VECTORS OF VETERINARY IMPORTANCE IN MALAYSIA: A SURVEY OF BITING FLIES IN RELATION TO TRYPANOSOMIASIS IN PERAK

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ABSTRACT. Biting flies are important vectors for disease transmission in ruminants such as cattle and buffaloes where pathogenic organisms such as *Trypanosoma evansi* are transmitted. The NZI and Vavoua traps were applied to survey the population of biting flies such as stable flies (Muscidae: Stomoxyinae) and horse flies (Tabanidae) which are the vectors for this disease. Results indicated the presence of biting flies as well as *Musca* sp. and *Haematobia* sp. in several livestock farms in Perak that can transmit Trypanosomiasis. Vector control has been suggested to limit the occurrence of the disease especially during rainy season when there is high morbidity and mortality in herds. These traps can be used as an eco friendly method to control flies in farms as well as a tool to collect vectors of veterinary importancefor research purposes.

Keywords: biting flies, vector control, *Tabanus* sp., *Stomoxys* sp., *Haematobia* sp.

INTRODUCTION

Flies are significant as disease vectors for human and animal health (Truc *et al*., 2013). Malaysia in endemic with Trypanosomiasis caused by *Trypanosoma evansi* (Cheah *et al*., 1999). Cheah *et al*. (1996 and 1997) reported that Trypanosomiasis in cattle can cause severe losses in milk production and body weight. Stable flies (*Stomoxys calcitrans*) and horse flies (*Tabanus* sp.) can transmit Trypanosomiasis (*Trypanosoma evansi*) or commonly known as Surra, in cattle, buffalo and deer in Malaysia.

In 2013, there was a disease outbreak with high mortality and mobidity reported in Perak state at Infoternak, Sungai Siput and several private pig farms in surrounding areas (Nurulaini *et al*., 2013) and Sani *et al*. (1990 and 1995) has also reported Trypanosomiasis in cattle. The outbreak of trypanosomiasis was diagnosed by the Parasitology Section of the Veterinary Research Institute (VRI) in early 2012. A total of 86 whole blood samples from deer, cattle and buffaloes from a government farm near Sungai Siput, Perak and 16 whole blood samples from a commercial pig farm in Jalong, Perak were submitted for analysis. Sixty-three samples were found to be positive for the *Trypanosoma* sp.

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A detailed epidemiological investigation was conducted to prevent future outbreaks (Nurulaini *et al*., 2007). In the South-east Asian countries, vectors for surra have been long studied. In a study based on horse flies and deerflies collected by the Phillipine Zoological Society Phillipines, a total of 50 species of Tabanus have been recorded (Philip, 1959). In Thailand, Papp *et al.* (2006) reported 99 Diptera families. Tumrasvin in 1978 reported the Tabanus species and its distribution as it was deemed important in the transmission of trypanosomiasis in livestock. In Malaysia, Philip also reported in 1960 on Tabanidae and new species of Haematopota. As such there is keen interest in local species of biting flies as trypanosomiasis in livestock is a continuing threat causing low productivity.

Life cycle of Tabanidae

After a blood meal the female lays batches of several hundred creamy-white or greyish cigar-shaped eggs, 1.0–2.5 mm long, on the underside of vegetation or on stones, generally in muddy or marshy areas. The eggs hatch in 1–2 weeks and the cylindrical, poorly differentiated larvae drop into the mud or water. The larvae, 1.0–6.0 cm long, are recognized as tabanids by their small black retractable heads, the prominent raised rings around the segments. Optimally, larval development takes three months, but it hibernation occurs, may extend for up to three years. Mature larvae pupate partially buried in mud or soil and the adult fly emerges after 1–3 weeks. The whole life cycle takes a minimum of 4–5 months or longer if larval development is prolonged (Taylor *et al*., 2007).

Life cycle of Muscidae

Female flies lay batches of up to 100 creamy-white, 1.0 mm long, banana-shaped eggs in faeces or rotting organic material. Eggs hatch, under optimal temperatures, in 12–24 hours to produce whitish, segmented, cylindrical larvae (maggots), which, anteriorly, are pointed and have a pair of small hooks. The three larval instars feed on decomposing organic material and mature to 1.0–1.5 cm long maggots in 3–7 days under suitable conditions. These then move to drier areas around the larval habitat and pupate in the final larval skin which contracts and becomes rigid and dark brown, to form the 6.0 mm long barrel-shaped puparium or pupal case. The adult fly emerges after 3–26 days depending on temperature (Taylor *et al*., 2007).

Annual report of VRI shows Trypanosomiasis to be commonly isolated in diagnostic cases. In 2012 there were 58 samples of blood positive for *Trypanosoma evansi*.

The aim of this study is to show the common vectors found in Malaysia that can be a vector for the transmission of Surra in ruminants.

MATERIALS & METHODS

Survey location

The survey was conducted in 5 districts in Perak. The fly traps were set up in several farms in Perak State and during the trapping activities, the following flies were identified according to keys by Masmeatathip *et al.* (2006); Foil, 1989; and Phasuk *et al.*, 2013. A total of 29 *Tabanus* sp. flies, 646 *Stomoxys* sp. flies, 50 *Haematobia* sp. flies and 1176 *Musca* sp. flies were collected using the Nzi and Vavoua trap (see Table 1). The weather during the trapping activities, carried out in several districts in Perak was bright and sunny which is suitable for trapping activities.

Table 1. Location of animal farms in Perak where the fly traps were set up.

Farm Location	Animals
VRI, Ipoh	Goat, Sheep, Cattle
VRI, Ipoh	Buffalo, Cattle, Goat
Infoternak Sg. Siput	Buffalo, Deer
Gopeng, Perak	Goat, Cattle, Chicken
VRI, Ipoh	Goat, Sheep, Cattle
VRI, Ipoh	Goat, Sheep, Cattle
Infoternak Sg. Siput	Cattle
Ampang, Kinta	Cattle
Kampar, Perak	Pig, Chicken
Tambun	Horse
Lawan Kuda, Gopeng	Horse
Jalong, Sg.Siput	Pig
Chemor	Cattle, Chicken
Tg. Rambutan	Cattle
Taiping	Horse
Jeram	Cattle
Lenggong	Deer
Parit	Cattle

Trapping methods

Two traps were used to evaluate the effectiveness in catching the flies in the farm environment. The traps were set up close to animal facilities in VRI and in several commercial livestock farms in Perak State.

Nzi Trap

The Nzi trap (Figure 1) is a simple, safe and economical cloth trap for the capture of biting flies. It is a passive killing device that works through the attraction of flies to large blue and black objects. Flies enter at the bottom front, fly into the transparent netting, and are then trapped when they fly up through the gap between the netting shelf and the back of the trap. Two blue rectangular "wings" extend out at an angle from the front framing the lower entrance. The top is closed by a netting "cone", made by cutting a wedge out of a square piece of netting and sewing up the sides. These results in a tetrahedron: a 3-d shape with three triangles joining at the apex (Mihok *et al.*, 2006).

VAVOUA Trap

The Vavoua trap (Figure 2) was designed as an economical alternative to the pyramidal

Figure 1. NZI flytrap

Figure 2. Vavoua flytrap

trap in widespread use for the control of stable flies. There are many similar designs that employ hanging blue-black screens. These open designs are excellent for stable flies. Traps like the Vavoua are straight forward to sew and assemble. They can be hung from simple wooden supports or trees. Unfortunately, trap styles with this open design are poor for tabanids according to Laveissière & Grébaut (1990).

Identification of flies

For identification, keys according to Masmeatathip *et al*. (2006)**;** Foil, 1989; Phasuk *et al.,* (2013) were used.

Method for Trypanosomiasis diagnosis is by MAFF, 1978

Blood samples were subjected to buffycoat and thin blood smear examination for detection of any blood protozoa (Wahab *et al*., 2002). Chandrawathani *et al*., (1998) has screened Trypanosomiasis using ELISA and CATT (Wahab, 2012) to measure the antibody against Trypanosomes. Whereas Cheah *et al*., (1999) has seen success in conducting PCR for the detection of Trypanosomiasis in a cattle blood. A capillary tube (1/4 of blood) was filled with blood from EDTA tube and one side of the capillary tube was sealed with plasticine. The capillary tube was placed in a micro-centrifuge and the sealed side must face out. Each capillary tube was balanced before centrifuging at

12,000 rpm for 5 minutes. The buffy-coat was examined under 10× of magnification objective by using compound microscope and trypanosomes were observed moving. *Trypanosoma evansi* was also identified in Giemsa stained thin blood smears as a monomorphic organism with a mean length of 24 µm, with subterminal kinetoplast, undulating membrane and free flagellum (Soulsby, 1982).

RESULTS

Analysis of flies captured from local farms.

Results obtained are shown in Figure 3 and Table 2.

From January to December 2013, a total number of 18 animal farms were screened for biting and sucking flies whereby four species of them were collected using both Vavoua and Nzi traps. The flies that were found are *Tabanus* sp., *Stomoxys* sp., *Haematobia* sp. and *Musca* sp. The results indicated that the farm which has cattle, buffalo and deer harboured four species of flies which can be a vector for Trypanosomiasis. The fly traps were set up around VRI animal facilities, Ipoh, two (2) animal farms in Kuala Kangsar districts including Infoternak Government farm in Sungai Siput (U), four (4) animal farms in Kinta districts, four (4) animal farms in Kampar, one (1) farm in Perak Tengah districts, one (1) farm in Hulu Perak districts and (1) one farm in Larut, Matang & Selama districts. During the trapping activities, a total of 1176 *Musca* sp. flies, 646 *Stomoxys* sp. flies, 50 *Haematobia* sp. flies and 29 *Tabanus* sp. flies were collected by using the Nzi and Vavoua traps. During the time of collection, the weather was sunny and the traps were set up in an open area about 50 metres from farm animals such as buffalo, pig, horse or cattle.

DISCUSSION & CONCLUSION

Results indicated the presence of biting flies as well as *Tabanus* sp., *Stomoxys* sp., *Musca* sp. and *Haematobia* sp. in the farms that can transmit Trypanosomiasis. Infoternak Sg. Siput farm has annual outbreaks of Surra in the deer and cattle herd, and control of these flies is an important aspect of the epidemiology of Surra. The flies that were found are *Musca* sp., *Stomoxys* sp. and *Haematobia* sp. Rainfall fluctuations may be the main climatic factor correlated with population changes of the most abundant species (Phasuk *et al*., 2011). In conclusion, fly trapping can be an alternative fly control technique as a sizeable number of flies were caught. Other management methods such as reducing water puddles and clearing damp fodder to prevent fly breeding habitats will further help to alleviate the problem. Many farmers still tend to rely on pesticides as pour on or spray to prevent the fly nuisance but a more sustainable, green method would be the use of a mechanical fly trap such as Nzi or Vavuoa in farms.

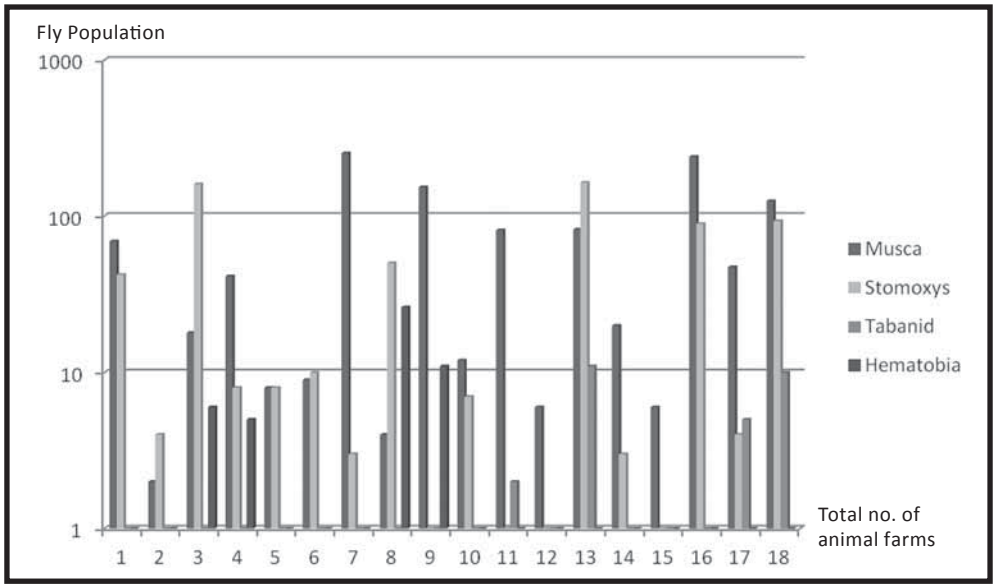

Figure 3. Population of flies collected from 18 animal farms.

Table 2. Fly species that were collected in each farm location.

No.	Farm Location	Species	Musca	Stomoxys	Tabanid	Haematobia
1	VRI, Ipoh	Goat, Sheep, Cattle	69	42	0	0
2	VRI, Ipoh	Buffalo, Cattle, Goat	2	4	0	0
3	Infoternak Sg. Siput	Buffalo, Deer	18	160	0	6
4	Gopeng, Perak	Goat, Cattle, Chicken	41	8	0	5
5	VRI, Ipoh	Goat, Sheep, Cattle	8	8	0	0
6	VRI, Ipoh	Goat, Sheep, Cattle	9	10	0	0
7	Infoternak Sg. Siput	Cattle	253	3	0	0
8	Ampang, Kinta	Cattle	4	50	0	26
9	Kampar, Perak	Pig, Chicken	153	0	0	11
10	Tambun	Horse	12	7	0	0
11	Lawan Kuda, Gopeng	Horse	81	0	2	1
12	Jalong, Sg.Siput	Pig	6	1	0	0
13	Chemor	Cattle, Chicken	82	164	11	0
14	Tg. Rambutan	Cattle	20	3	0	0
15	Taiping	Horse	6	0	0	0
16	Jeram	Cattle	240	89	1	0
17	Lengggong	Deer	47	4	5	1
18	Parit	Cattle	125	93	10	0
		Total	**1176**	**646**	**29**	**50**

Table 3. Positive cases of Trypanosomiasis diagnosed in VRI from 2000-2013

Year	Species	No. of Sample	No. of Positive
2000	Buffalo	4	2
2001	Cattle	19	6
2003	Deer	160	35
2006	Deer	21	12
2007	Deer	22	3
2007	Cattle	13	5
2008	Cattle	3	1
2008	Deer	80	7
2009	Deer	155	23
2010	Cattle	100	9
2010	Deer	94	9
2011	Deer	28	5
2012	Deer	105	41
2012	Cattle	105	19
2012	Buffalo	20	5
2012	Pig	16	13
2013	Deer	35	3
2013	Cattle	8	3
2013	Buffalo	17	7

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