Investigation of *Trypanosoma evansi* in Sumatran Elephant (*Elephas maximus sumatranus*) in Indonesia Using Various Methods

Kartika Purnamasari[1]*, Wisnu Nurcahyo[2], Muhammad Tauhid Nursalim[2], Lucia Tri Suwanti[3], Agnes Theresia Soelih Estoepangestie[3]
[1]Faculty of Veterinary Medicine, Wijaya Kusuma Surabaya University, Surabaya, Indonesia, [2]Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta, Indonesia, [3]Faculty of Veterinary Medicine, Airlangga University, Surabaya, Indonesia.
*Corresponding author: kartika_fkh@uwks.ac.id

Abstract

Trypanosoma evansi is a widespread hemoprotozoa that causes trypanosomiasis in both livestock and wildlife. One of the susceptible animals is the Sumatran elephant, which is included in the endangered species category. The infection of this parasite often develops into chronic and sub-clinical forms in elephants, therefore it may become unnoticed and hard to diagnose. This study aimed to analyze and evaluate the infection of *T. evansi* in semi-captive Sumatran elephants in Way Kambas National Park, Indonesia, using various diagnostic tests. The prevalence of *T. evansi* in a total of 53 Sumatran elephants was estimated using a card agglutination test for trypanosomiasis (CATT) in 2016. A longitudinal study was later conducted in 2019 using Giemsa stained blood smear (GSBS) and polymerase chain reaction (PCR). The results showed that 26.4% of samples (14/53) were positively detected by both CATT in 2016 and PCR in 2019, while GSBS was unable to detect the parasites in all samples. Furthermore, four individuals were confirmed to have persistent infections. This study concluded that the ability of CATT and PCR were more convincing over GSBS for the diagnosis of sub-clinical trypanosomiasis in Sumatran elephants. However, it is recommended to use a combination of CATT as a screening tool and PCR as a confirmatory test for reliable results.

Keywords: card agglutination test, giemsa stain, polymerase chain reaction, Sumatran elephant

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INTRODUCTION

Trypanosoma evansi (Kinetoplastida, Trypanosomatidae) is a hemoflagellate parasite whose infection can lead to trypanosomiasis or surra disease. The disease is prominently transmitted by vectors namely blood-sucking flies, such as Tabanids and Stomoxys, to almost all terrestrial mammals (Desquesnes *et al*., 2013). The disease spread throughout Southeast Asia and some other regions causing major economic impacts. This impact is mostly due to the infection in livestock, such as horses and cattle (Nurcahyo *et al*., 2019; Mossaad *et al*., 2020), thus the report of the disease is still limited to these animals. However, the parasite can also infect and become a dreadful disease in wildlife yet it has not been thoroughly investigated (Camoin *et al*., 2018; Purnama *et al*., 2021; Panjaitan *et al*., 2021).

Vellayan *et al*. (2004) reported that an outbreak of trypanosomiasis caused the fatal death of five captive Sumatran rhinoceros in Selangor Malaysia. The infection was allegedly obtained from buffaloes located close to the rhino. Moreover, outbreaks of trypanosomiasis also happened in two elephant camps in Thailand, which led to severe clinical signs in some elephants in those camps. This species commonly showed non-pathognomonic signs including fever, anemia, weakness, edema, and weight loss (Camoin *et al*., 2018). These reports caused many conservation facilities to become more aware and try to prevent this disease from spreading, especially to endangered species.

Sumatran elephant, one of the sub-species of Asian elephant that is in endangered status, can be also fatally affected by trypanosomiasis (EFSA AHAW Panel *et al*., 2017). Though trypanosomiasis can be acute and fatal in

elephants, it can evolve into chronic and sub-clinical which symptoms are not clear or even asymptomatic (Desquesnes *et al*., 2013). Individuals with unclear clinical signs may act as reservoirs and spread the infection to other elephants or wildlife (Anderson *et al*., 2011). The persistent infection can be a threat to the sustainability of wildlife inside the conservation area. Thus, to ensure the infection of *T. evansi* in elephants, the diagnosis should be confirmed by some laboratory tests (Camoin *et al*., 2018; Syah *et al*., 2020).

There are several diagnostic tests for the detection of *T. evansi* based on parasitological, serological, and molecular methods. Generally, parasitological methods are used to confirm this parasite and dubbed as a gold standard despite having low sensitivity (Aslam *et al*., 2010). On the other hand, a serological test that can detect antibodies of *T. evansi*, card agglutination test for trypanosomiasis (CATT), is also frequently used for the epidemiological study of trypanosomiasis (Nurcahyo *et al*., 2019). At the same time, molecular diagnostic tests have been widely used for the detection of trypanosomiasis in various animals (Sudan *et al*., 2015). However, there was no report yet about the assessment of each method mentioned above to be applied in Sumatran elephant. Therefore, the objective of this study was to estimate the prevalence and evaluate several diagnostic tests for *T. evansi* infection in the Sumatran elephants.

MATERIALS AND METHODS

Ethical Approval

All procedures of this study received approval from the Research Ethics Committee, Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta, with Ethical Clearance Number: 0020/EC-FKH/Int./2019.

Study Period and Location

This study was conducted at five elephant camps in Way Kambas National Park, Indonesia (4.917ºS 105.750ºE) from 2016 to 2019. This site was one of the conservation areas for Sumatran elephants which is managed by the Indonesian Ministry of Environment and Forestry.

Sample Collection and Preparation

A total of 53 semi-captive Sumatran elephants were used as the objects for this study. The collection of blood samples was conducted twice in the same individuals, i.e. in 2016 for a card agglutination test for trypanosomiasis (CATT) and in 2019 for a follow-up study using Giemsa stained blood smear (GSBS) and polymerase chain reaction (PCR). Approximately, 10 mL of blood samples were collected through auricular veins. These whole-blood samples were stored and prepared differently depending on the diagnostic methods used. The samples were kept on an ice pack in a thermally-insulated box where the temperature was maintained at around 4ºC, then transferred to the laboratory for further examination process.

For the serological test in 2016, the blood samples from each elephant were collected in plain tubes and allowed to clot for 1–2 hours in the refrigerator until serum separated. Furthermore, these serum samples were stored at -20ºC until further use for CATT. For a follow-up study in 2019, the blood samples were collected in tubes containing ethylene diamine tetraacetic acid (EDTA). These samples were, then, centrifuged at 3,300 × g for 10 minutes to obtain a buffy coat. For the parasitological test, buffy coat samples were processed into GSBS for six hours of sample collection, while buffy coat samples for the molecular test were stored at -20ºC until further used for extraction of *T. evansi* DNA for PCR amplification.

Card Agglutination Test for Trypanosomiasis (CATT)

The procedure used was according to the Institute of Tropical Medicine, Antwerp, Belgium. Briefly, the steps were described as follows. A total of 20 µL serum was diluted in 60 µL phosphate buffer saline (1:4) at pH 7.2 on the enzyme-linked immunosorbent assay plate. Then, 25 µL diluted serum was put on the circular test area of CATT and about 45 µL CATT antigen was added. The mixture was homogenized using a

stirring rod. The CATT card was placed on the rotator for 5 minutes at 70 rpm. After 5 minutes, the results were read by observing the agglutination level (Figure 1).

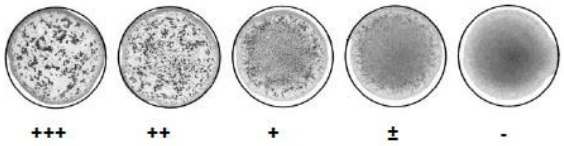

Figure 1. Interpretation of CATT result based on the agglutination level by the Institute of Tropical Medicine, Antwerp, Belgium: (+++) strongly positive, (++) and (+) positive, (±) weakly positive, (-) negative.

Giemsa Stained Blood Smear (GSBS)

The following method was based on Abdel-Rady (2008) with some modifications. Buffy coats from each sample were thin-smeared on a glass slide, fixed in methyl alcohol for 2 minutes, and allowed to dry, then stained by Giemsa 10%. The slides were examined under a light microscope at a magnification of 400–1000×.

Polymerase Chain Reaction (PCR)

The genomic DNA of each sample was extracted from buffy coat using FavorPrep™ Blood Genomic DNA Extraction Mini Kit (Biotech) following the Favorgen® protocol. The DNA concentration (ng/µL) was assessed from 1 µL of this extract using MaestroNano Pro Spectrophotometer. The blood of experimental mice at peak parasitemia from the Laboratory of Parasitology, Universitas Gadjah Mada was used as the positive control of *T. evansi* DNA. Later, the DNA was kept at -20ºC until used as a template for PCR.

The PCR amplification was performed using two sets of primers (Table 1), i.e. TBR1/2 (Masiga *et al*., 1992) and ITS1 CF/BR (Njiru *et al*., 2005). The PCR reaction was performed in a 20 µL reaction mixture containing 10 µL of MyTaq™ HS Red Mix (Bioline), 1 µL of each primer set, 2 µL of extracted DNA, and 6 µL of ddH2O. Geneaid 100 bp DNA Ladder (Biotech, Taiwan) was used as a molecular size marker. About 3 µL of amplified products and DNA ladder were loaded in a 1.5% agarose gel with ethidium bromide and run for 30 min at 100 V by electrophoresis. The genomic DNA was visualized on a UV transilluminator.

Data Analysis

Data were tabulated and analyzed using Microsoft Excel 2016. Then to explain the data, it was presented descriptively and sequentially.

RESULTS AND DISCUSSION

In this study, the prevalence of *T. evansi* in Sumatran elephants was estimated using the CATT in 2016. This method confirmed that antibodies of *T. evansi* were present in 14 blood samples. Furthermore, the longitudinal study in 2019 showed there were no positive samples detected by Giemsa stained blood smear, but DNA amplicons of *T. evansi* were detected in 14 of all parasitological negative samples using TBR1/2 primers with the expected size of 164 bp. However, ITS1 CF/BR primers were unable to detect the parasite and showed negative results in all samples. Therefore, the percentage of detection was 26.4% for CATT, 0% for GSBS, and 26.4% and 0% for PCR using TBR1/2 and ITS1 CF/BR primers respectively (Table 2).

Table 1. Procedures used for PCR amplification

Primers	Sequences	Size	Procedures
TBR1/2	5’-GAATATTAAACAATGCGCAG-3’ 5’-CCATTTATTAGCTTTGTTGC-3'	164 bp (multiples)	3' at 94°C, 35 cycles: [1' at 94°C, 2' at 57°C, 30" at 72°C], and 5' at 72°C
ITS1 CF/BR	5’-CCGGAAGTTCACCGATATTG-3’ 5’-TGCTGCGTTCTTCAACGAA-3’	480 bp	5' at 94°C, 35 cycles: [40" at 94°C, 40" at 57°C, 90" at 72°C], and 5' at 72°C

Table 2. Prevalence of *T. evansi* infection in Sumatran elephant using different diagnostic tests

Test		Year	Positive Samples		Negative Samples	
			Number	%	Number	%
CATT		2016	14	26.4	39	73.6
GSBS			0	0	53	100
PCR	TBR1/2	2019	14	26.4	39	73.6
	ITS1 CF/BR		0	0	53	100

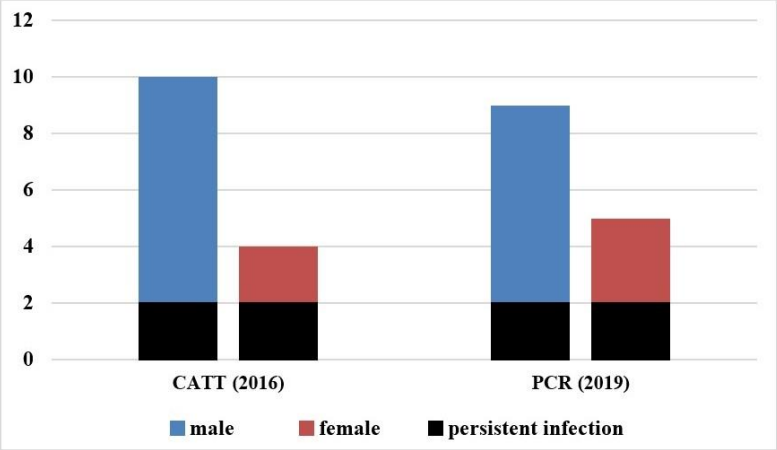

Figure 2. Persistent infection of *T. evansi* in Sumatran elephant population.

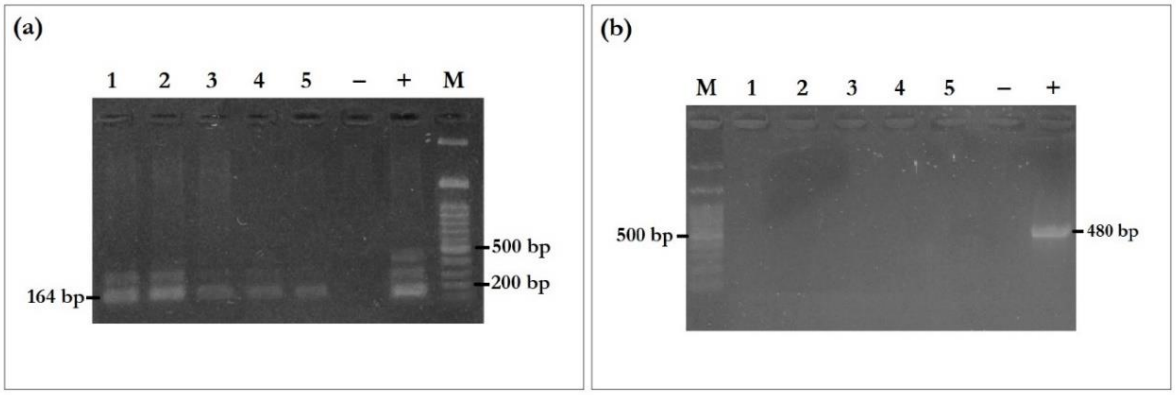

Figure 3. PCR products using different sets of primers: (a) TBR1/2 (b) ITS1 CF/BR.

The results of CATT in 2016 showed 14 positive blood samples which obtained from ten males and four females of Sumatran elephants. Furthermore, 14 positive blood samples that were positively detected by PCR using TBR1/2 in 2019 consisted of nine males and five females of Sumatran elephants. Data showed that there were four persistent infections among elephant herds. The persistent infection of *T. evansi* was observed in two males and two females of Sumatran elephants (Figure 2). Generally, all infected Sumatran elephants did not show any clinical symptoms, whether with recent or persistent infections.

There are several possible reasons for the occurrence of *T. evansi* in Sumatran elephants. In the study area, livestock and wildlife live together nearby, as in several other conservation areas in Indonesia. Several buffaloes were often seen grazing together with elephants during the dry season in Way Kambas National Park (Purnamasari *et al*., 2021). Moreover, the presence of blood-sucking flies around the elephant camps, notably, *Tabanus* sp., increases the risk of transmission (Kuncoro *et al*., 2017). These conditions are similar to Dobson *et al*. (2009) who reported that livestock does have the potential as a reservoir for vectors to spread the

disease to other animals and enhances the risk of interspecies infection by this parasite.

The four persistent infections detected in Sumatran elephants suggest that this parasite may have been underdiagnosed all this time. According to Desquesnes *et al*. (2013), the infection of *T. evansi* often develops into chronic and sub-clinical form in elephants, therefore it may become unnoticed and hard to diagnose. The persistence of this parasite might be caused by reinfection, which is mostly due to the existence of a reservoir followed by the increasing biting rate of vectors that have an important role in carrying out repeated transmission to the host (Reid, 2002). Moreover, the probability of repeated infection of *T. evansi* can rise if the vector and reservoir co-exist around the susceptible host, hence maintaining the presence of the parasite in this circle (Desquesnes *et al*., 2013).

Furthermore, the persistent infection among elephant herds can increase the possibility of further transmission or even an outbreak. The outbreak may occur due to several conditions, such as stressful situations related to malnutrition, concurrent infections, increase of vector populations, immunodepression, and the presence of infected animals (Ramírez-Iglesias *et al*., 2017; Arifin *et al*., 2019). Therefore, infection of this parasite should be monitored in Sumatran elephants and routine diagnostics should be performed to detect the disease as it could pose a threat to the conservation of the Sumatran elephant.

Several diagnostic methods for the detection of *T. evansi* in animals have been available and demonstrated. The GSBS test is considered as gold standard despite some weaknesses in its application i.e. low sensitivity and a higher chance of giving false negative results (Abdel-Rady, 2008). False negative can occur when the host has low parasitemia since the intensity of parasites in the blood circulation affects the sensitivity of the parasitological test. This condition frequently happens in the chronic infection (Elhaig *et al*., 2013). Moreover, the samples must be examined immediately after collection (within 4–6 hours) since parasites will be dead and hard to detect under a microscope, thus it seems to be inconvenient to use in the field and often gives equivocal results (Aslam *et al*., 2010). Due to these reasons, the GSBS test is not recommended as a routine diagnostic test in Sumatran elephants.

Serological test based upon the detection of either antigen or antibody is generally used for epidemiological application. This test, particularly CATT, claimed to be able to detect antibodies of *T. evansi* with a simple procedure and is considered low-cost. It has been applied widely to study the seroprevalence of this parasite in horses, cattle, sheep, goats, camels, water buffalo, and donkeys (Tehseen *et al*., 2017; Abdel-Gawad *et al*., 2019; Mossaad *et al*., 2020; Tapdasan *et al*., 2020; Kyari *et al*., 2021). Based on this study, CATT has also proven useful for the detection of trypanosomiasis in the Sumatran elephant population since it is practical to be used in the field. However, the serological tests based on antibody detection lacked specificity and were unable to distinguish a current infection from a previous infection. It showed unsatisfactory results due to the prolonged persistence of antibodies in the blood of the host (Aslam *et al*., 2010). Therefore, CATT is more suitable as a screening test and then it can be continued with a molecular test to confirm the parasites' existence in the blood (Nurcahyo, 2017).

The development and application of PCR, which detects parasite nucleic acids, have improved the diagnosis of trypanosomiasis so far. It is a very sensitive method that can detect even 1 pg of the parasite DNA in the blood samples. However, the sensitivity of PCR depends on several factors, such as the quality and quantity of the target DNA present in the samples, DNA extraction method, specificity of primers, and the copies number of the target sequence in the genome of the organism from the primers employed (Eleizalde *et al*., 2021). This study showed that TBR1/2 was more sensitive than ITS1 CF/BR to detect *T. evansi* in Sumatran elephant blood (Figure 3). This is due to TBR1/2, which amplifies multicopy satellite regions, having a higher number of copies compared to ITS1 CF/BR in the genome of the parasite.

Moreover, the minimum amount of DNA detectable by PCR using TBR1/2 primers was 0.001 ng (Fernández *et al*., 2009). Therefore, despite the simplicity and low cost of the serological and parasitological methods, it is recommended to use them in combination with the PCR method for accurate and reliable results (Abdel-Gawad *et al*., 2019).

CONCLUSION

This study revealed that there were 14 asymptomatic Sumatran elephants infected by *T. evansi* in 2016. The follow-up study indicated the presence of four persistent infections among the 14 positively infected Sumatran elephants in 2019. Moreover, the evaluation obtained from this study shows that the CATT and PCR tests are more convenient and sensitive than GSBS as a diagnostic test for *T. evansi* infection in the Sumatran elephants, especially in individuals with low parasitemia. However, the CATT is only recommended as a screening method to detect the antibody of *T. evansi* and should be followed by PCR to confirm the current infection of this parasite.

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AUTHORS' CONTRIBUTIONS

KP and WN: Conceptualization and drafted the manuscript. MTN, LTS, and ATSE: Collected and evaluated samples. WN and LTS: Validation, supervision, and formal analysis. KP, WN, LTS, and ATSE: Performed the statistical analysis and the preparation of tables and figures. All authors have read, reviewed, and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

Abdel-Gawad, A., Nassar, A., & Abdel-Wahab, A. (2019). Evaluation of the Card Agglutination Test (CATT/*T. Evansi*) in comparison with PCR for detection of *Trypanosoma evansi* infection in donkeys in Egypt. *Journal of the Egyptian Society of Parasitology*, 49(3), 539–542.

Abdel-Rady, A. (2008). Epidemiological studies (parasitological, serological and molecular techniques) of *Trypanosoma evansi* infection in camels (*Camelus dromedarius*) in Egypt. *Veterinary World*, 1(11), 325–328.

Anderson, N. E., Mubanga, J., Fevre, E. M., Picozzi, K., Eisler, M. C., Thomas, R., & Welburn, S. C. (2011). Characterisation of the wildlife reservoir community for human and animal trypanosomiasis in the Luangwa Valley, Zambia. *PLOS Neglected Tropical Disease*, 5(6), 1–16.

Arifin, K., Kusnoto, K., Yudhana, A., Sunarso, A., Purnama, M. T. E., & Praja, R. N. (2019). Prevalence of Haemonchiasis on Etawah Crossbreed Goat in Kalipuro, Banyuwangi. *Jurnal Medik Veteriner*, 2(2), 108.

Aslam, A., Chaudhary, Z. I., Rehman, H., Ashraf, K., Ahmad, N., Yaqub, T., Maqbool, A., & Shakoori, A. R. (2010). Comparative evaluation of parasitological, serological, and DNA amplification methods for diagnosis of natural trypanosomal infection in equines. *Pakistan Journal of Zoology*, 42(4), 371–376.

Camoin, M., Kocher, A., Chalermwong, P., Yangtarra, S., Thongtip, N., Jittapalapong, S., & Desquesnes, M. (2018). Adaptation and evaluation of an ELISA for *Trypanosoma evansi* infection (surra) in elephants and its application to a serological survey in Thailand. *Parasitology*, 145(3), 371–377.

Desquesnes, M., Holzmuller, P., Lai, D., Dargantes, A., Lun, Z., & Jittapalapong, S. (2013). *Trypanosoma evansi* and surra: a review and perspectives on origin, history, distribution, taxonomy, morphology, hosts, and pathogenic effects. *BioMed Research International*, 2013, 1–22.

Dobson, R. J., Dargantes, A. P., Mercado, R. T., & Reid, S. A. (2009). Models for *Trypanosoma evansi* (surra), its control and economic impact on small-hold livestock owners in the Philippines. *International Journal for Parasitology*, 39(10), 1115–1123.

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), More, S., Bøtner, A., Butterworth, A., Calistri, P., Depner, K., Edwards, S., Garin-Bastuji, B., Good, M., Schmidt, C. G., Michel, V., Miranda, M. A., Nielsen, S. S., Raj, M., Sihvonen, L., Spoolder, H., Stegeman, J. A., Thulke, H. H., Velarde, A., Willeberg, P., Winckler, C., Baldinelli, F., Broglia, A., Candiani, D., Beck, B. B., Kohnle, L., Morgado, J., & Bicout, D. (2017). Scientific opinion on the assessment of listing and categorisation of animal diseases within the framework of the Animal Health Law (Regulation (EU) No 2016/429): *Trypanosoma evansi* infections (including surra). *EFSA (European Food Safety Authority) Journal*, 15(7), 1–34.

Eleizalde, M. C., Gómez-Piñeres, E., Ramírez-Iglesias, J. R., & Mendoza, M. (2021). Evaluation of five primer sets for molecular detection of *Trypanosoma vivax* by polymerase chain reaction (PCR) and their implementation for diagnosis in naturally infected ruminants from Venezuela. *Veterinary Parasitology: Regional Studies and Reports*, 25, 1–7.

Elhaig, M. M., Youssef, A. I., & El-Gayar, A. K. (2013). Molecular and parasitological detection of *Trypanosoma evansi* in camels in Ismailia, Egypt. *Veterinary Parasitology*, 198(1–2), 214–218.

Fernández, D., Baradat, B. G., Eleizalde, M., Marcano, E. G., Perrone, T., & Mendoza, M. (2009). *Trypanosoma evansi*: a comparison of PCR and parasitological diagnostic tests in experimentally infected mice. *Experimental Parasitology*, 121(1), 1–7.

Kuncoro, P., Rosa, E., Rustiati, E. L., & Candra, D. (2017). Identifikasi ektoparasit pada gajah Sumatera (*Elephas maximus sumatranus*) di Pusat Latihan Gajah Taman Nasional Way Kambas. *Jurnal Biologi Eksperimen dan Keanekaragaman Hayati*, 4(2), 51–54.

Kyari, F., Mbaya, A. W., Biu, A. A., Adamu, L., & Dennis, O. O. (2021). Seroprevalence of *Trypanosoma evansi* in camels using CATT/*T. evansi* technique in Borno and Yobe states, Nigeria. *Parasite Epidemiology and Control*, 13, 1–26.

Masiga, D. K., Smyth, A. J., Hayes, P., Bromidge, T. J., & Gibson, W.C. (1992). Sensitive detection of trypanosomes in tsetse flies by DNA amplification. *International Journal for Parasitology*, 22(7), 909–918.

Mossaad, E., Ismail, A. A., Ibrahim, A. M., Musinguzi, P., Angara, T. E. E., Xuan, X., Inoue, N., & Suganuma, K. (2020). Prevalence of different trypanosomes in livestock in Blue Nile and West Kordofan States, Sudan. *Acta Tropica*, 203, 1–6.

Njiru, Z. K., Constantine, C. C., Guya, S., Crowther, J., Kiragu, J. M., Thompson, R. C.

A., & Dávila, A. M. R. (2005). The use of ITS1 rDNA PCR in detecting pathogenic African trypanosomes. *Parasitology Research*, 95, 186–192.

Nurcahyo, W. (2017). Penyakit surra pada hewan dan ternak. Samudra Biru, pp: 87–90.

Nurcahyo, W., Yowi, M. R. K., Hartati, S., & Prastowo, J. (2019). The prevalence of horse trypanosomiasis in Sumba Island, Indonesia and its detection using Card-Agglutination Tests. *Veterinary World*, 12(5), 646–652.

Panjaitan, C., Suprihati, E., Yudhana, A., Hastutiek, P., Wibawati, P. A., & Purnama, M. T. E. (2021). Acanthochephaliasis in White-lipped Green Pitviper (*Trimeresurus insularis*). *Jurnal Medik Veteriner*, 2021, 4(1), 155–159.

Purnamasari, K., Nursalim, M. T., Anggraeni, D. E., Suwanti, L. T., Estoepangestie, A. T. S., Raharjo, H. M., Wibowo, D. S., & Nurcahyo, W. (2021). Detection of *Trypanosoma evansi* in Sumatran elephant (*Elephas maximus sumatranus*) in Way Kambas National Park, Indonesia. Bangkok, Thailand, 29–30 April 2021, pp: 11–13.

Purnama, M. T. E., Dewi, W. K., Triana, N. M., & Ooi, H. K. (2021). Serum liver enzyme profile in Timor deer (*Cervus timorensis*) with fascioliasis in Indonesia. *Tropical Biomedicine*, 38(1), 57–61.

Ramírez-Iglesias, J. R., Eleizalde, M. C., Reyna-Bello, A., & Mendoza, M. (2017). Molecular diagnosis of cattle trypanosomes in Venezuela: evidences of *Trypanosoma evansi* and *Trypanosoma vivax* infections. *Journal of Parasitic Diseases*, 41(2), 450–458.

Reid, S. A. (2002). *Trypanosoma evansi* control and containment in Australasia. *TRENDS in Parasitology*, 18(5), 219–224.

Sudan, V., Jaiswal, A. K., Parashar, R., & Shanker, D. (2015). A duplex PCR-based assay for simultaneous detection of *Trypanosoma evansi* and *Theileria annulate* infections in water buffaloes. *Tropical Animal Health and Production*, 47, 915–919.

Syah, A., Basri, C., & Wicaksono, A. (2020). Incidence of Surra Disease on Beef Cattle in Barru Regency, South Sulawesi Province in 2015–2017. *Jurnal Medik Veteriner*, 3(2), 145–153.

Tapdasan, E. P., Baldrias, L. R., Mingala, C. N., Divina, B. P., Bajenting, G. P., & Salces, C. B. (2020). Prevalence and risk factors of *Trypanosoma evansi* infection in water buffaloes (*Bubalus bubalis*) of Ubay, Bohol, Philippines. *Philippine Journal of Veterinary Medicine*, 57(2), 164–175.

Tehseen, S., Jahan, N., Desquesnes, M., Shahzad, M. I., & Qamar, M. F. (2017). Field investigation of *Trypanosoma evansi* and comparative analysis of diagnostic test in horses from Bahawalpur, Pakistan. *Turkish Journal of Veterinary and Animal Sciences*, 41, 288–293.

Vellayan, S., Mohamad, A., Radcliffe, R. W., Lowenstine, L. J., Epstein, J., Reid, S. A., Paglia, D. E., Radcliffe, R. M., Roth, T. L., Foose, T. J., Khan, M. K. M., Jayam, V., Reza, S., & Abraham, M. (2004). Trypanosomiasis (surra) in the captive Sumatran rhinoceros (*Dicerorhinus sumatrensis sumatrensis*) in Peninsular Malaysia. Kuala Lumpur, Malaysia, 23–27 August 2004, pp: 187–189.

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