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The Pattern of Biological Character, Morphometry, and Genetic Information of *Trypanosoma evansi* Isolated from Bogor District, Indonesia

(Pola Karakter Biologis, Morfometri, dan Informasi Genetik *Trypanosoma evansi* yang Diisolasi dari Kabupaten Bogor, Indonesia)

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ABSTRAK

Trypanosoma evansi (*T. evansi*) merupakan penyebab utama trypanosomiasis pada hewan ternak. Penelitian ini dilakukan untuk menggambarkan variasi virulensi, morfometri, dan karakter genetik empat isolat *T. evansi* yang berasal dari sapi dan kerbau di Kabupaten Bogor, Jawa Barat. Seluruh isolat diperbanyak pada mencit untuk mengamati periode prepatent, perkembangan parasitemia, ciri morfologi, serta hubungan genetiknya. Hasil penelitian menunjukkan adanya tiga pola virulensi. Isolat dengan virulensi sedang berasal dari sapi dan kerbau menyebabkan kematian mencit pada hari ke-14 pascainfeksi. Dua isolat lain dari sapi menunjukkan virulensi rendah (kematian pada hari ke-24) dan virulensi tinggi (hari ke-4). Perbedaan ukuran tubuh juga terlihat jelas, masing-masing 22,05 µm pada isolat virulensi rendah, 24,1 µm pada virulensi sedang, dan 24,6 µm pada virulensi tinggi. Analisis sekuensing menunjukkan bahwa isolat dengan virulensi rendah dan sedang berada dalam kelompok yang sama, sedangkan isolat virulensi tinggi berkelompok dengan isolat dari Thailand dan Tiongkok. Studi ini menunjukkan bahwa isolat *T. evansi* di lapangan memiliki keragaman biologis dan genetik yang cukup lebar, sehingga pemantauan terhadap penyebarannya tetap diperlukan, termasuk pada isolat yang tampak kurang virulen.
Kata kunci: Bogor; informasi genetic; morfologi; *Trypanosoma evansi*

INTRODUCTION

Trypanosomiasis, or Surra's disease, is a strategic disease caused by *Trypanosoma evansi*, which can infect cattle and domestic animals worldwide. The disease can be acute or chronic and is transmitted mechanically by blood-sucking fly vectors such as *Tabanus* sp. and *Stomoxys* sp. (Desquesnes et al. 2013). Anemia occurs in animals infected with Trypanosoma because this parasite is an extracellular parasite in blood plasma. This parasite will take up the glucose in the

host's blood and produce trypanotoxin, which affects the host's immune system (Desquesnes et al. 2022).

T. evansi infection in livestock can cause significant economic losses, such as declining production of meat and milk, reproductive disorders (abortion), and livestock death (Perrone et al. 2018). Trypanosomosis outbreaks in East Sumba, Indonesia, in 2011 caused the death of 4268 livestock, including 1608 horses, 2646 buffaloes, and 196 cows. Economic losses at that time reached 167.5 billion rupiahs, not only the loss caused by the death of livestock but also the costs incurred for the program prevention and control (Dewi et al. 2020).

Trypanosoma control is done by administering anti-drug Trypanosomes such as isometamedium and diminazene aceturate. However, the problem of Trypanosoma resistance to drugs arises from Trypanosoma. As reported by Nuryady et al. (2021) in Indonesia, almost all isolates from several provinces were proven resistant to isometamedium, and some isolates were resistant to diminazen aceturate. Besides, various reports prove the existence of various varieties of *T. evansi,* which is related to sensitivity to anti-trypanosoma drugs, Subekti et al. (2015) reported the diversity of *T.evansi* against anti-trypanosoma drugs in Indonesia.

Different patterns of parasitemia and virulence are also present in the diversity of *Trypanosoma evansi* in some provinces of Indonesia Subekti et al. (2013). Classify the level of virulence into three biotypes. Biotype 1 is the most virulent, with the ability to kill infected hosts ≤ 8 days after infection. Biotypes 2 and 3 have lower levels of virulence compared to biotype 1. The characteristic of biotype 2 is parasitemia undulant, while the characteristic of biotype 3 is high parasitemia over a long time with the ability to kill mice ≥ 14 days. Based on our knowledge, there is no information about the pattern of morphometry of Trypanosoma, including the size of Trypanosoma during propagation on mice and the genetic information of the different virulent of *T. evansi*, mainly isolated from Indonesia. Besides, this study was observed about the number of different patterns of *Trypanosoma evansi* from infected animals or the heterogenicity of *Trypanosoma evansi*. Research from other regions has demonstrated that *T. evansi* isolates can exhibit diverse levels of virulence. For instance, Kamidi et al. (2018) and Barghash (2020) showed notable differences in parasitemia dynamics and pathogenicity across strains from Africa and the Middle East. However, studies that link virulence levels with morphometric characteristics and genetic profiles are still scarce. In Indonesia, although Surra cases are widely reported in cattle, buffaloes, and horses, most investigations have been limited to molecular detection or basic parasitological identification, without

exploring deeper biological variation among isolates.

Therefore, this research was conducted to determine the virulence and pattern of morphometry of *Trypanosoma evansi* in mice from buffalo and cattle isolates in Bogor, Indonesia.

MATERIALS AND METHODS

Ethical Statement

This research has received approval from the Ethics Committee School of Veterinary Medicine and Biomedical Sciences, IPB University, with number 008/KEH/SKE/I/2023.

Parasite and Propagation on Mice

A total of 20 mice males of the Deutschland, Denken, and Yoken (DDY) line aged 10-12 weeks (body weight 26-30 g) were used in this research. Feed and drinking water are provided ad libitum. Before use for the propagation of *T. evansi*, mice were adapted. Four *T. evansi* isolates were examined, consisting of three isolates from cattle (BGR-SP1, BGR-SP2, BGR-SP3) and one isolate from buffalo (BGR-KB), all obtained from naturally infected animals in the Ciapus area, Bogor Regency.the isolates came from three infected cows (BGR-SP1, BGR-SP2, and BGR-SP3) and buffalo (BGR-KB) infected in the Ciapus area, Bogor Regency. The isolate passage was carried out on mice and stored in the freezer at -80 ºC. Before the isolates used were thawed first at a temperature (of 25-28 ºC) for 10 minutes, then diluted with PBS-G 1% (Phosphate Buffer Saline with Glucose 1%) to 0.2 mL and injected intraperitoneally (i.p). The parasitemia level of the mice was checked every two days. If the level of parasitemia had reached a peak (10^8 *Trypanosoma*/mL blood), mice were anesthetized using ketamine and sacrificed. The blood was collected intracardially for use as a source of infection (Bal et al. 2012).

The biological character of *T. evansi* on mice

The biological character of *T. evansi* was observed in two stages (two passages). The mice were inoculated with 10^6 *Trypanosoma* /ml intraperitoneally. Each stage is divided into four groups (5 mice/group), four infected groups of cattle (BGR-SP1, BGR-SP2, and BGR-SP3) and buffalo (BGR-KB). The biological characteristics of field isolates, including the mortality of mice, were observed every day, while the level of parasitemia was observed twice a day. The level of parasitemia was calculated using the *Neubauer* chamber (Bal et al. 2012).

Morphometry of *T. evansi*

In addition, observations were also made by observing the morphology of *T. evansi* under a microscope at 1000x magnification. The morphology of *Trypanosoma* was observed from a blood smear, which was stained using Giemsa 10%. The morphology of the *Trypanosoma* was observed and

measured based on several parameters, including size (length) and shape of the *Trypanosoma*. The length of *Trypanosoma*: total length of the trypomastigote, including the free flagella. The number of *Trypanosoma* measured was 10 of *Trypanosoma* from different days post-infection (Khalafalla and Al Mawly 2020)**.**

Polymerase Chain Reaction

When the parasitemia reached at least 10^6 *Trypanosoma* /ml, the 10 µl of blood collected from the tail of mice was then diluted with PBS 1X up to 200 µl. The blood was then extracted using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) and the procedures from the manufactured protocol. The extracted DNA was stored at -20ºC until use. The PCR was performed using 25 µl PCR mixture for each PCR step, containing one µl DNA template, 1X MyTaq HS Buffer (Bioline's, London, UK), 0.4 µM of each primer of Internal Transcribed Spacer (ITS-1) Forward: 5'-CCGGAAGTTCACCGATATTG-3'; Reverse: 3'-TTGCTGCGTTCTTCAACGAA-5', 1 U/µl MyTaq™ HS DNA Polymerase (Bio line's, London, UK), and nuclease-free water (NFW). The PCR conditions used consisted of a pre-denaturation stage at 95ºC for 1 minute, followed by denaturation at 95ºC for 15 seconds, annealing at 55ºC for 15 seconds, extension at 72ºC for 1 minute 30 seconds, and final extension at 72ºC for 1 minute. The denaturation to extension stages was carried out in 35 cycles. The PCR product was performed on 1.5% Agarose gel using electrophoresis for 30 minutes on 100 volts. The expected size of the DNA product was 419 bp (Setiawan et al. 2021)

Sequencing Analysis

The National Center for Biotechnology Information database was queried to identify sequence similarities using the Basic Local Alignment Search Tool (BLAST) based on positive PCR sequences. The ClustalW and MEGA (version X) software programs were utilized to analyze the aligned sample sequences against homologous sequences deposited in GenBank. Phylogenetic trees were constructed based on the sequence data of the ITS-1 regions of the samples and reference GenBank sequences. Neighbour-joining was utilized to conduct the analysis, and 1000 bootstrap replications were performed for each branch point.

RESULTS AND DISCUSSION

Parasitemia level and mortality rate

Based on the parasitemia observation of mice, the parasitemia pattern was different from each isolate and divided into three levels of virulent (low, moderate, and high virulent). Low virulence was observed on BGR-SP1, and the parasitemia level fluctuated from 2 to

24 days post-infection (d.pi). The parasitemia level reached 10^5 to 10^8 *Trypanosoma*/ml on 4, 12, and 16 d.p.i, while the parasitemia level was low (10^2 *Trypanosoma*/ml) on 6 and 18 d.p.i. The parasitemia level was constant from 18 to 24 d.p.i (10^2 *Trypanosoma*/ml). The moderate virulent was observed on BGR-SP2 and BGR-KB. The parasitemia also fluctuated similarly with BGR-SP1, gradually increasing from 2 to 6 d.pi (the highest parasitemia reached 10^6 *Trypanosoma*/ml) and decreased at eight d.p.i (10^1 *Trypanosoma*/ml). However, the day after, the parasitemia increased to 12 d.pi (10^6 *Trypanosoma*/ml) (Figure 1). The extracellular trypanosome undergoes morphological changes throughout its life cycle, which could be an indication of a particular adaptation to the host environment. *T. evansi* only has a trypomastigote bloodstream form, and in order to shield itself from the host immune system, it needs to constantly modify its variable surface glycoprotein (VSG) coat (Duarte et al. 2018). *Trypanosoma evansi* is the most common trypanosome species, affecting domestic animals and producing a variety of trypanosomiases due to its high mortality and morbidity rates (Kamidi et al. 2018). The three levels of virulent parasitemia (low, moderate, and high) were derived from observing parasitemia in mice, and each isolate had a distinct parasitemia pattern. The different types of *T. evansi* found in some Indonesian areas also show different patterns of parasitemia and virulence. The level of virulence has been divided by into three biotypes. Biotype 1 (high virulence) is the most severe; it can kill infected hosts eight days after they are affected. Compared to biotype 1, biotypes 2 (moderate virulence) and 3 (low virulence) are less likely to cause disease. Parasite levels rise and fall in biotype 2, while high parasitemia remains long and can kill mice for more than 14 days in biotype 3. The low and moderate virulence also shows a parasitemia pattern of "high - low - high" (rapidly growing then falling quickly for a while until it also disappears from the peripheral blood and increases sharply again/"undulating parasitemia"). It can occur once or frequently in the biological cycle of *T. evansi* biotype 2 in mice. Undulating parasitemia is also seen in African trypanosomes and Salivarian trypanosomiasis [12]. Another interesting finding in this study is that the isolates utilized came from the exact location, yet the results differ depending on the prepatent period and long life. This finding implies that the presence of isolate has a different strain of *T. evansi*. Bloodstream form parasites have a wavy appearance, with flagella attached to the cell body and with kinetoplast DNA positioned between the nucleus and the flagellar pocket (Figure 2). Despite their close evolutionary relationship, *T. evansi* morphometric alterations result in considerable parasitemia disparities between mouse groups. Different

degrees of parasitemia among *T. evansi* isolates based on motility and mortality (Barghash 2020).

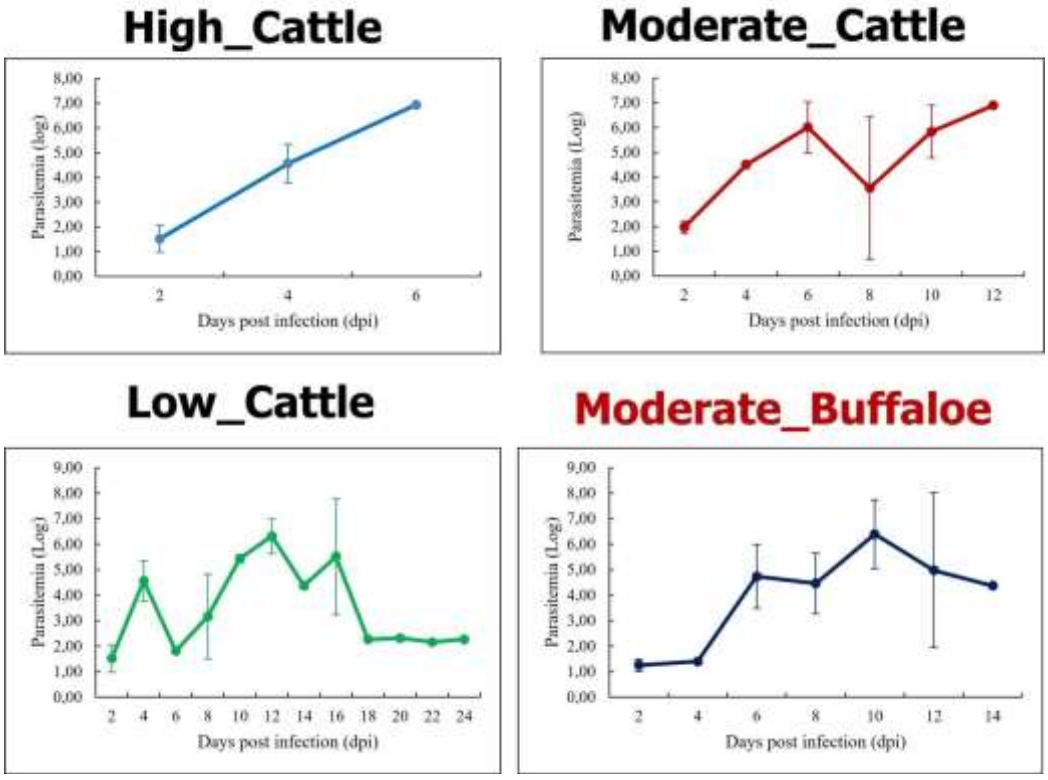

Figure 1. The parasitemia pattern from four different isolates of four infected mice, namely cattle (BGR-SP1, BGR-SP2, and BGR-SP3) and buffalo (BGR-KB). The pattern of parasitemia was divided into low, moderate, and high virulence.

Parasitemia was estimated daily by microscopic examination of blood smears (Duarte et al. 2018). Morphometry indicated multiple morphologies in Giemsa 10%-stained blood smears. The morphological pattern of low-virulent *T. evansi* from BGR-SP1 showed an average size of 22.5 µm (intermediate form). In propagation from 2 to 16 d.p.i., the average size was 23.8 µm (intermediate form). During propagation from 18 to 24 d.p.i., the *Trypanosoma* size was marginally smaller, averaging 18.4 µm (stumpy form). *T. evansi* morphology from BGR-SP2 and BGR-KB (moderate virulent) was similar, with an average size of 24.1µm (intermediate form). A distinctive pattern was found during early propagation four d.p.i. The *Trypanosoma* was smaller than other stages in the isolate (20.2 µm). Morphology of *T. evansi* from BGR-SP3 (high virulent) showed an average size of 24.3 µm. This study found that the morphology varied between stumpy and intermediate forms from three different virulence patterns. *T. evansi* examined had a maximum size of 25-35 µm, was

elongated and thin, and had a kinetoplast at the end.

Biomorphometry of *Trypanosoma*

The morphometry showed several different morphologies during our observations from blood smear stained with Giemsa 10%. The average size of the morphology pattern of *T. evansi* from BGR-SP1 for low virulent isolate was 22.5 ±3.2 µm. During propagation from 2 to 16 d.p.i, the average size was 23.8 µm. However, when the propagation from 18 to 24 d.p.i, the *Trypanosoma* size was slightly small, and the average size was 18.4 µm. The morphology pattern of *T. evansi* from BGR-SP2 and BGR-KB (moderate virulent) was almost similar, and the average size was 24.1±1.9 µm. The unique pattern was observed during the early-stage propagation four d.p.i. The size of the *Trypanosoma* was smaller than other stage propagation within the same isolate (20.2 ±1.8 µm). The morphology pattern of *T. evansi* from BGR-SP3 (high virulent), the average size was 24.3 ±1.0 µm (Figure 2 and Figure 3).

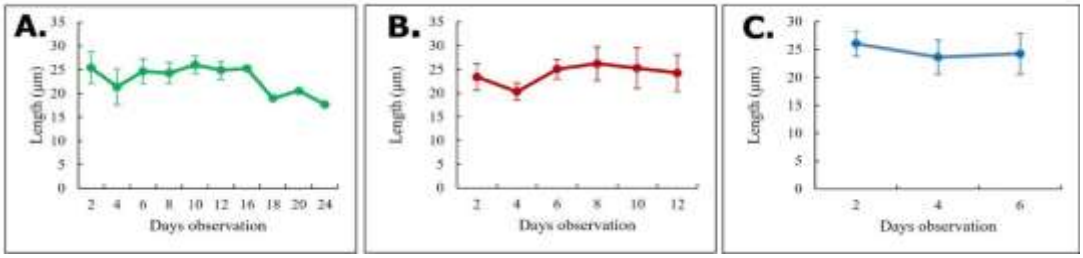

Figure 2. The size of *T. evansi* from different pattern virulence. The morphology pattern of *T. evansi* from BGR-SP1 for low virulent isolate, the average size was 22.5 ±3.2 µm (A). The morphology pattern of *T. evansi* from BGR-SP2 and BGR-KB (moderate virulent) was almost similar, and the average size was 24.1±1.9 µm (B, C).

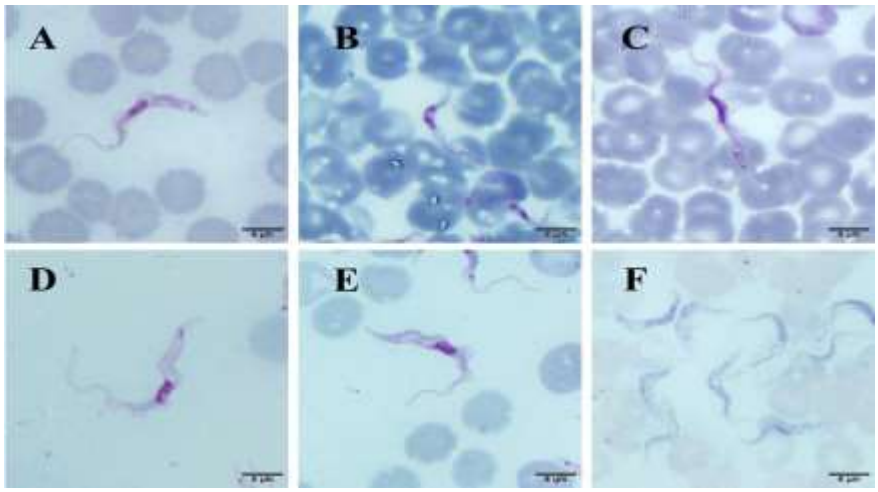

Figure 3. The morphology of *T. evansi* from three different virulence patterns varies between stumpy (B and F) and intermediate forms (A, C, D, and E). The size of stumpy and intermediate forms was 18.4 and 24.3 µm, respectively.

Polymerase Chain Reaction and Sequencing Analysis

The sequencing result showed that the low and moderate virulent were in the same group. However, the highly virulent isolate was with another isolate from Thailand and China (Figure 4).

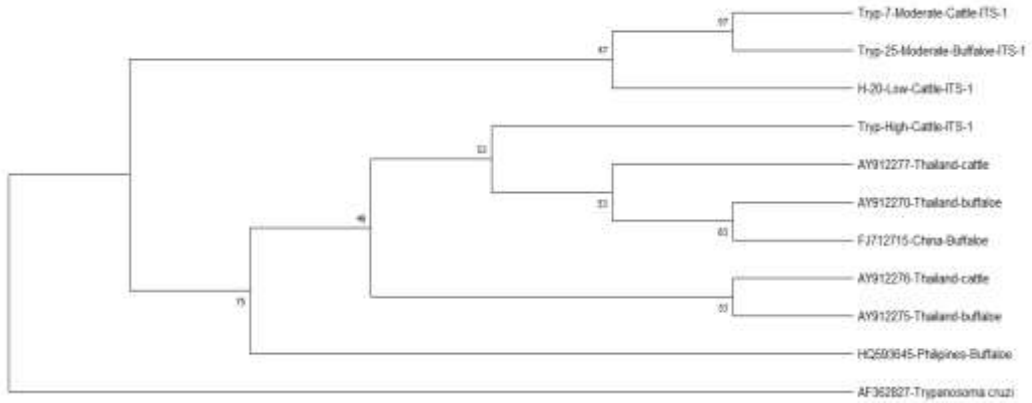

Figure 4. The phylogenetic tree of four different isolates using the ITS-1 gene. The low and moderate virulent were in the same group together (H-20-Low-Cattle-ITS-1; Tryp-7-Moderate-Cattle-ITS-1; Tryp-25-Moderate-Buffaloes). The highly virulent (Tryp-High-Cattle-ITS-1) isolate was together with another isolate from Thailand and China.

The sequencing findings indicated that the low and moderate virulent strains were grouped. Nevertheless, the highly virulent strain was joined with another strain from Thailand and China. The DNA sequencing analysis reveals significant genetic heterogeneity among parasites from the same host and area. Hence, these data hold significant importance in elucidating the variety of patterns of *Trypanosoma* virulence in Indonesia. In addition, this knowledge is crucial for designing strategies to prevent and cure Surra disease in Indonesia.

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CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

AUTHOR CONTRIBUTION

ABN: devised the project, the main conceptual ideas, and the proof outline, analysis and interpretation of data, and final approved the manuscript
UCH: analysis and interpretation of data, drafting of the manuscript, and final approval of the manuscript.

REFERENCES

Bal MS, Singla LD, Kumar H, Vasudev A, Gupta K, Juyal PD. (2012). Pathological studies on experimental *Trypanosoma evansi* infection in Swiss albino mice. *J Parasit Dis.* 36(2):260–264. DOI:10.1007/s12639-012-0120-5

Dewi RS, Damajanti R, Wardhana AH, Mulatsih S, Poetri ON, Steeneveld W, Hogeveen H. (2020). The economic losses of surra outbreak in Sumba Timur, Nusa Tenggara Timur–Indonesia. *Trop Anim Sci J* 43(1):77–85. DOI:10.5398/tasj.2020.43.1.77

Desquesnes M, Gonzatti M, Sazmand A, Thévenon S, Bossard G, Boulangé A. (2022). A review on the diagnosis of animal trypanosomoses. *Parasit Vectors* 5(1):64. DOI:10.1186/s13071-022-05190-1

Desquesnes M, Holzmuller P, Lai DH, Dargantes A, Lun ZR, Jittaplapong S. (2013). *Trypanosoma evansi* and surra: A review and perspectives on origin, history, distribution, taxonomy, morphology, hosts, and pathogenic effects. *Biomed Res Int* 1–22. DOI:10.1155/2013/194176

Khalafalla RE, Al Mawly J. (2020). Biometrical and morphological description of *Trypanosoma evansi* among one-humped camel (*Camelus dromedarius*) in Oman. *J Saudi Soc Agric Sci* 9(5):326–331. DOI:10.1016/j.jssas.2020.03.002

Njiru ZK, Constantine CC, Masiga DK, Reid SA, Thompson RCA, Gibson WC. (2006). Characterization of *Trypanosoma evansi* type B. *Infect Genet Evol* 6(4):292–300. DOI:10.1016/j.meegid.2005.08.002

Nuryady MohM, Nurcahyo RW, Hindun I, Fatmawati D. (2021). Multidrug resistance protein structure of *Trypanosoma evansi* isolated from buffaloes in Ngawi District, Indonesia: A bioinformatics analysis. *Vet World* 14(1):33–39. DOI:10.14202/vetworld.2021.33-39

Perrone T, Aso PM, Mijares A, Holzmuller P, Gonzatti M, Parra N. (2018). Comparison of infectivity and virulence of clones of *Trypanosoma evansi* and *Trypanosoma equiperdum* Venezuelan strains in mice. *Vet Parasitol* 253:60–64. DOI:10.1016/j.vetpar.2018.0

2.024

Queiroz AO, Cabello PH, Jansen AM. (2000). Biological and biochemical characterization of isolates of *Trypanosoma evansi* from Pantanal of Matogrosso, Brazil. *Vet Parasitol* 92(2):107–118. DOI:10.1016/S0304-4017(00)00286-7

Setiawan A, Nurcahyo W, Priyowidodo D, Budiati RT, Susanti DS. (2021). Genetic and parasitological identification of *Trypanosoma evansi* infecting cattle in South Sulawesi, Indonesia. *Vet World* 14(1):113–119. DOI:10.14202/vetworld.2021.113-119

Subekti DT, Sawitri DH, Wardhana AH, Suhardono. (2013). Parasitaemia pattern and mortality of mice infected by Indonesian isolate of *Trypanosoma evansi*. *J Ilmu Ternak Vet* 18(4). DOI:10.14334/jitv.v18i4.334

Subekti DT, Yuniarto I, Sulinawati, Susiani H, Amaliah F, Santosa B. (2015). Trypanocidals effectivity against some isolates of *Trypanosoma evansi* propagated in mice. *J Ilmu Ternak Vet* 20(4). DOI:10.14334/jitv.v20i4.1246