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Outbreaks of trypanosomiasis and the seroprevalence of *T. evansi* in a deer breeding centre in Perak, Malaysia

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Introduction

Trypanosoma evansi causes the disease called trypanosomiasis or "surra" in many species of both domestic and wild animals (Luckins and Dwinger 2004). The protozoa are transmitted mechanically by biting flies such as *Tabanus* and *Stomoxys spp*. (Luckins 1988). Trypanosomiasis generally causes anaemia, progressive emaciation and weakness in affected animals. In Malaysia, trypanosomiasis has been reported in institutional farms of cattle and buffaloes (Abas-Mazni and Zainal-Abidin 1985; Cheah et al. 1999) and in a rhinocerous centre (Vellayan et al. 2004). The disease is considered endemic in Southeast Asia, and has been reported in Thailand (Lohr et al. 1985), Indonesia (Payne et al. 1991) and Vietnam (Pham Si Lang 1991).

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Deer are susceptible to *T. evansi*, however, reports of trypanosomiasis in deer is not common. Within the region of Asia, only Gu et al., and Chen et al., as cited by Lun et al. (1993) have reported the occurrence of trypanosomiasis in deer in several provinces in China. In Malaysia, deer farming is gaining popularity due to its potential to cater for the meat market and for agro-based tourism and industry. Therefore, any diseases such as trypanosomiasis that result in large numbers of deaths of deer should strategically be controlled or prevented. In 2006, a large deer breeding centre in Lenggong in the state of Perak suffered an episode of deaths of the *Cervus timorensis russa* that was later confirmed as due to trypanosome (Nurulaini et al. 2007). Within a span of one week in March 2006, the deaths of 17 adult female deer between the ages of 12–13 years were recorded. During that period, 8 out of 28 blood samples submitted to the Veterinary Research Institute (VRI) in Ipoh, Perak were found positive for *T. evansi*. The present study was conducted to examine the trypanosomiasis outbreaks, and to determine the prevalence of trypanosomes among the deer population since the first outbreak in 2006.

Materials and methods

Background of the farm

The present study was conducted in November 2007 at a government livestock breeding centre located in

the forest reserve in Lenggong, Hulu Perak. The centre was established in 2000. The deer were used for breeding and research while a small proportion was slaughtered for meat and meat by-products. The deer population originated from a farm in Behrang Ulu, which has since ceased its operations. The animals were raised in an extensive system where they were allowed to roam freely. The centre practiced a scheduled deworming programme for all deer and occasional fogging to control flies. At the time of the study, the total number of deer was 1106. *Timorensis* deer made up the majority number with 939 heads. The *Axis* and the *Sambar* deer accounted for 112 and 55 heads, respectively. No identification system was used in the centre. Other than deer, the centre also had a few resident buffaloes, cattle, eland (*Taurofragus oryx)* and mousedeer (*Tragulus napu*).

The centre is surrounded by the Lenggong forest where wild pigs (*Sus scrofa* Linnaeus) and other wild animals such as wild eland and barking deer (*Muntiacus muntjak)* are frequently spotted. At the time of the study, there were other deer farms located within 25 km of the centre.

Investigation of the outbreak

Definition of cases

In the present study, death due to trypanosomiasis was defined as such according to center records stating that death was due to the disease. *T. evansi* infection was confirmed based on the clinical signs manifested by the animal prior to its death and the ensuing post-mortem findings and laboratory confirmations.

Data collection

A set of open-ended questionnaires was prepared to collect information about the outbreaks. The questionnaires were administered through face-to-face interviews with farm personnel. Farm records between January, 2006 and October, 2007 were also examined, which included laboratory and post-mortem reports of dead animals (when available).

Prevalence of trypanosome

A total of 30 adult males (> 24 months old), 30 young males (6– 24 months old) and 40 young females (6–24 months old) were non-randomly selected from the *Timorensis* deer. Other breeds of deer were not sampled due to technical difficulties in handling. Adult female *Timorensis* deer were not sampled to avoid causing stress that could result in abortions among any pregnant deer.

Blood was collected via jugular venipuncture using EDTA and plain vacutainer tubes. Blood from EDTA tubes were subjected to the Haematocrit Concentration Technique (H.C.T.) test (Woo 1969). Serum samples were subjected to the Card Agglutination Test for Trypanosomiasis (CATT/ *T. evansi*) according to Bajyana Songa and Hamers (1988). The CATT/*T. evansi* test has been proven suitable for testing *T. evansi* in various species of animals such as horses (Claes et al. 2005) and buffaloes (Verloo et al. 2000) with a high level of sensitivity (between 80–100%) and specificity (98%).

Data analysis

Data were analysed using SPSS version 15.0 (SPSS Inc, Chicago). Comparison between proportions was carried out using Chi-square test or Fischer Exact Test. All tests were performed at a significance level of $\alpha=0.05$. The mortality rates were calculated using the formula based on Gertsman (2003) as follows:

Crude mortality rate:

(No. of deaths/midyear total population) x 100

Cause-specific mortality rate:

(No. of death due to cause/midyear total population) x 100

Age-specific mortality rate (similar for gender-specific):

(No of death in age group/midyear no. of deer in age group) x 100

Proportional mortality rate:

(No. of death due to cause/total deaths in the population) x 100

Results

Trypanosomiasis outbreaks began when four female deer died acutely in March, 2006. This was followed by another 18 deaths in April, 2006. At the time, a

team of investigators from VRI visited the farm and samples were collected from the remaining living animals where the presence of *T. evansi* was detected. Given the suggestive post-mortem findings along with the parasitological diagnosis, trypanosomiasis was determined to be the cause of death (Nurulaini et al. 2007).

The cases subsided after April (2006) and emerged later when 10 deaths in October and one in November were recorded. Infected animals were weak, inappetant, self-isolated, with tilted heads and blindness. For a proportion of the animals, their blood samples were sent to VRI prior to their death which revealed *T. evansi*. Among the post-mortem findings were pale mucous membrane, watery blood, sunken eyes, consolidation of lungs, presence of chicken fat clot in the blood vessels of the heart and hepatic congestion. The presence of *T. evansi* was detected from organ (such as spleen, lung, liver, heart and kidneys) impression smears. In 2007, deaths due to trypanosomiasis occurred every month until October. An increased number of cases were recorded for March and June (Fig. 1). There were no cases of trypanosomiasis in other breeds of deer (i.e. *Axis* and *Sambar* deer) or in other animals present at the centre.

All *Timorensis* deer, the only group that appeared to be affected, were treated with Berenil®, containing 7% diminazene aceturate, given at 3.5 mg/kg intramuscularly. Treatment was repeated within 3 months after the initial treatment. After the outbreak, fogging was done with lambda cyhalothrin 2.8% w/w every month in 2006 to control flies. For the first 10 months of 2007, three fogging sessions were carried out.

The crude mortality rate among deer at the centre for 2006 and 2007 was 9.2% (119/1281) and 9.5% (111/1165), respectively. Trypanosomiasis resulted in 27.7% and 29.7% of all deaths (proportionate mortality) that occurred at the centre in 2006 and 2007, respectively. The cause-specific death rates due to trypanosomiasis were 2.6% (3/1281) and 4.6% (54/1165) for the years 2006 and 2007, respectively, and the difference between the rates was significant (χ^2= 6.953, p=0.008). The trypanasomisis case-fatality rate cannot be computed as information on deer showing clinical signs was not recorded. The gender-specific mortality rate among *Timorensis* male deer in 2006 and 2007 was 1.2% (4/340) and 7% (18/255), respectively; while among female deer the rate was 4.1% (29/711) and 5% (36/720), respectively (Table 1). The difference between the male and female mortality rates for the two years was not significant (χ^2=0.912, p=0.066). Deaths due to trypanosomiasis appeared to occur more in adult animals (Table 1) as compared to younger ones (p=0.01). Adult animals were about 2.5 times more likely to die of trypanasomiasis as compared to younger animals (i.e. age <12–24 months) (OR=1.9, 95 CI= 1.2–3.1). No deaths were seen in deer less than 12 months old.

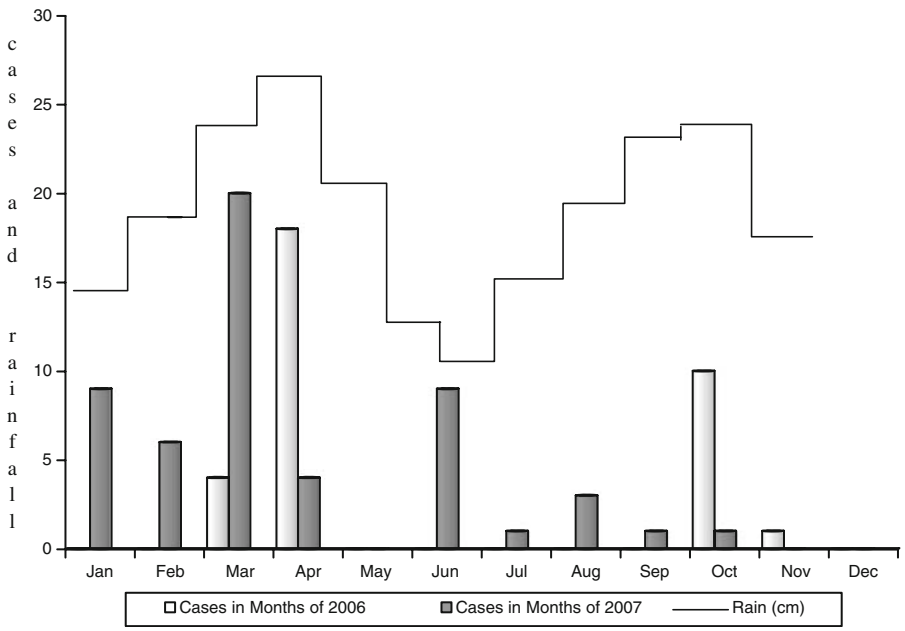

Fig. 1 Epidemic curve of trypanosomiasis that occurred in a deer breeding centre at Lenggong, Perak between January 2006 and Oct 2007 (number of cases) and the average monthly rainfall (cm) for the two years in Peninsular Malaysia (source: Malaysian Meteorological Centre). □ Cases in the months of 2006; ▩ Cases in the months of 2007; ─rainfall (cm)

Table 1 Deaths due to trypanosomiasis in deer based on age and sex between 2006 and 2007 at a deer breeding centre in Lenggong, Perak

Age												
	<12 months				12–24 months				>24 months			
	male	n	female	n	male	n	female	n	male	n	female	n
*2006	0	122	0	117	0	141	18	134	4	77	11	460
*2007	0	103	0	110	5	107	4	110	13	69	32	513

* n; total population at midyear

It appeared that the occurrence of surra in this centre followed the general rainfall pattern (Fig. 1). Most deaths occurred between January and June with a peak in March and April, coinciding with the wettest months of the year.

Prevalence of *T. evansi*

T. evansi was detected in 23% of blood samples using the HCT, while antibodies against the parasite were detected in 78% of the samples. All but one of the samples that tested positive for HCT also tested positive using CATT/*T.evansi.*

The prevalence of trypanosome based on the HCT was similar between deer of different age categories (χ^2=0.971, p=0.325) and gender (χ^2=0.762, p=0.383) (Table 2).

Using CATT/*T.evansi*, the seroprevalence was similar across age groups (χ^2=0.710, p=0.399). Young female deer appeared to have a lower seroprevalance to *T. evansi* compared to male deer of similar age group, however the difference was not significant (χ^2=3.345, p=0.064) (Table 2).

Discussion

The outbreak of trypanosomiasis among the deer population in Lenggong was the first to be reported in Malaysia. Between 2006 and 2007, more than a quarter of deaths that occurred in the centre were due to trypanosomiasis. This signifies a considerable economic loss as approximately one in four deaths on the farm were attributed to *T. evansi* infection. The source of *T. evansi* remains speculative as the common vector for the disease, *Tabanus* and *Stomoxys* flies, were not observed in the centre. Nevertheless, their role in *T. evansi* transmission cannot be overruled as the centre is surrounded by thick jungle and their presence might have been missed. The presence of tabanids was previously reported in an outbreak of trypanosomiasis which killed five Sumatran rhinoceros (*Dicerorhinus sumatrensis*) at a conservation facility in Selangor, Malaysia (Vellayan et al. 2004) indicating that the fly is present within this part of peninsular Malaysia. Other than *Tabanus* and *Stomoxys* flies, *T. evansi* can also be transmitted by other flies (Hilali and Fahmy 1993; Lun et al. 1993) including *Musca* and *Haemoptobia* (Jones et al. 1996).

According to one officer at the centre, prior to the outbreak in March 2006, there were anecdotes of trypanosomiasis occurrence in another deer farm located approximately 25 km away (H. Razman, pers. comm.). Since the farm is surrounded by forest, it is possible that wild animals may have spread the agent from the affected farm to the centre. Wild pigs were seen in the farm's vicinity. According to Reid et al. (1999), wild pigs are

Table 2 Prevalence of *T. evansi* in a 100 deer at a breeding centre in Lenggong, Perak based on HCT and CATT methods

	Number of positive cases (%)	
	HCT	CATT
Adult males >24 months old (n=30)	5 (16.7)	25 (83.33)
Young males 12–24 months old (n=30)	7 (23)	26 (86.67)
Young females 12–24 months old (n=40)	11 (27.5)	27 (67.50)

capable of spreading the protozoa. In addition, buffaloes (also present at the centre), which can be severely affected by surra can also become the reservoir of the agent (Luckins 1988; Taylor and Authié 2004).

Deer may tolerate a heavy burden of *T. evansi* without showing any clinical signs; therefore, they can be an efficient reservoir of the organism (Reid et al. 1999). Nevertheless, deer may succumb to surra when aggravated by stress. Transport, handling, and poor nutrition can lead to profound immunosuppression in deer. According to Behnke (1990), lactation and pregnancy may reduce host immunity to *T. evansi.* During lactation, it is possible that the hormone prolactin has an adverse effect on the differentiation of lymphoid cells, hence leading to a lower immune-defence (Behnke 1990). This observation is consistent with the findings in the present study where a high proportion of adult females' deaths occurred during lactation period (data not shown). The peak for the outbreak occurred in March and April which coincided with the wettest months of the year (Malaysian Meteorological Department). Heavy rain may further aggravate the animal stress level making it easier for them to succumb to the infection.

Although Berenil® (diminazene aceturate) was used since the first incidence of trypanosomiasis, treatment was confined to the affected deer groups. Luckins and Dwinger (2004) in an experiment in water buffaloes in Indonesia observed that untreated animals may serve as the reservoir of trypanasomiasis infection. The increase in the reservoir of infection may partially explain why the cause-specific mortality rate was higher in 2007 as compared to 2006. The absence of an identification system in the farm may have further resulted in some of the affected deer not being treated.

A high percentage of sampled deer at the centre were positive for *T. evansi* and its antibodies. The majority (78%) of the deer at the centre had been exposed to *T. evansi* at some point in their life and 23% were positive for the parasite in their blood. The findings signify a high infection rate among the deer, which suggests an ongoing, active infection among the deer population at the centre.

Conclusion

The outbreak of trypanosomiasis in a deer breeding centre in Perak started in March 2006 and continued until the end of 2007 when the present study took place. Death due to the disease contributed to more than 27% of all deaths at the centre, denoting an important financial implication. The high infection rate of *T. evansi* in the deer population suggests that specific management of the disease is required to reduce the attributable deaths.

Selective treatment of deer may not be the best solution to reduce the incidence of the disease during an outbreak as asymptomatic infected animals may act as a reservoir for *T. evansi*. Prophylactic treatment of adult animals at risk in the farm before the risk period (i.e. before rainy seasons) may prove to be more effective in reducing incidence and propagation of the disease, and reduce overall deaths due to trypanosomiasis in the population. In addition, fumigation to control the vector at the right time, i.e. before and during rainy seasons, will no doubt help in mitigation of disease spread.

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