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BITING FLIES AND TRYPANOSOMIASIS IN SAHOM LIVESTOCK FARM: 'THE MISSING LINK'

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ABSTRACT. Trypanosomiasis positive cases were reported in Sahom Farm Retreat in Gopeng, Perak; with multispecies livestock animals. Nzi and Vavoua traps were applied to survey the population of biting flies; stable flies (Muscidae: Stomoxyinae) and horse flies (Tabanidae) as the vector for surra. Results indicated the presence of Trypanosomiasis infection diagnosed by buffy coat examination, thin blood stained smears and serological test (Surra Sero K-Set test) and identification of its insect vectors. The presence of both biting flies provides the missing link between the occurrence of the disease and host or environmental factors precipitating the disease. Besides trypanosomiasis in cattle, other parasitic infections were also recorded with heavy infections for liver fluke (*Fasciola gigantica* ova) and coccidia oocysts. Therefore, some control measures are recommended to eradicate the vectors and to treat infected animals in order to prevent the dissemination of the trypanosmiasis.

Keywords: biting flies, trypanosomiasis, *Tabanus* sp., *Stomoxys* sp., blood protozoa

INTRODUCTION

The Veterinary Research Institute has consistently diagnosed parasitic infections in a variety of livestock and wild animals over the past eight decades. Findings from Chandrawathani *et al*., 2014, has shown that positive cases of trypanosomiasis have been diagnosed since 1931. Perry *et al*., 2002 reported that Trypanosomiasis is ranked in the top 20 diseases found globally to have an impact on the poor especially in pastoral and mixed crop-livestock systems. In Malaysia's efforts to achieve first world status and be self-sufficient in livestock products, the National Key Economic Areas (NKEA) Programme has encouraged farmers and veterinarians to improve productivity and management so as to maximize benefits. As such continuous research and diagnostic activity is needed to update information

on the occurrence of trypanosomiasis and concurrent diseases.

Surra, caused by *Trypanosoma evansi* has been diagnosed in cattle regularly in Malaysia; almost annually since 1931 (Chandrawathani *et al*., 2014). Geographically, it is the most widely distributed haemoprotozoan parasite. In addition, the disease can affect a very large range of domestic and wild hosts including camelids, equines, cattle, buffaloes, sheep, goats, pigs, dogs and other carnivores, deer, gazelles, and elephants (Marc *et al.,* 2013) and can also be transmitted mechanically by several blood-sucking flies like stable fly (*Stomoxys calcitrans*), buffalo fly (*Haematobia* sp.), deer fly (*Chrysops* sp.) and mosquitoes (Manuel, 1998; Soulsby, 1982). Some clinical signs caused by Surra are severe anemia, oedema and various neurological disorders; leading to increased susceptibility to other diseases or vaccination failure (Zayed *et al*., 2010). immunodeficiency and death of the infected animal.

It is important to note that trypanosomiasis outbreaks are common in Malaysia and the *Trypanosomes* like *T. evansi* infections among animals were suspected to be transmitted by biting flies (Nurulaini *et al*., 2007). Infection leads to poor reproductive performance, low meat and milk yield production as well as high mortality (Manuel, 1998). Trypanosomiasis can be treated with medications such as Berenil/Suramin or Trypamidium (Cheah *et al*., 1997). The cattle also need to be treated with antibiotic, anti-stress medication and supportive supplement like vitamins. The environment surrounding the farm and bushes must be removed. Wet and marshy grounds should be dried out to reduce potential vector breeding spots. In December 2013, the Veterinary Research Institute (VRI) diagnosed a case of trypanosomiasis in pig blood sample. The sample was from a private pig farm situated near cattle and buffalo grazing areas (Premaalatha *et al.,* 2014). Following this episode, several cattle and deer farms in the vicinity have reported mortalities, suspecting Trypanosomiasis. In order to establish this trend of disease outbreak, the VRI is seriously viewing all potential mortality cases and supporting full investigations to help reduce losses among farmers.

The aim of this study, thus, is to identify the cause of morbidity in the Sahom Farm retreat, with special empahasis on fly vectors and parasitic infection, both blood protozoans and gastrointestinal parasites.

MATERIALS AND METHODS

A total of 20 adult cattle of the Kedah-Kelantan Brahman crosses were sampled for blood (EDTA and serum) and faecal samples and sent for laboratory diagnosis of blood protozoan and gastrointestinal infections. The methods being used for diagnosis are thin blood smear, Buffy coat and Surra Sero K-Set test. The faecal samples were examined by using McMaster method and sedimentation to identify the presence of worm eggs (MAFF, 1978).

The blood samples collected from the 20 cattle were for screening of blood parasites such as trypanosomiasis, babesiosis, anaplasmosis and theileriosis. Other than that, fly trapping was conducted to trap flies of veterinary importance that may carry or be a vector for trypanosomiasis.

Thin Blood Smear

A drop of blood was placed at one end of a clean grease-free glass slide. By holding the spreader at an angle of 45 degrees, the blood drop was pushed along the slide, drawing the blood behind it until it has been smeared. The blood film was allowed to dry for 1-2 minutes. A few drops of methyl alcohol was applied onto the smear for three minutes (or left overnight) to fix the smear onto the slide. The slide was then washed off and stained with 8% of Giemsa stain (diluted 1/20 I buffered distilled water pH7.2) for 30 minutes. The excess stain was discarded and the slide was washed with running tap water. The slide was allowed to dry and observed under the microscope in 1000× magnification (MAFF, 1978).

Haematocrit Buffy Coat Examination

Buffy coat examination was conducted for the identification of trypanosomes. The blood in the EDTA tube was mixed well by gently inverting the tube by blood rocker machine. Blood was drawn 2/3 of the way into a 75 mm × 1.00 mm microhaematocrit capillary tube. Blood at the tip was wiped off and carefully plugged at the end with the plasticine. After that, the capillary tube was then placed into the microhaematocrit centrifuge machine with closed ends outwards, and spun at 12,000 rpm for 5 minutes. The capillary tube was removed from the centrifuge machine and the Buffy coat was viewed under the light microscope at 100× magnification for *Trypanosoma* sp. (MAFF, 1978).

Surra Sero K-SeT test

The kit components, in unopened packaging, and specimens were allowed to reach room temperature (15-30 °C) before performing the test. The pouch was opened and the device was removed. The test was run immediately once opened. The specimen number was indicated on the device. A total of 15 µL of serum was dispensed into inner side of the device sample by using a micropipette. A volume of 2 drops of ST-A buffer was added into the outer side of the sample well. The device was left to react for 15 minutes. The results were observed in the reading window as a reddish line (Zayed *et al*., 2010).

Mcmaster Method And Sedimentation For Faecal Samples

The McMaster technique was done for all faecal samples to estimate the number of helminth eggs in faeces. Saturated salt water was used to float the eggs and then observed on a McMaster slide under

Table 1a. Samples collected and identification of faecal and blood parasites

Sample	No. of samples	Technique used	Percentage positive	Results
Blood (EDTA)	20	Thin blood film stained with Giemsa	20%	*Trypanosoma* sp.
Serum	20	Surra Sero K-Set test	40%	*Trypanosoma* sp.
Faecal	10	McMaster Sedimentation	100%	Coccidian oocyst fluke egg

Table 1b. Detailed results of blood and faecal screening for parasites

	Trypanosomiasis Diagnosis			Faecal Diagnosis		
No.	Buffy Coat	Thin Smear	Serology	Coccidiosis	Fluke	Helminthiasis
1	-	-	-	-	+	0
2	-	-	+	-	+	0
3	-	-	+	-	+	0
4	-	-	-	-	+	0
5	-	-	-	-	+	0
6	-	-	+	+	+	0
7	-	-	-	-	+	0
8	+	-	-	-	+	0
9	-	-	-	-	+	0
10	-	-	-	-	+	100
11	-	-	+			
12	-	-	-			
13	-	-	+			
14	-	-	+			
15	-	+	-			
16	-	+	-			
17	-	-	+			
18	-	-	-			
19	-	+	-			
20	-	-	-			

Table 2. Number and species of flies collected from Sahom Farm Retreat

Species of flies	No. collected from Nzi trap
Tabanus sp.	2
Stomoxys sp.	67
Haematobia sp.	13
Musca sp.	128

Figures 1-5. Pictures showing various stages of the study

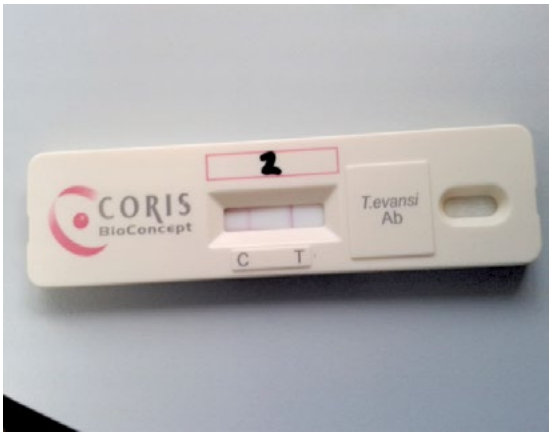

Figure 2: *Positive result for Trypanosoma evansi from cattle tested using Surra Sero K-SeT test. This is indicated by the* visible reddish-purple band at the Test line position (T). The pink line at the (C) denotes the test is valid and in good working order.

Figure 4: Several flies perch on the skin of the cattle simultaneously biting and irritating the cattle *as shown in the red circle.*

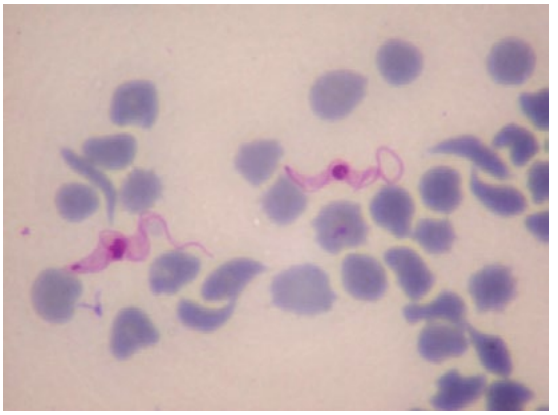

Figure 1: *Trypanosoma evansi* (as shown in the red circle)

Figure 3: Numerous flies trapped over a 2 hour period in the Nzi Trap in the vicinity of the farm. Identifications were done as shown in Table 2.

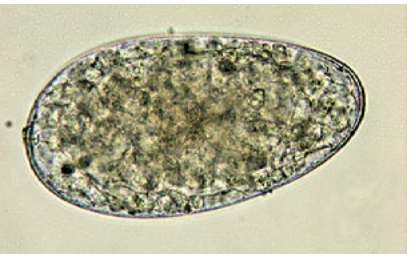

Figure 5: Fluke egg obtained from Sedimentation Technique (10x magnification)

microscope at 10× magnification. The faecal sample was mixed with a dilution of 1:15. For instance, 3 mg of faecal was mixed with 15 ml of salt water. The mixture was then ground by using mortar and pestle. Next, the mixture was filtered by 85 mesh screen into a bottle. The filtrate was then transferred by using a pipette into the chambers of a McMaster Slide. Eggs that reside within the chambers were counted.

The sedimentation method was used to detect the liver fluke egg in the faeces of the cattle. The liver fluke, being denser than water, would sediment after adding the water. The sediment was then added into a petri dish and mixed with methylene blue before observing under a stereo-microscope for golden brown eggs of flukes. All techniques pertaining to faecal and blood samples were according to protocol (MAFF, 1978).

Fly Identification

All flies caught in the farm were identified according to Masmeatathip *et al*., (2006). The flies were caught by using the NZi trap (Erwanas *et al*., 2014).

RESULTS

Out of 20 blood samples, 4 were positive for *Trypanosoma* sp. examined from thin blood stained smears and 8 were positive for *Trypanosoma* sp. by using serological Surra Sero K-SeT test. All 10 faecal samples were positive for fluke egg and coccidian oocyst. The faecal samples were negative for helminth eggs. Data of blood and faecal parasites are shown in Tables 1a and b. A total of 2 *Tabanus* sp. flies, 67 *Stomoxys* sp. flies, 13 *Haematobia* sp. flies and 128 *Musca* sp. flies were collected using the Nzi and Vavoua trap as shown in Table 2. Among the *Musca* sp. there were 32 *Musca crassirostris* identified. According to Taylor *et al*., 2007, *M. crassrostris* can be identified based on the four longitudinal dark stripes on the thorax and they use the prestomal teeth to rasp the skin and draw blood which is then ingested. From the results, it can be observed that different animals were noted to have trypanosomiasis according to the various tests. No one animal was found to be positive for surra with all three tests. However all animals were positive for flukes.

Figures 1 to 5 show the various stages during the project; diagnosis of parasites such as trypanosomiasis and flukes, flies trapped, and on the animal and the test kit used.

DISCUSSION AND CONCLUSION

Animal trypanosomiasis caused by *Trypanosoma evansi* is endemic throughout Southeast Asia, where it is an important constraint on the productivity of smallholder livestock. In the past decade, *T. evansi* has emerged as a serious threat to the viability of smallholder livestock industries in Malaysia and has caused severe disease outbreaks with high mortality especially

in deer, pigs and cattle (Premaalatha *et al*., 2014). It is difficult to estimate the economic loss attributable to *T. evansi* infection. Most estimates are based on the cost of mortality and chemotherapeutic interventions. Manuel (1998), estimated that in the Philippines, from death alone, *T. evansi* had caused losses of >US$1.1 million in nine years. However, true losses as a result of *T. evansi* might be at least five times higher than official figures with under-report cases, or US$7.9 million, for the same period. In this study, the farm reported the presence of biting flies such as *Tabanus* sp. and *Stomoxys* sp. which are efficient vectors of trypanosomes. In addition, *Musca crassirostris*, a haematophagous fly was also identified. Trypanosomiasis infection in cattle was observed via direct examination (20%) as well as serologically (40%). It is generally believed that trypanosomiasis causes immunosuppression which may allow the animals to be at risk for other diseases. In this case, the animals were also infected with flukes. As the results indicate that different animals were diagnosed with surra with different tests, it is important to harmonise these tests to enable diagnosis to be conducted as efficiently and accurately as possible. Further studies need to be done over a longer period of time and using more animals for screening.

Constant monitoring of the animals is recommended to evade serious outbreaks and mortality in the herd. Furthermore, fly control and improving the animal's immune status by good nutrition and care will be highly beneficial in reducing losses due to poor weight gain, poor reproductive performance and death. There is insufficient information on the determinants of Surra to enable a prediction on the consequence of infection. Given that parasite prevalence is often not related to the occurrence of clinical disease, there must be other factors involved. Such factors include concurrent disease, poor nutrition, work stress, innate and acquired resistance, parasite pathogenicity and strain of parasite.

REFERENCES

4. Perry B. D., Randolph T.F., McDermott J.J, Sones K.R. and Thornton P.K. (2002). Investing in Animal Health Research to Alleviate Poverty. International Livestock Research Institute. ISBN 92-9146-108-3
5. Chandrawathani P., Tan T.K., Adnan M., Erwanas A.I., Premaalatha B., Low K.N., Moo K.S., Soon X.Q., Yvonne A.L. Lim and Ramlan M. (July, 2014). Significant Blood Protozoan Infections, Their Host Range and Trend of Infections in Domestic Animals of Malaysia Diagnosed by the Department of Veterinary Services and Veterinary Research Institute (VRI) from 1931 to 2010 – A Historical Preview. *Malaysian Journal of Veterinary Research (MJVR)* **5(2)**: 47-61.
6. Cheah T.S., Sani R.A., Chandrawathani P., Sansul B. and Dahlan I. (1997). Natural Infection of *T. evansi* in crossbred calves in a farm in Peninsular Malaysia. In Proc: *9th Veterinary Association Malaysia Scientific Congress,* 3-5 October 1997, Penang, p.38-40
7. Erwanas A.I., Masrin A., Chandrawathani P., Jamnah O., Premaalatha B. and Ramlan M. (2014). Vectors of Veterinary Importance in Malaysia: A Survey of Biting Flies in Relation to Trypanosomiasis in Perak. *Malaysian Journal of Veterinary Research (MJVR)* **6(1):** 89-96
8. Manual of Veterinary Investigation laboratory Techniques, part 4 Haematology: Ministry of Agriculture, Fisheries and Food (MAFF) 2nd Edition (1978) p. 7-18.
9. Manuel M. F. (1998). Sporadic outbreaks of Surra in the Philippines and its economic impact. *J. Protozool. Res.* **8:** 131-138.

10. Marc Desquesnes, Philippe Holzmuller, De-Hua Lai, Alan Dargantes, Zhao-Rong Lun and Sathaporn Jittaplapong (2013). *Trypanosoma evansi* and Surra: A Review and Perspectives on Origin, History, Distribution, Taxonomy, Morphology, Hosts, and Pathogenic Effects. *BioMed Research International* Volume 2013 (2013), Article ID 194176, 22 pages
11. Nurulaini R., Jamnah O., Adnan M., Zaini C.M., Khadijah S., Rafiah A. and Chandrawathani P. (2007). Mortality of domesticated java deer attributed to Surra. *Tropical Biomedicine* **24** (2): 67-50.
12. Premaalatha B., Chandrawathani P., Erwanas A.I., Jamnah O. and Lily Rozita M.H. (July, 2014). Trypanosomiasis in Pigs. *Malaysian Journal of Veterinary Research (MJVR)* **5(2)**: 69-71.
13. Roungthip Masmeatathip, Chitapa Ketavan and Gérard Duvallet (2006). Morphological Studies of *Stomoxys* spp. (Diptera: Muscidae) *Kasetsart J. (Nat. Sci.)* **40:** 872-881
14. Soulsby E.J.L. (1982). Helminths, Arthropods, and Protozoa of Domesticated Animals. 7th edition.
15. Taylor M.A., Coop R. L., Wall R. L. (2007). Veterinary Parasitology. Blackwell Publishing. pp 874.
16. Zayed A.A., Habeeb S.M., Allam N.A.T., Ashry H.M.Z., Mohamed A.H.H., Ashour A.A., Taha H.A. (2010). A Critical Comparative Study of Parasitological and Serological Differential Diagnostic Methods of *Trypanosoma evansi* Infections in Some Farm Animals in Egypt. *American-Eurasian Journal of Agricultural & Environment Sciences*, **8(6)**:633-642.

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