THE OCCURRENCE OF TRYPANOSOMIASIS IN CATTLE AND DEER IN PERAK, MALAYSIA FROM 2017 TO 2022

FAZLY ANN, Z.*, AISYAH MUNIRA, M.A., PREMAALATHA, B., JUBREY, M., AND ERWANAS ASMAR, I.
Veterinary Research Institute, Department of Veterinary Services, 59, Jalan Sultan Azlan Shah, 31400 Ipoh, Perak, Malaysia
**Corresponding author:* fazly@dvs.gov.my

ABSTRACT. The vector-borne disease of trypanosomiasis is cyclically or mechanically transmitted by biting flies. The flies carried a unicellular hemoflagellate protozoan known as *Trypanosoma* spp. that infects livestock and wildlife. This study aims to determine the status of trypanosomiasis in cattle and deer in Perak, Malaysia through retrospective assessment of the cases submitted to the Veterinary Research Institute (VRI), Ipoh, Perak from 2017 to 2022. A total of 3,434 cattle and deer blood samples comprising of 291 cases were received and examined by thin blood smear examination, buffy coat, and haematocrit concentration technique in the Parasitology Laboratory, VRI. Results showed that 7.22% (21 out of 291 cases) detected positive for trypanosomiasis, with 1.72% (5 cases) in cattle and 5.50% (16 cases) in deer. Based on the total number of samples, 1.98% (68 out of 3,434) were positive for trypanosomiasis, with 0.29%(10) and 1.69% (58) from cattle and deer, respectively. Among the positive cases samples, 18.84% (68 out of 361) blood samples were detected with *Trypanosoma* spp., with 2.77% (10) from cattle and 16.07% (58) from deer. The PCV values for positive samples were recorded at 19.50±6.84 and 36.59±5.83 for cattle and deer, respectively. This study demonstrated a low occurrence of trypanosomiasis in Perak for cattle and deer over six years. Understanding the current disease status is important for developing future mitigating strategies. Controlling the vector is one of the efforts to minimise the transmission of the disease.

Keywords: Trypanosomiasis, cattle, deer, biting flies

INTRODUCTION

Trypanosomiasis is a vector-borne disease caused by a protozoan parasite known as *Trypanosoma* spp. which ubiquitously infects wide range of domestic livestock and wild animals in many tropical and subtropical counties including Africa and Asia (Zecharias and Zaryehun, 2012; Da Silva *et al*., 2010). Trypanosomiasis caused by *Trypanosoma evansi* is known as Surra (Desquesnes *et al*., 2013). The other names of trypanosomiasis are Chagas disease (i.e. American trypanosomiasis) which was caused by *T. cruzi* (Gomes *et al*., 2019), the sleeping sickness disease (i.e. African trypanosomiasis) caused by *T. brucei* and dourine caused by *T. equiperdum* (Papagni *et al*., 2023).

The aetiological agent is a unicellular hemoflagellate protozoan from the class of Kinetoplastea, order Trypanosomatida, family Trypanosomatidae, and genus *Trypanosoma* identified by the flagella that attached to the undulating membrane. The organism is elongated and tapered at both ends with the size of the parasite varying from 8 to over 50 µm with the mean length of 24 µm (Soulsby, 1982). The kinetoplast at the posterior end, differ in size and position according to species.

The parasite is cyclically transmitted by tsetse flies, or mechanically by tabanid and other biting flies. Apart from transmitting the parasite cyclically, tsetse flies can also act as mechanical vectors (Kumar *et al*., 2012; Desquesnes *et al*., 2009; Uilenberg, 1998). The disease is commonly

found at the environment with moist substrates such as stagnant water, wet soil, mud, ponds or swamps, which is suitable for fly vector breeding (Bhatia *et al*., 2006). Study by Erwanas *et al*. (2015) have recorded the presence of biting flies such as *Tabanus* spp., *Stomoxys* spp., and *Haematobia* spp., in the farms with positive cases of trypanosmiasis. Trypanosome exists in two categories; salivaria and stercoraria. The salivarian species (e.g. *T. evansi*) develops in the anterior part of biting flies and transmitted to the host by insect feed. Whereas, the stercorarian species (e.g. *T. cruzi*) develops in posterior part of vector digestive tract and then deposited on the host's skin and infection occurs through penetration of the parasite.

In Malaysia, trypanosomiasis cases have been reported in cattle (Jesse *et al*, 2016; Cheah *et al*., 1999; Sani *et al*., 1995; Abas-Mazni and Zainal-Abidin, 1985), horses (Rajdi *et al*., 2021; Elshafie *et al*., 2013), deer (Nurulaini *et al*., 2007), and multiple species including deer, cattle, buffaloes and pigs (Nurulaini *et al*., 2013). According to Chau *et al.* (2016), the most common species of *Trypanosoma* detected in cattle were *T. congolense, T. brucei, T. vivax* and occasionally *T. evansi*. In deer, besides *T. evansi* and *T. vivax*, two other species namely *T. mazamarum* and *T. cervi* has also been reported by Stuht *et al*. (1975).

This study aimed to determine the status of trypanosomiasis in cattle and deer in the state of Perak, Malaysia through retrospective assessment of the suspected cases submitted to the Veterinary Research Institute (VRI), Ipoh, Perak.

MATERIALS AND METHOD

Collection of Samples

The screening for trypanosomiasis was conducted in the Parasitology Laboratory of Veterinary Research Institute (VRI), Ipoh, Perak (4° 35'32.9" N, 101° 07'12.6" E). A total of 3,434 fresh blood samples were received for blood protozoal detection, consisting of 291 cases from 1,761 cattle samples (186 cases) and 1,673 deer samples (105 cases). The samples were submitted from all the districts in Perak commencing from 2017 to 2022. The samples were collected either from a jugular or coccygeal vein and kept in vacutainer blood tube containing anti-coagulant at chilled temperature upon storage and transportation to the laboratory for diagnosis.

Thin Blood Smear Examination

The blood parasite examination was performed by using the thin blood smear technique as described in the Manual of Veterinary Laboratory Testing for Parasitology (Chandrawathani *et al.*, 2016). The dried smears were fixed with methanol and then stained using 8% Giemsa in phosphate-buffered saline (PBS) at pH 7.2 for 45 minutes. Stained thin blood smears were examined specifically around the tail end. The parasites were observed under a compound microscope (Olympus CX31, Japan) at high-power magnification (×1000) with oil immersion.

Buffy Coat Examination

Blood samples that were received in less than 48 hours of sampling were subjected to buffy coat examination. The microhaematocrit tube was filled with approximately 70 µl of whole blood prior to centrifugation at 12,000 g for 5 minutes. Following centrifugation, the motile *Trypanosoma* spp. was observed directly between the lower buffy coat and red blood cell (RBC) layer under a compound microscope (Olympus CX31, Japan) at ×100 magnifications.

Haematocrit Concentration Technique

The procedure was conducted to determine the packed cell volume (PCV) of the animals. The test was performed using the same microhaematocrit tube from the previous buffy coat examination. The PCV was then measured using a manual haematocrit reader (Hawksley GB850179, England).

RESULTS AND DISCUSSION

The result showed that 21 out of 291 cases (7.22%) were detected positive for trypanosomiasis with 5 cases (1.72%) in cattle and 16 cases (5.50%) in deer. Based on the number of samples, 68 (1.98%) out of 3,434 samples, were positive for trypanosomiasis in six consecutive years. Total number of *Trypanosoma* spp. in cattle and deer samples

Table 1. The number and percentage of positive trypanosomiasis samples according to the year, district and types of animals.

Year	Case ID	District	Type of animals	No. of samples	Positive trypanosomiasis	
					No. of samples	Percentage (%)
2017	Case 1	Kuala Kangsar	Cattle	43	3	6.98
	Case 2	Hulu Perak	Deer	28	1	3.57
	Case 3	Kuala Kangsar	Deer	11	6	54.55
2018	Case 4	Kuala Kangsar	Deer	21	7	33.33
	Case 5	Kampar	Cattle	3	1	33.33
	Case 6	Kuala Kangsar	Deer	15	3	20.00
2019	Case 7	Kuala Kangsar	Deer	5	2	40.00
	Case 8	Kuala Kangsar	Deer	46	4	8.70
	Case 9	Kuala Kangsar	Deer	20	1	5.00
	Case 10	Kuala Kangsar	Cattle	7	3	42.86
	Case 11	Hulu Perak	Deer	15	4	26.67
	Case 12	Hulu Perak	Deer	20	1	5.00
2020	Case 13	Hilir Perak	Cattle	6	1	16.67
2021	Case 14	Kuala Kangsar	Deer	15	9	60.00
2022	Case 15	Kuala Kangsar	Cattle	16	2	12.50
	Case 16	Kuala Kangsar	Deer	15	2	13.33
	Case 17	Kuala Kangsar	Deer	4	4	100.00
	Case 18	Kuala Kangsar	Deer	10	2	20.00
	Case 19	Kuala Kangsar	Deer	27	7	25.93
	Case 20	Kuala Kangsar	Deer	4	1	25.00
	Case 21	Kuala Kangsar	Deer	30	4	13.33
Total				**361**	**68**	**18.84**

detected were 10 (0.57%) out of 1,761 and 58 (3.47%) out of 1,673 each, as in **Table 1**.

The study revealed deer exhibit higher trypanosomiasis compared to cattle. This is most probably due to several factors including host specificity, vector dynamics, environmental and stress factors. According to *Reid et al.* (1999), deer can be an efficient reservoir for Trypanosomes and may tolerate heavy burden of the parasite without showing any clinical signs. The disease can also be influenced by the abundance and distribution of the vector especially surrounding the bushes and any potential breeding grounds such as marshy and wet area. In Perak, the deer were raised in an extensive system where they were allowed to roam freely which increase the exposure to potential vector. Besides that, transportation, handling and poor nutrition also may lead to profound immunosuppression in deer.

All the positive samples were received from four out of twelve districts in Perak with 80.06% from Kuala Kangsar, 17.45% from Hulu Perak, 1.66% from Hilir Perak, and 0.83% from Kampar. Presented in Figure 1 was the trend of positive trypanosomiasis cases from 2017 to 2022. The results showed highest positive cases in deer (60%, 9 out of 15) and cattle (42.86%, 3 out of 7) occurred in 2021 and 2019 respectively.

In this study, most of the positive samples were detected by combination of both thin blood smear and buffy coat examination methods (58.82%), followed by buffy coat examination method (25.00%) and thin blood smear method (16.18%), as presented in Table 2.

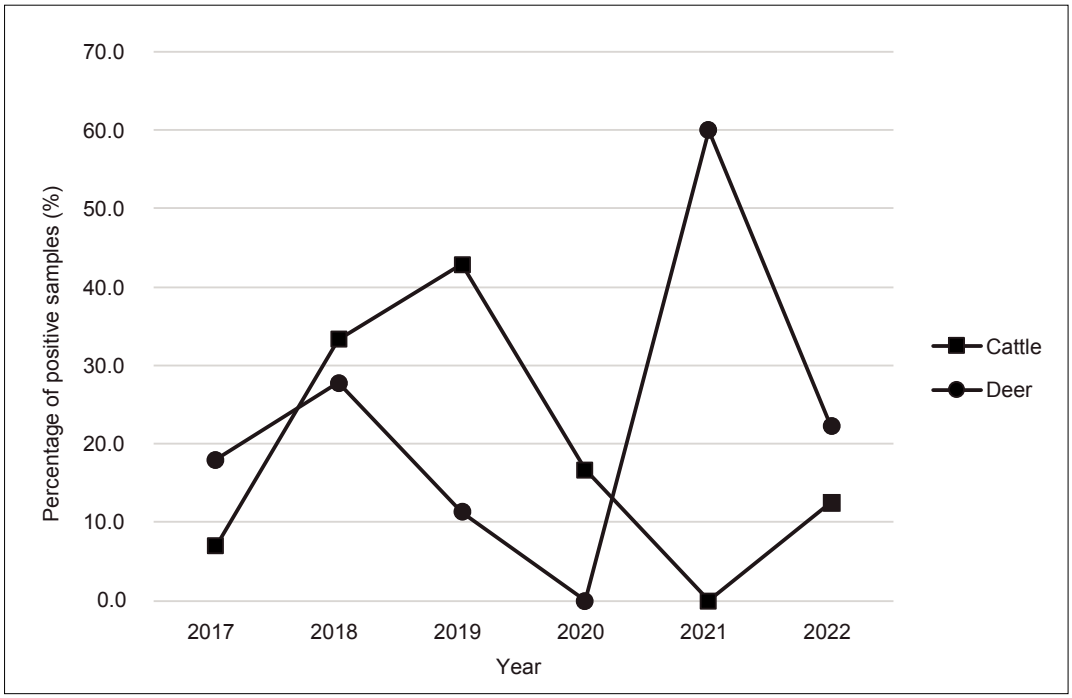


Figure 1. The trend of positive trypanosomiasis samples according to the year in cattle and deer.

Table 2. The number of cattle and deer positive for trypanosomiasis according to the different diagnostic techniques.

Type of animals	Diagnostic techniques			Total
	Thin blood smear	Buffy coat examination	Thin blood smear and buffy coat examination	
Cattle	3	3	4	10
Deer	8	14	36	58
Total	**11**	**17**	**40**	**68**

The results showed that highest positive samples were detected using both thin blood smear and buffy coat examination. The thin blood smear technique requires only a small blood sample to detect parasites, while the buffy coat examination involves filling three-quarters of a microhaematocrit tube for parasite detection. Nevertheless, only fresh blood samples, less than 48 hours old, are suitable for observing living and motile Trypanosomes. Other techniques that can be employed include wet blood smear for fresh samples and thick blood smear for detecting dead intracellular parasites (Moody and Chiodini, 2000).

The assessment in this study relied solely on the detection of parasites in blood samples submitted for protozoal screening in Veterinary Research Institute. It is anticipated that the use of serological techniques, which do not necessitate the presence of the parasite, would yield higher prevalence rates. However, as indicated by Sivajothi *et al.* (2016), blood smear analysis has limited sensitivity, diagnosing less than half of infected animals. Therefore, it is more effective in cases of acute trypanosomiasis with high parasitaemia, compared to chronic cases (Rhaymah and Al-Badrani, 2012).

The morphological detection of *Trypanosoma* spp. at the peripheral thin smear showed the nucleus of the protozoa at the central position with a small kinetoplast visible at the posterior end. Figure 2 showed the blood film of *Trypanosoma* spp. in cattle and deer under ×1000 magnification.

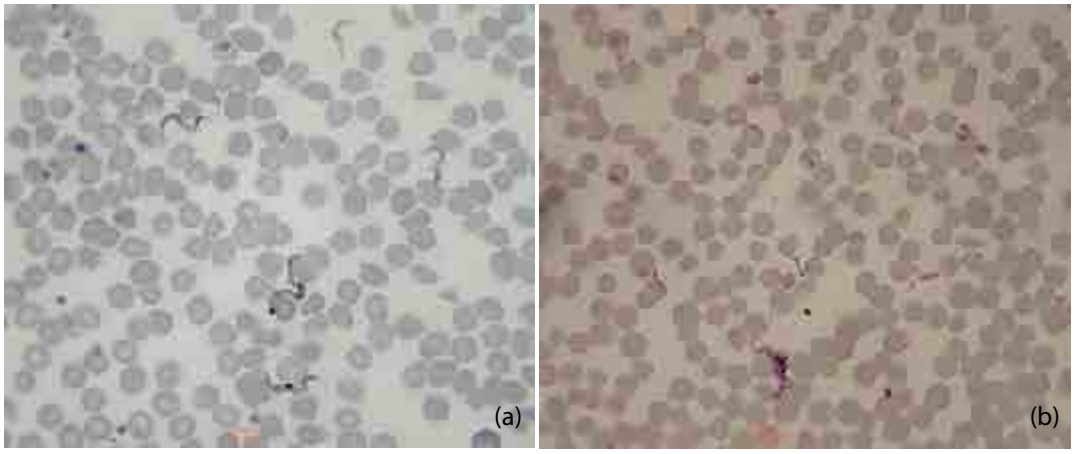
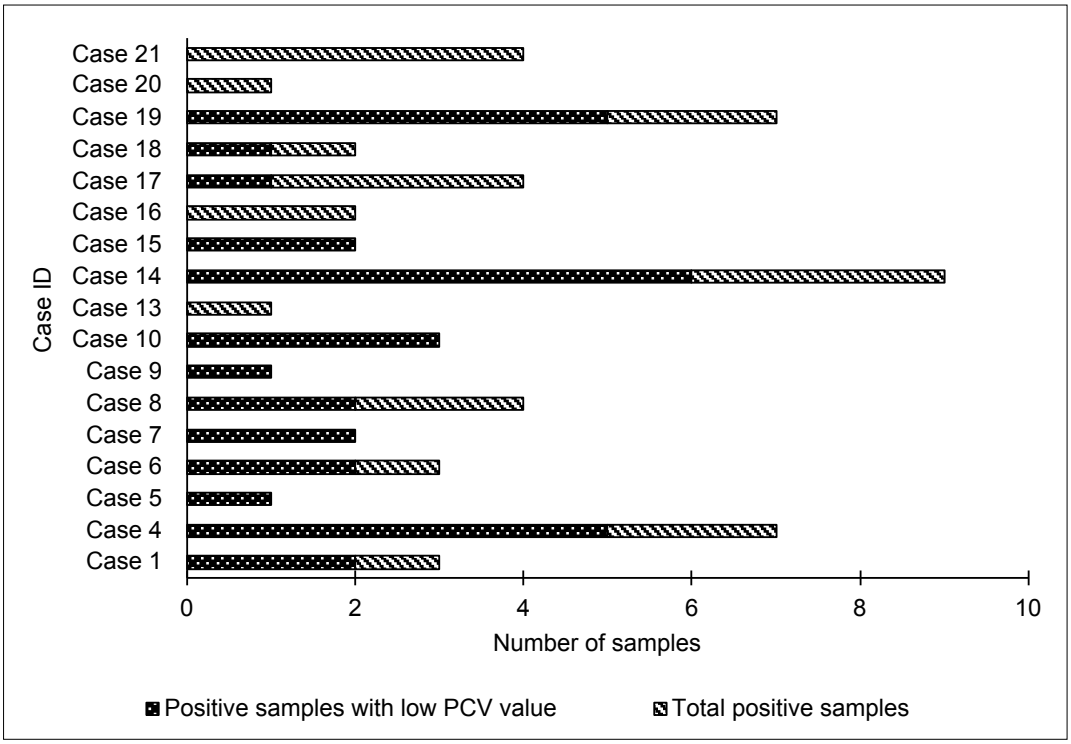


Figure 2. Giemsa-stained blood film showing *Trypanosoma* spp. under ×1000 magnification in cattle (a) and deer (b).

The mean PCV values (%) for cattle and deer positive with *Trypanosoma* spp. were 19.50±6.84 and 36.59±5.83, respectively. In contrast, cattle not infected with *Trypanosoma* spp. had a mean PCV value of 26.34±6.38 and for deer at 38.05±7.02. From the results, it was shown that trypanosomiasis affected the PCV value in which animals infected with trypanosomiasis tend to have lower PCV values outside of the normal range. The normal packed cell volume (PCV) values for cattle generally fall within a range of 24% to 46% (Radostits *et al.,* 2000). In contrast, deer show slightly higher PCV values, which usually range from 39% to 43% (Zawida *et al.*, 2012). Figure 3 is the number of positive trypanosomiasis samples with low PCV values according to positive cases.

The pathogenesis of trypanosomiasis by salivarian species begins when blood-sucking flies inject parasites during feeding (Radwanska *et al*., 2018). The trypanosomes actively multiply in the bloodstream of mammals, elevating the host's body temperature and causing fever (Vickerman, 1985). Furthermore, the parasites consume the host's plasma glucose, resulting in hypoglycaemia, weakness, lethargy, and emaciation (Michels *et al*., 2021). Trypanosomes also produce metabolites that coat erythrocytes and leukocytes, leading to phagocytosis of red and white blood cells due to the unrecognised foreign cell by the macrophage, resulting in anaemia and leukopenia (Radwanska *et al*., 2018).



Notes: Normal PCV value for cattle from 24-46%, and for deer from 39-43%; The data for positive Case 2, 3, 11 and 12 were not available.

Figure 3. The number of positive trypanosomiasis samples with low PCV value according to Case ID.

The painful bites of blood-sucking flies also induce discomfort and interrupt the animals' feeding. To control the flies, insecticides, environmental management, and physical barriers such as fly traps can be employed (Okello *et al*., 2021; Hargrove *et al*., 2012). As for the treatment of trypanosomiasis, the intramuscular injection of the trypanocide Diaminazine aceturate is recommended at a dosage of 3.5 to 7.0 mg/kg body weight (Giordani *et al*., 2016).

CONCLUSION

Findings in this study demonstrated that the occurrence of trypanosomiasis in cattle and deer over the past 6 years (2017-2022) in Perak was low, at only 1.98% incidence rate. This suggests that trypanosomiasis infection in cattle and deer was under control. Proper strategies to mitigate this disease must be designed and implemented, such as controlling biting flies to reduce disease transmission and safeguard the health and productivity of cattle and deer in Malaysia.

REFERENCES

1. Abas-Mazni, O., Zainal-Abidin, A.H. (1985). The infectivity and virulence of *Trypanosoma* evansi in local Indian dairy X Kedah-Kelantan cattle. *MARDI Research Bulletin*, 13: 291-301.
2. Bhatia, B.B., Pathak, K.M.L., Banerjee, D.P. (2006). Text book of veterinary parasitology, 2nd Ed. Kalyani Publishers (pp. 304-315). Ludhiana: India.
3. Chandrawathani, P., Premaalatha, B., Jamnah, O., Zaini, C.M. (2016). *Manual on parasitology*. Department of Veterinary Services: Malaysia.
4. Chau, N.V., Desquesnes, M., Herder, S., Lan, N.P., Campbell, J.I., Van Cuong, N., Yimming, B., Chalermwong, P., Jittapalapong, S., Franco, J.R., Tue, N.T. (2016). A clinical and epidemiological investigation of the first reported human infection with the zoonotic parasite *Trypanosoma evansi* in Southeast Asia. *Clinical Infectious Diseases*, 62: 1002-1008.
5. Cheah, T.S., Sani, R.A., Chandrawathani, P., Bahri, S., Dahlan, I. (1999). Epidemiology of *Trypanosoma evansi* infection in crossbred dairy cattle in Malaysia. *Tropical Animal Health and Production*, 31: 25-31.
6. Da Silva, A.S., Wolkmer, P., Costa, M.M., Tonin, A.I.A., Eilers, T.L., Gressle, L.T., Otto, M.A., Zanette, R.A., Santurio, J.M., Lopes, S.T.A., et al. (2010). Biochemical changes in cats infected with *Trypanosoma evansi*. *Veterinary Parasitology*, 17: 4852.
7. Desquesnes, M., Biteau-Coroller, F., Bouyer, J., Dia, M.L., Foil, L.D. (2009). Development of a mathematical model for mechanical transmission of *trypanosomes* and other pathogens of cattle transmitted by tabanids. *International Journal of Parasitology*, 39: 333-346.
8. Desquesnes, M., Holzmuller, P., Lai, D.H., Dargantes, A., Lun, Z.R., Jittaplapong, S. (2013). *Trypanosoma evansi* and *surra*: a review and perspectives on origin, history, distribution, taxonomy, morphology, hosts, and pathogenic effects. *BioMed Research International*, 2013: 194176.
9. Elshafie, E.I., Sani, R.A., Hassan, L., Sharma, R., Bashir, A., Abubakar, I.A. (2013). Seroprevalence and risk factors of *Trypanosoma evansi* infection in horses in Peninsular Malaysia. *Research in Veterinary Science*, 94: 285-289.
10. Erwanas, A.I., Masrin, A., Chandrawathani, P., Jamnah, O., Premaalatha, B., Ramlan, M. (2015). Vectors of veterinary importance in Malaysia: a survey of biting flies in relation to *trypanosomiasis* in Perak. *Malaysian Journal of Veterinary Research*, 6: 89-96.
11. Giordani F., Morrison, L.J., Rowan, T.G., Koning, H.P.D.E., Barrett, M.P. (2016). The animal *trypanosomiases* and their chemotherapy: a review. *Parasitology*, 143: 1862-1889.
12. Gomes, C., Almeida, A.B., Rosa, A.C., Araujo, P.F., Teixeira, A.R.L. (2019). American *trypanosomiasis* and Chagas disease: sexual transmission. *International Journal of Infectious Diseases*, 81: 81-84.
13. Hargrove, J.W., Ouifki, R., Kajunguri, D., Vale, G.A., Torr, S.J. (2012). Modeling the control of *trypanosomiasis* using trypanocides or insecticide-treated livestock. *PLoS Neglected Tropical Diseases*, 6: e1615.

14. Jesse, F.F.A., Bitrus, A.A., Abba, Y., Sadiq, M.A., Hambali, I.U., Chung, E.L.T., Ping, F.L.P., Haron, A.W., Lila, M.A.M., Saharee, A.A., Norsidin, M.J. (2016). A clinical case of bovine trypanosomosis in an endemic farm in Malaysia. *Journal of Advanced Veterinary and Animal Research*, 3(3): 286-291.
15. Kumar, H., Gupta, H.P., Sidhu, P.K., Mahajan, V., Bal, M.S., Kaur, K., Ashuma, Verma, S., Singla, L.D. (2012). An outbreak of acute *Trypanosoma* evansi infection in crossbred cattle in Punjab, India, *Journal of Applied Animal Research*, 40(3): 256-259.
16. Michels, P.A.M., Villafraz, O., Pineda, E., Alencar, M.B., Caceres, A.J., Silber, A.M., Bringaud, F. (2021). Carbohydrate metabolism in trypanosomatids: new insights revealing novel complexity, diversity and species-unique features. *Experimental Parasitology*, 224: 108102.
17. Moody, A.H., Chiodini, P.L. (2000). Methods for the detection of blood parasites. *Clinical and Laboratory Haematology*, 22: 189-202.
18. Nurulaini, R., Jamnah, O., Adnan, M., Zaini, C.M., Khadijah, S., Rafiah, A., Chandrawathani, P. (2007). Mortality of domesticated java deer attributed to surra. *Tropical Biomedicine*, 24(2): 67-70.
19. Nurulaini, R., Premaalatha, B., Zaini, C.M., Adnan, M., Chandrawathani, P., Fazly Ann, Z.A., Enie Aryanti, A., Ramlan, M. (2013). *Trypanosomiasis* outbreak in deer, cattle, buffaloes and pigs in Perak. *Malaysian Journal of Veterinary Research*, 4: 55-58.
20. Okello, W.O., MacLeod, E.T., Muhanguzi, D., Waiswa, C., Welburn, S.C. (2021). Controlling tsetse flies and ticks using insecticide treatment of cattle in Tororo District Uganda: cost benefit analysis. *Frontiers in Veterinary Science*. 8: 616865.
21. Papagni, R., Novara, R., Minardi, M.L., Frallonardo, L., Panico, G.G., Pallara, E., Cotugno, S., Ascoli Bartoli, T., Guido, G., De Vita, E., Ricciardi, A., Totaro, V., Camporeale, M., Segala, F.V., Bavaro, D.F., Patti, G., Brindicci, G., Pellegrino, C., Mariani, M.F., Putoto, G., Sarmati, L., Castellani, C., Saracino, A., Di Gennaro, F., Nicastri, E. (2023) Human African *trypanosomiasis* (sleeping sickness): Current knowledge and future challenges. *Frontiers in Tropical Diseases*, 4:1087003.
22. Radostits, O.M., Gay, C.C., Blood, D.C., Hinchcliff, K.W. (2000). *Veterinary medicine*, *9th Ed*., pp: 1819-1822. W.B. Saunders, London: United Kingdom.
23. Radwanska, M., Vereecke, N., Deleeuw, V., Pinto, J., Magez, S. (2018). *Salivarian trypanosomosis*: a review of parasites involved, their global distribution and their interaction with the innate and adaptive mammalian host immune system. *Frontiers in Immunology*, 9: 2253.
24. Rajdi, N.Z.I.M., Mohamad, M.A., Tan, L.P., Choong, S.S., Reduan, M.F.H., Hamdan, R.H., Zalati, C.W.S.C.W. (2021). First *case* report on the occurrence of *Trypanosoma evansi* in a Siam B Mare in Kelantan, Malaysia. *Veterinary Medicine and Science*, 7: 303-309.
25. Reid, S.A., Husein, A., Hutchinson, G.W. and Copeman, D.B., 1999. A possible role for Rusa deer (*Cervus timorensis russa*) and wild pigs in spread of *Trypanosoma* evansi from Indonesia to Papua New Guinea. *Memórias do Instituto Oswaldo Cruz*, Rio de Janeiro, 94: 195–197.
26. Rhaymah, M.S.H., Al-Badrani, B.A. (2012). *Clinical and diagnostic study of trypanosomiasis* of cattle in Mosul, Iraq. Research Opinions in Animal & Veterinary Sciences, 2: 23-26.
27. Sani, R.A., Chandrawathani, P., Salim, N.B. (1995). *Diagnosis of T. evansi infection in cattle and buffaloes in W. Malaysia*. Paper presented at 7th National *Biotechnology* Seminar, Langkawi, Malaysia, 20-22 November 1995.
28. Sivajothi, S., Rayulu, V.C., Malakondaiah, P., Sreenivasulu, D. (2016). Diagnosis of *Trypanosoma evansi* in bovines by indirect ELISA. *Journal of Parasitic Diseases*, 40: 141-144.
29. Soulsby, E.J. (1982). *Helminths, arthropods and protozoa of domesticated animals*, 7th Ed., Bailliere Tindall, London: United Kingdom.
30. Stuht, J.N. (1975). Morphology of trypanosomes from white-tailed deer and wapiti in Michigan. Journal of Wildlife Diseases, 11: 256-262.
31. Uilenberg, G. (1998). A field guide for the diagnosis, treatment and prevention of African animal *trypanosomosis*. *Food and Agriculture Organization of the United Nations*: Rome, Italy.

32. Vickerman, K. (1985). Developmental cycles and biology of *pathogenic trypanosomes. British Medical Bulletin*, 41: 105-114.
33. Zawida, Z., Adnan, M., Premaalatha, B., Zaini, C.M., Jamnah, O., Chandrawathani, P., Nurul Aini, R., Norlindawati, A.P., Norazura, A.H., Mohd Bohari, J. (2012). Haematology and serum biochemistry profile of physically restrained *Cervus timorensis*. *Malaysian Journal of Veterinary Research*, 3(2): 29-34.
34. Zecharias, A., Zeryehun, T. (2012). Prevalence of bovine *trypanosomosis* in selected district of Arba Minch, Snnpr, Southern Ethiopia. *Global Veterinaria*, 8: 168-173.

ACKNOWLEDGEMENT. The authors express their gratitude to the Director General of the Department of Veterinary Services (DVS) Malaysia for their kind permission to publish this paper. The authors would also like to thank the Director of the Veterinary Research Institute (VRI) for their encouragement, as well as to the Parasitology and Haematology Section for their valuable contribution and support to this study.