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Seroprevalence and risk factors of *Trypanosoma evansi* infection in horses in Peninsular Malaysia

E.I. Elshafie [a,b], R.A. Sani [a,*], L. Hassan [a], R. Sharma [a], A. Bashir [a], I.A. Abubakar [a]

[a] Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Selangor, Malaysia
[b] Central Veterinary Research Laboratories, Al Amarat, P.O. Box 8067, Khartoum, Sudan

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ABSTRACT

A cross-sectional study was designed to assess the seroprevalence and risk factors associated with *Trypanosoma evansi* infection among horses, using a total of 527 blood samples obtained from eight states in Peninsular Malaysia. A structured questionnaire was used to collect data on risk factors associated with *T. evansi* seroprevalence. The overall seroprevalence detected by card agglutination test for *T. evansi* (CATT/*T. evansi*) was 13.90% (73/527, CI: 11.2–17.1%). Female and exogenous horses showed a higher risk in association with the disease seroprevalence compared to other groups. The majority of the horse owners were not familiar with surra (85.30%). However, most of them were very cautious with the health of their animals. In conclusion, this study showed that *T. evansi* occurred in low frequency among horses in Peninsular Malaysia, and the good management system adopted by horse owners was probably responsible for the low *T. evansi* occurrence.

1. Introduction

Trypanosoma evansi is a flagellated protozoon that causes a devastating disease called surra. It is the most prevalent pathogenic trypanosome throughout the tropical and subtropical areas of the world, owing to its ability of mechanical transmission by biting flies (Luckins, 1988). An Australian mare formed the focus of *T. evansi* outbreak in Peninsular Malaysia about a century ago (Fraser and Symonds, 1909). Following this report, a series of outbreaks occurred sporadically and affected other mammalian hosts. A recent report indicated a seroprevalence of 78% in a deer farm (Adrian et al., 2010). The occurrence of *T. evansi* infection in horses in Peninsular Malaysia has been poorly studied and the last publication on natural infection was reported by Ikede et al. (1983).

T. evansi infects a wide range of domestic and wild animals. Effects of the infection in different geographical locations vary according to the strain of parasites and species of the host (Losos, 1980). The risk factors for trypanosomiasis can be the environment, as observed in the high seroprevalence of *T. evansi* in camels in a wooded area adjacent to watering points (Dia et al., 1997), or host particularly the association between seroprevalence and age of camels (Gutierrez et al., 2000; Njiru et al., 2004). Therefore, an assessment of the risk factors can enhance the control program of *T. evansi* infection since it can determine the factors associated with disease either at management or at host levels.

Increasing interest in equestrian sports in Peninsular Malaysia enhanced the importation of exogenous horses from *T. evansi*-free geographical regions such as Australia, New Zealand, United Kingdom and America (Bashir, 1993). The introduction of naive horses to endemic *T. evansi* areas can greatly affect this animal species by developing a severe sickness. In addition, the climate of the country is favourable for the disease vectors to propagate and increases the mobility of *T. evansi* between susceptible and reservoir hosts (Luckins, 1988).

No previous assessment of the disease prevalence and risk factors has been conducted for *T. evansi* infection in horses in Peninsular Malaysia. Thus, our study investigated the seroprevalence of *T. evansi* using CATT/*T. evansi* and determines a number of hypothesized risk factors associated with the seroprevalence among horses in Peninsular Malaysia.

2. Materials and methods

2.1. Study design and sample collection

A total of 527 blood samples were obtained from 66 horse stables located in eight out of the 11 states of Peninsular Malaysia namely Kedah, Kelantan, Terengganu, Pahang, Selangor, Negeri Sembilan, Melaka and Johor from October 2007 to August 2009. Horses were selected based on the convenience and willingness of animal owners. The minimum sample size required in this study was calculated as suggested by Thrusfield (2005) for the estimation of prevalence in a large population. Calculation was done based on

* Corresponding author.
E-mail address: rehana@vet.upm.edu.my (R.A. Sani).

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the expected prevalence of 43% (Sani et al., 1995), absolute precision of ±5% and 95% confidence interval. Each animal was physically examined, and 5 ml of blood was collected via jugular venipuncture into ethylenediaminetetraacetic acid (EDTA) tube. All samples were kept in an icebox containing ice packs and transported to the laboratory within half-a-day.

2.2. Packed cell volume (PCV)

Measurement of PCV was used as an indication for anaemia. Each capillary tube was filled with approximately 70 μl of EDTA whole blood, centrifuged at 12,000*g* for 5 min and then PCV was measured using a manual haematocrit reader.

2.3. Card agglutination test for T. evansi (CATT/T. evansi)

Plasma was separated from collected blood samples by centrifuging at 1000*g* for 3 min and then tested for the presence of anti-*T. evansi* antibodies using the CATT/*T. evansi* kit (Institute of Tropical Medicine, Antwerp, Belgium). Approximately, 45 μl of the antigen was poured into the circular reaction zones of the supplied plastic card and mixed with 25 μl of the test plasma diluted at 1:4 with PBS pH 7.2 according to the manufacturer's instructions. The antigen/plasma mixture was then spread with a clean plastic stirring rod. Each plastic card contained two reaction zone for the positive and negative controls provided by the manufacturer. The card was agitated at 70 rpm for 5 min on an electric rotator (Ika Labortechnik®, KS 130 basic, Germany) and the reaction was checked in visible light. Positive reactions were confirmed by the presence of blue granular agglutination. Weak positive samples were considered as seropositive because horses are highly susceptible to *T. evansi* infection.

2.4. Hypothesized risk factors and questionnaire

Two questionnaires were designed to generate information on the animal and environment. Firstly, information on the individual animal such as, age, sex, breed, purpose, distance covered during rides, herd structure, trypanocidal treatment and insect control program were gathered. A second questionnaire was performed at stable level which includes questions on the stable location, exercise regime of the horses, housing, source of horses, owner action on diseased horses, and type of neighbouring farms. The knowledge of the horse owners about surra, clinical signs and occurrence of the disease were also obtained.

2.5. Data analysis

The seroprevalence of *T. evansi* was calculated based on the number of seropositive animals divided by the total population at risk (Thrusfield, 2005). The univariate association between the seroprevalence and the categorical risk factors with two levels such as gender, herd structure and insect control program were investigated individually by testing its significance using the Pearson Chi-square or Fisher Exact test. Risk factors with more than two categorical levels such as the stable location, age, breed, purpose and distance covered during rides were investigated individually using univariate logistic regression. Binary logistic regression was performed to test the significance of the variables in the model and to test the significance revealed by the univariate analysis. Mean packed cell volume for the seropositive and seronegative samples was computed and compared using independent *T*-test. All statistical tests were conducted using SPSS version 18 (SPSS Inc., Chicago) at $\alpha = 0.05$ significance level.

3. Results

3.1. Questionnaire analysis

Descriptive statistics of the stables sampled are summarized in Table 1. Data were obtained from 34 out of 66 stables in the target population. The seroprevalence of *T. evansi* in the 34 stables was 15% (48/319, CI: 11.54–19.39) while the seroprevalence calculated from the stables which did not submit their questionnaires (32 stables) revealed a prevalence of 12% (25/208, CI: 8.28–17.14). The seroprevalence of the latter group of stables appeared within the confidence interval of that of the 34 stables. None of the horse owners report a trypanocidal treatment for the last 6 months prior sample collection.

Table 1
Summary of questionnaire survey responses on risk factors of *T. evansi* infection by horse owners.

Subjects	Frequency	Per cent
Do you routinely exercise your horse?		
(a) Yes	33	97.1
(b) No	1	2.9
Total	34	100
Where do you keep your horses most of the time?		
(a) Indoor	31	91.2
(b) Outdoor	3	8.8
Total	34	100
Are there other species of animals in your farm?		
(a) Yes	10	29.4
(b) No	24	70.6
Total	34	100
Where is the source of your horses?		
(a) Own breeding	4	11.8
(b) Bought from neighbouring stable	5	14.7
(c) Bought from another state	8	23.5
(d) It imported overseas.	17	50
Total	34	100
What is your action when a horse comes down with a disease?		
(a) Treat it by yourself	4	11.8
(b) Contact experienced friend	3	8.8
(c) Contact a regional veterinary service	19	55.9
(d) Contact UPM clinic	8	23.5
Total	34	100
Is there any farm located 5 km from your stable?		
(a) Yes	16	47.1
(b) No	18	52.9
(c) Not sure	0	0
Total	34	100
If yes, what type of animal on the neighbouring farm?		
(a) Cattle	5	14.7
(b) Sheep/goat	1	2.9
(c) Mixed ruminants	10	29.4
(d) Do not have	18	52.9
Total	34	100
Have you heard of the disease trypanosomiasis?		
(a) Yes	5	14.7
(b) No	29	85.3
Total	34	100
If yes, what are the signs of the disease?		
(a) Weakness, pale mucus membrane and jaundice	5	14.7
(b) Do not know	29	85.3
Total	34	100
Have you seen these signs in your horses?		
(a) Yes	0	0
(b) No	34	100
Total	34	100