration of trypanosomes were done by ion exchange chromatography and finally washed

several times before trypnosomas were lyophilised. The lyophilised antigen was reconstituted in distilled water and diluted to the working strength in VB. Antigen is standardised against a 1:5 and 1:10 dilution of antisera.

Antisera: Freeze-dried form of antisera was reconstituted with sterile distilled water. Sera was produced in experimentally infected equines at BGVV (Berlin). Before use, serum were inactivated at 58°C for 30 minutes.

Normal Control Serum: Inactivated healthy horse serum, screened free from glanders, dourine and piroplasmosis antibodies was used. All the sera were inactivated at 58°C for 30 minutes.

Field sera: In all 288 horse sera, samples were obtained from animal quarantine stations and private stables.

Complement fixation test: The test was done in microtitration plates using an eight channel micro diluter. The plates were labeled. Serum at 1:10 dilution with 25 µl volume, antigen containing two units with 25 µl volume and 25 µl of complement having 1½ minimum haemolytic Dose (MHD) were used. All suitable controls were included. Test plates were covered with cling film and incubated in a water bath at 37°C for 60 minutes with occasional shaking. Subsequently, 50 µl of sensitised RBCs with uniform suspension were added to all the wells. Plates were further incubated for 30 minutes and removed for reading the results. The degree of haemolysis was judged visually. Samples were regarded as positive which showed complement fixation of more than 50%. Anti-complementary sera were retested after heating at 62°C for 30 minutes.

RESULTS AND DISCUSSION

Malaysia has never reported any clinical case of dourine amongst horses. To demonstrate the freedom against dourine, it is pertinent that sero-monitoring studies are in place. In view of this, a total of 288 horse sera samples obtained from animal quarantine stations and equine establishments, were tested for dourine using complement fixation test which is a prescribed test for international trade of horses (OIE Terrestrial Manual Trade 2008). It was reported that there is cross reactions resulted from different protozoal infections occurring in the test due to presence in some countries of other trypanosomes (Cencek *et al.*, 2008). Most of the countries have eradicated dourine using complement fixation test. Similar test was used in India for the movement of thoroughbred horses within India and abroad (Uppal, 2003 and 2009).

There has been apprehension that there is some cross reaction with washed dourine antigen because rat blood cannot be completely removed from trypanosoma preparations. Therefore, in the present studies, ion exchange chromatography antigen developed was used. Similarly, control negative sera used was free from glanders, dourine, and piroplasmosis antibodies to minimise cross reactions. In addition "warm" fixation technique rather

than "cold" fixation technique was used. Dr C. Staak working at BGVV, Berlin demonstrated that cold fixation technique was disastrous. In his experiment done on 65 German horses with certainly no past history of dourine showed more than 50% of horses were positive at 1:10 dilution of serum. However, by warm technique only one horse out of 65 showed positivity at 1:10 while the rest were negative (Personal communication). Therefore, warm fixation technique was used in the present studies on 288 horse sera samples. None of the sera samples were found positive against dourine antigen. The result shows authenticity that horses in Malaysia which are clinically free from dourine are also free serologically. Similarly, in India, more than twenty thousand horses from various agro-geographical conditions were tested with this antigen in order to obtained country freedom against Dourine. All the sera samples have been found negative at Diagnostic Research Laboratories (Pune) approved by government of India and dossier has been developed (Uppal, personal communication). The present antigen used is relatively much better and had not shown any false positive reaction either on sera used in India or on sera samples originated in Malaysia. This study reinforces the existing information on the absence of Dourine in Malaysia

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