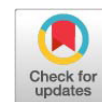


Research Article



Molecular Identification and Phylogenetic Analysis of Equine Trypanosomiasis in Malaysia

NUR ZUL IZZATI MOHD RAJDI¹, RUMAIZI SHAARI^{1*}, TAN LI PENG², MOHD FARHAN HANIF REDUAN², MOHAMMAD SABRI ABDUL RAHMAN¹, MOHAMMAD DAUDA GONI¹, MIMI ARMILADIANA MOHAMAD¹

¹Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, 16100 Kota Bharu, Kelantan; ²Department of Veterinary Paraclinical Studies, Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, 16100 Kota Bharu, Kelantan

Abstract | *Trypanosoma evansi*, the causative agent of equine trypanosomiasis (surra), is an economically important haemoprotozoan parasite that compromises equine health and productivity. Despite its veterinary significance, current information on molecular epidemiology and genetic diversity of *T. evansi* in Malaysian horses remains limited. This study aimed to investigate the molecular prevalence and phylogenetic relationships of *T. evansi* isolates among horses in Malaysia. A cross-sectional study was conducted between January 2023 and January 2024 involving 440 horses sampled from equine facilities across Peninsular Malaysia and Malaysian Borneo. Genomic DNA extracted from whole blood was analysed by PCR targeting the internal transcribed spacer 1 (ITS1) region of *T. evansi*. Representative PCR-positive amplicons were confirmed by Sanger sequencing. Phylogenetic analysis was performed using the Maximum Likelihood method based on ITS1 sequences together with global reference isolates retrieved from GenBank. *Trypanosoma evansi* DNA was detected in 24 of 440 horses, corresponding to an overall molecular prevalence of 5.5% (24/440; 95% CI: 3.6–7.9%). Most infections were identified in Peninsular Malaysia (83.3%), particularly in the Southern and Central regions, whereas only sporadic cases were detected in Malaysian Borneo. Phylogenetic analysis of 23 Malaysian isolates demonstrated that all sequences clustered within the established *T. evansi* clade and showed close genetic relationships with isolates from Asia, Africa, the Middle East, and South America. Malaysian isolates did not form a distinct lineage, although one isolate from Sarawak occupied a separate phylogenetic position closely related to strains from Saudi Arabia and Paraguay, indicating the presence of genetic diversity within the local parasite population. This study provides the first nationwide molecular and phylogenetic characterization of equine *T. evansi* infection in Malaysia. The predominance of infections in Peninsular Malaysia and the close genetic relatedness of Malaysian isolates to globally circulating strains highlight the influence of transboundary animal movement on parasite dissemination. These findings provide updated baseline data for molecular surveillance and support the implementation of strengthened biosecurity, quarantine, and vector control strategies to reduce the impact of equine trypanosomiasis in Malaysia.

Keywords | *Trypanosoma evansi*, Surra, Horse, Molecular detection, Phylogenetic analysis, Its1; Malaysia.

Received | August 04, 2025; **Accepted** | August 11, 2026; **Published** | September 15, 2026

***Correspondence** | Rumaizi Shaari, Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, 16100 Kota Bharu, Kelantan; **Email:** rumaizi@umk.edu.my

Citation | Rajdi NZIM, Shaari R, Peng TL, Reduan MFH, Rahman MSA, Goni MD, Mohamad MA (2026). Molecular identification and phylogenetic analysis of equine trypanosomiasis in Malaysia Vet. Sci., 14(10):2226–2233.

DOI | <https://dx.doi.org/10.17582/journal.aavs/2026/14.10.2226.2233>

ISSN (Online) | 2307-8316



Copyright: 2026 by the authors. Licensee ResearchersLinks Ltd, England, UK.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Equine trypanosomiasis (ET), commonly known as surra, is a neglected vector-borne disease caused by *Trypanosoma evansi*, a haemoflagellate protozoan belonging to the subgenus *Trypanozoon* (Hoare, 1964). Unlike other pathogenic African trypanosomes that require cyclical development within tsetse flies, *T. evansi* is transmitted predominantly through mechanical transmission by hematophagous flies, particularly *Tabanus* and *Stomoxys* species, without undergoing developmental transformation within the insect vector (Desquesnes *et al.*, 2013; Eyob and Matios, 2013; Kamidi *et al.*, 2017). This unique adaptation has enabled the parasite to expand beyond the tsetse belt of Africa, making *T. evansi* one of the most widely distributed animal trypanosomes and the causative agent of surra in horses and numerous domestic and wild mammals throughout tropical and subtropical regions of Asia, Africa, the Middle East, and South America (Desquesnes *et al.*, 2013).

Horses are among the most susceptible hosts to *T. evansi*, and infection is frequently associated with severe clinical disease. Affected animals commonly exhibit intermittent fever, progressive anaemia, weight loss, ventral oedema, lymphadenopathy, reduced athletic performance, reproductive disorders, ocular lesions, and neurological manifestations resulting from central nervous system involvement (Berlin and Baneth, 2009; Desquesnes *et al.*, 2013; Jaimes-Dueñez *et al.*, 2023). In the absence of timely diagnosis and treatment, the disease may progress to chronic debilitation or death, leading to substantial economic losses through reduced performance, decreased productivity, restrictions on animal movement, and increased veterinary costs.

The epidemiology of *T. evansi* is closely influenced by the interaction between susceptible hosts, competent vectors, and favourable environmental conditions. Malaysia's tropical climate, characterized by consistently high temperatures and humidity, supports the survival and proliferation of hematophagous flies, including *Tabanus* and *Stomoxys* species, the principal mechanical vectors of *T. evansi* (Mohammed *et al.*, 2016; Kazim *et al.*, 2022; Ola-Fadunsin *et al.*, 2020). In addition, increasing cross-border movement of livestock, particularly through illegal animal smuggling along the Malaysia–Thailand border, poses a significant biosecurity concern. Cattle and other livestock can act as asymptomatic reservoir hosts, facilitating parasite persistence and increasing the risk of spillover transmission to horses (Kamyngkird *et al.*, 2020; Thi Nguyen *et al.*, 2025). This concern is supported by the report of clinical equine trypanosomiasis associated with horse smuggling activities in Kelantan, Malaysia (Mohd Rajdi *et al.*, 2021).

Furthermore, the continued expansion of Malaysia's equine industry through domestic breeding programmes and international horse movement may further increase the risk of pathogen introduction and dissemination despite existing biosecurity measures.

Although *T. evansi* has been recognized in Malaysia for several decades, contemporary epidemiological information on equine infections remains scarce. Previous investigations were largely restricted to selected regions of Peninsular Malaysia and relied predominantly on conventional parasitological methods, particularly microscopic examination of blood smears, which has limited sensitivity for detecting chronic infections with low parasitaemia (Chandrawathani *et al.*, 1998; Zawida *et al.*, 2010; Elshafie *et al.*, 2013a; Elshafie *et al.*, 2013b; Naama *et al.*, 2021). Consequently, the true prevalence of *T. evansi* infection may have been underestimated, particularly among subclinically infected horses that serve as important reservoirs for parasite transmission.

Molecular diagnostic methods have substantially improved the detection and characterization of *Trypanosoma* infections by providing greater sensitivity and specificity than conventional parasitological techniques. Because *Trypanosoma* species exhibit considerable morphological similarity and mixed infections frequently occur in endemic areas, molecular markers are indispensable for accurate species identification and epidemiological investigations. Among the available genetic targets, internal transcribed spacer 1 (ITS1) regions have become widely used for the detection and characterization of *Trypanosoma* species. The ITS1 region is a multicopy genetic marker located between the 18S and 5.8S rRNA genes that exhibits sufficient sequence variability and species-specific size polymorphisms to enable sensitive detection and reliable differentiation of *Trypanosoma* species, including *T. evansi* (Desquesnes and Dávila, 2002; Njiru *et al.*, 2005). In addition to its diagnostic utility, ITS1 sequence analysis has been widely applied to investigate genetic diversity and evolutionary relationships among *T. evansi* isolates from different geographical regions, making it a valuable marker for molecular epidemiological and phylogenetic studies (Gaithuma *et al.*, 2019; Suprihati *et al.*, 2022).

Beyond disease detection, molecular characterization has become an essential component of modern epidemiological investigations. Phylogenetic analysis provides valuable insights into the genetic diversity, evolutionary relationships, and geographical distribution of parasite populations, thereby improving understanding of transmission dynamics and the emergence or introduction of novel strains. Comparative molecular studies have demonstrated that *T. evansi* is genetically closely related

to *T. brucei*, representing a derivative lineage within the *Trypanozoon* subgenus that evolved following the loss of kinetoplast DNA (Stevens and Rambaut, 2001; Lai *et al.*, 2008). Furthermore, two principal molecular lineages, designated Type A and Type B, have been identified, with Type A exhibiting a broad global distribution while Type B has been reported predominantly in eastern Africa (Birhanu *et al.*, 2016). Despite the increasing application of molecular epidemiology worldwide, information regarding the genetic diversity and phylogenetic relationships of Malaysian *T. evansi* isolates remains limited.

Given the favourable ecological conditions for vector survival, increasing animal movement, and the paucity of contemporary molecular epidemiological data, there is a need for updated information on the occurrence and genetic diversity of *T. evansi* in Malaysian horses. Therefore, the present study aimed to determine the molecular prevalence of *T. evansi* infection among horses in Malaysia using PCR targeting the ITS1 gene and to characterize the genetic relationships of Malaysian isolates through phylogenetic analysis. The findings will provide updated evidence on the molecular epidemiology of *T. evansi* in Malaysia, strengthen national surveillance programmes, facilitate early detection of transboundary infections, and support the development of effective prevention and control strategies for equine trypanosomiasis.

MATERIALS AND METHODS

STUDY DESIGN AND SAMPLE COLLECTION

A cross-sectional study was conducted between January 2023 and January 2024 involving horses from equine facilities across six geographical zones in Malaysia, comprising the Central (Kuala Lumpur, Selangor, Putrajaya), Northern (Perlis, Perak, Kedah, Pulau Pinang), Southern (Melaka, Negeri Sembilan, Johor), and East Coast (Kelantan, Terengganu, Pahang) regions of Peninsular Malaysia, and Northeast (Sabah) and Northwest (Sarawak) in Malaysian Borneo. Horses were recruited using convenience sampling with owner consent.

A total of 440 horses representing different breeds, ages, sexes, management systems, and purposes of use were included in the study. Approximately 3 mL of whole blood was collected aseptically from the jugular vein into EDTA tubes by a licensed veterinarian. Samples were transported under cold-chain conditions and stored at 4 °C prior to molecular analysis. Demographic and management information was recorded for each horse. However, these variables were not evaluated for statistical association with *T. evansi* infection in the present study, as the primary focus was molecular detection and phylogenetic characterization.

All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC), Universiti Malaysia Kelantan (Approval No. UMK/FPV/ACUE/PG/001/2023).

DNA EXTRACTION AND PCR AMPLIFICATION

Genomic DNA was extracted from 200 µL of whole blood using the gSYNC™ DNA Extraction Kit (Geneaid Biotech Ltd., Taiwan) according to the manufacturer's instructions. DNA was stored at -20 °C until analysis. Molecular detection of *T. evansi* was performed by conventional PCR targeting the internal transcribed spacer 1 (ITS1) region using the universal ITS1 primers described by Desquesnes *et al.* (2001). These primers amplify an approximately 540-bp fragment, and the presence of the expected amplicon was considered suggestive of *T. evansi*. As the ITS1 region may also be amplified from other *Trypanosoma* species, species identity was subsequently confirmed by sequencing of the PCR products.

PCR amplification was carried out in a 25-µL reaction mixture containing 12.5 µL GoTaq Green Master Mix (Promega, USA), 1 µL each of forward and reverse primers (10 µM), 5 µL of genomic DNA, and nuclease-free water to the final volume. Positive controls consisted of previously confirmed *T. evansi* DNA archived at the Molecular Biology Laboratory, University Veterinary Diagnostic Centre, Universiti Malaysia Kelantan, while nuclease-free water served as the negative control.

Amplified products were separated by electrophoresis on 2% agarose gels stained with Midori Green and visualized using a Gel Doc™ EZ Gel Imager (Bio-Rad, USA).

DNA SEQUENCING AND SPECIES CONFIRMATION

Representative PCR-positive amplicons were purified and subjected to bidirectional Sanger sequencing (Apical Scientific Sdn. Bhd., Malaysia). Forward and reverse sequences were assembled into consensus sequences using BioEdit version 7.2 and compared with reference sequences in the National Center for Biotechnology Information (NCBI) database using BLASTn to confirm species identity.

PHYLOGENETIC ANALYSIS

High-quality ITS1 sequences generated in this study were deposited in GenBank and analysed together with representative *T. evansi* reference sequences retrieved from GenBank. Multiple sequence alignment was performed using the MUSCLE algorithm implemented in MEGA version 11.0. Phylogenetic relationships were inferred using the Maximum Likelihood method based on the Tamura–Nei nucleotide substitution model with 1,000 bootstrap replicates to assess branch support. Bootstrap

values $\geq 50\%$ were considered statistically supported. The resulting phylogenetic tree was visualized and edited using FigTree version 1.4.4.

STATISTICAL ANALYSIS

The molecular prevalence of *T. evansi* infection was calculated as the proportion of PCR-positive horses among the total number of horses examined. Descriptive statistical analyses were performed using IBM SPSS Statistics version 30 (IBM Corp., Armonk, NY, USA).

RESULTS

MOLECULAR DETECTION OF *TRYPANOSOMA EVANSI*

PCR amplification targeting the ITS1 region detected *T. evansi* DNA in 24 of 440 horses, yielding an overall molecular prevalence of 5.5% (95% CI: 3.6–7.9%). Most PCR-positive horses were detected in Peninsular Malaysia, accounting for 83.3% (20/24) of all positive cases, whereas 16.7% (4/24) originated from Malaysian Borneo (Table 1). Within Peninsular Malaysia, the highest molecular prevalence was observed in the Central region (17.4%; 8/46; 95% CI: 7.9–31.9%), followed by the Southern region (13.3%; 10/75; 95% CI: 6.6–23.0%), Northern region (2.6%; 2/76; 95% CI: 0.3–9.2%), and East Coast region (0/156; 95% CI: 0.0–2.3%). In Malaysian Borneo, *T. evansi* was detected in the Northeast (5.1%; 3/59; 95% CI: 1.1–14.1%) and Northwest (3.6%; 1/28; 95% CI: 0.1–18.3%) (Table 1).

PHYLOGENETIC ANALYSIS OF *TRYPANOSOMA EVANSI*

A total of 24 PCR-positive amplicons were submitted for Sanger sequencing, of which 23 high-quality ITS1 sequences were successfully obtained and deposited in GenBank. The corresponding GenBank accession numbers for all 23 sequences generated in this study are provided in the phylogenetic tree (Figure 1). These sequences were analysed together with 32 representative *T. evansi* reference sequences retrieved from GenBank, while *Leishmania mexicana* (GenBank accession no. AB558251.1) was included as the outgroup. The phylogenetic tree was reconstructed using the Maximum Likelihood method based on ITS1 sequences (Figure 1).

All Malaysian isolates clustered within the *T. evansi* lineage and were clearly separated from the outgroup, confirming the molecular identity of the PCR-positive samples. None of the Malaysian isolates formed a separate lineage outside the established *T. evansi* clade. The Malaysian isolates were interspersed among reference sequences from Asia, Africa, the Middle East, and South America rather than forming a distinct monophyletic cluster, indicating a high degree of genetic similarity with globally circulating *T. evansi* strains. Most isolates from the Central and Southern

regions of Peninsular Malaysia clustered within a well-supported subclade together with reference isolates from Africa and South America. In contrast, isolates from the Northern region of Peninsular Malaysia and Sabah formed a separate cluster closely related to Asian reference isolates. One isolate from Sarawak occupied a distinct phylogenetic position relative to the remaining Malaysian isolates and clustered most closely with reference isolates from Saudi Arabia and Paraguay, suggesting the presence of genetic diversity among *T. evansi* isolates circulating in Malaysia.

DISCUSSION

The present study demonstrated an overall molecular prevalence of *Trypanosoma evansi* of 5.5% among horses in Malaysia, with infections showing a distinct geographical bias towards Peninsular Malaysia. More than 80% of PCR-positive horses originated from Peninsular Malaysia, whereas only sporadic cases were detected in Malaysian Borneo. This uneven distribution suggests that *T. evansi* transmission is influenced by regional differences in livestock movement, vector ecology, and husbandry practices. However, these potential factors should be interpreted cautiously, as formal statistical risk factor analysis was beyond the scope of the present study.

Within Peninsular Malaysia, the Southern and Central regions recorded the highest prevalence of *T. evansi*. The Southern region accommodates numerous commercial livestock farms, increasing opportunities for contact between horses and potential reservoir hosts. Furthermore, livestock importation from endemic countries, including Argentina, may facilitate the introduction and persistence of *T. evansi* within the region (Bernama, 2003). Animal movement has long been recognised as a major driver of surra dissemination, contributing to the spread of infected animals across geographical boundaries and introducing the parasite into previously unaffected areas (Desquesnes et al., 2013). Nevertheless, these factors represent possible explanations for the observed regional pattern rather than statistically established risk factors in the present study.

Regional differences in horse management may have further influenced the observed distribution. Horses in the Southern and Central regions are predominantly maintained under intensive stable systems, where close confinement and the persistence of hematophagous flies around stables may enhance opportunities for mechanical transmission. Although *T. evansi* does not undergo biological development within its vectors, interrupted feeding by *Tabanus* and *Stomoxys* spp. enables efficient transfer of infected blood between susceptible hosts (Herrera et al., 2004; Desquesnes et al., 2013; Shorba et al., 2024).

Table 1 : Molecular prevalence of *trypanosoma evansi* among horses in Malaysia by geographical region.

Geographical region	Horses examined, n	PCR-positive, n	Molecular prevalence, % (95% CI)
Peninsular Malaysia			
East Coast	156	0	0.0 (0.0–2.3)
Northern	76	2	2.6 (0.3–9.2)
Central	46	8	17.4 (7.9–31.9)
Southern	75	10	13.3 (6.6–23.0)
Malaysian Borneo			
Northeast	59	3	5.1 (1.1–14.1)
Northwest	28	1	3.6 (0.1–18.3)
Total	440	24	5.5 (3.6–7.9)

Note. CI, confidence interval; PCR, polymerase chain reaction. Values in parentheses represent 95% confidence intervals calculated using the exact binomial method.

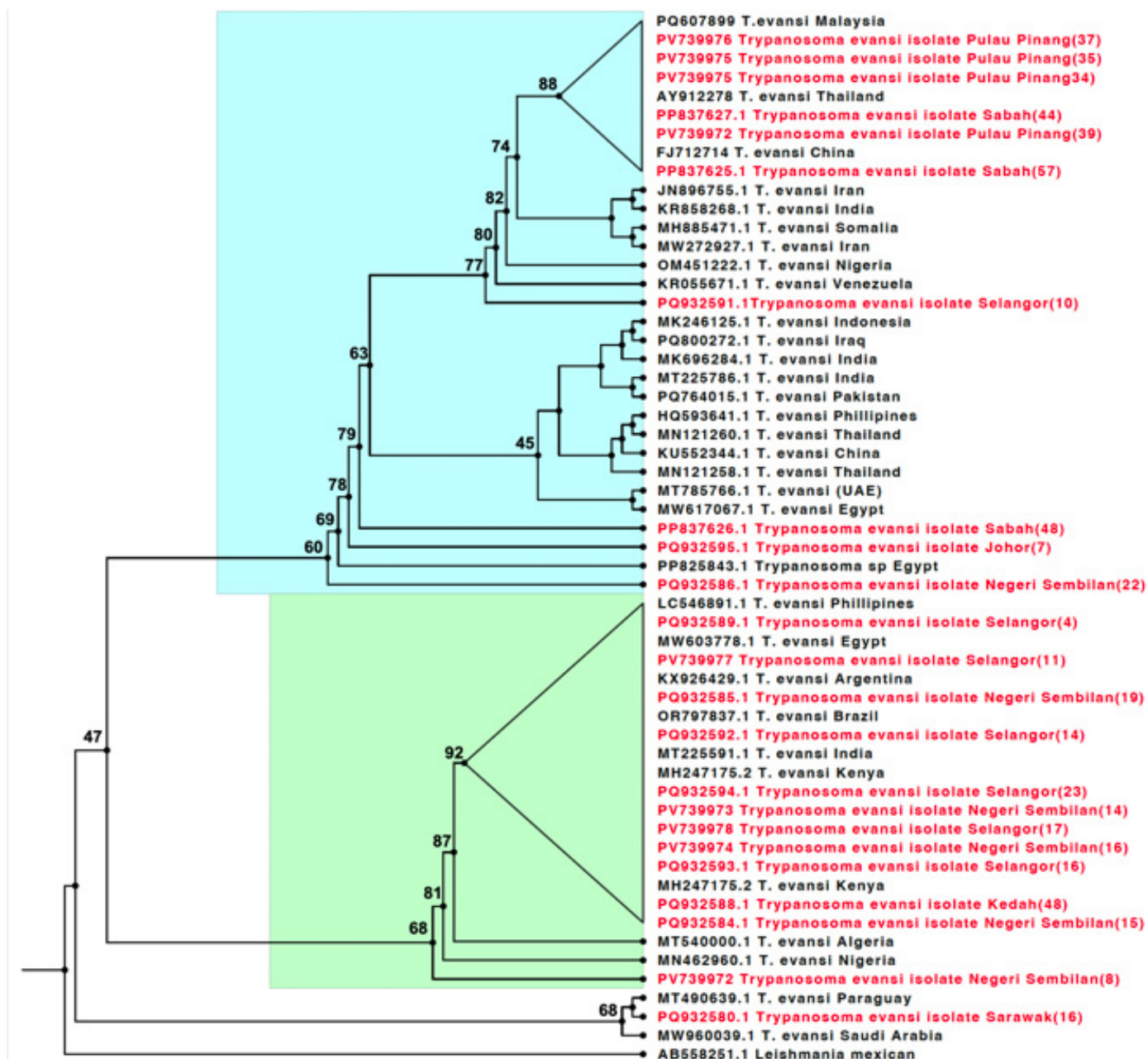


Figure 1 : Phylogenetic tree of *Trypanosoma evansi* based on partial ITS1 gene sequences, constructed using the maximum likelihood method with the Tamura – Nei model and 1,000 bootstrap replicates. Sequences highlighted in red represent novel isolates obtained in the current study.

Conversely, the comparatively lower prevalence observed in the East Coast, Northeast and Northwest may be associated with more extensive grazing systems, lower stocking densities, and reduced exposure to vectors commonly associated with stable environments. Similar relationships between management practices, vector abundance, and *T. evansi* transmission have been documented in other endemic regions (Desquesnes *et al.*, 2013). However, these observations remain descriptive in the present study, and the specific epidemiological and management-related risk factors for *T. evansi* infection will be evaluated separately using appropriate statistical analysis.

Phylogenetic analysis confirmed that all Malaysian isolates clustered within the established *T. evansi* clade and were clearly separated from the *Leishmania mexicana* outgroup, supporting accurate species identification. These findings further demonstrate the suitability of the ITS1 region as a reliable molecular marker for the detection and phylogenetic characterization of *T. evansi*, consistent with previous reports (Khuchareontaworn *et al.*, 2007; Zangoie *et al.*, 2018; Elobaid *et al.*, 2021; Verma *et al.*, 2024).

The Malaysian isolates did not form a distinct country-specific lineage but were interspersed among reference isolates from Asia, Africa, the Middle East, and South America, indicating a high degree of genetic similarity with globally circulating *T. evansi* strains. This observation is consistent with previous studies reporting limited phylogeographic structure in *T. evansi*, largely attributable to the parasite's broad host range and extensive dissemination through domestic and international animal movement (Desquesnes *et al.*, 2013; Javanshir *et al.*, 2023). Rather than evolving in geographical isolation, the Malaysian *T. evansi* population appears to comprise multiple lineages introduced through repeated transboundary transmission events.

A notable finding was the distinct phylogenetic placement of one isolate from Sarawak, which clustered closely with reference isolates from Saudi Arabia and Paraguay. This observation agrees with the findings of Javanshir *et al.* (2023), who reported complete nucleotide sequence identity among *T. evansi* isolates from Saudi Arabia, Malaysia, Pakistan, India, Egypt, and Kenya. Such close genetic relationships among isolates from geographically distant regions highlight the remarkable dispersal capacity of *T. evansi* and reinforce the role of international livestock movement in shaping parasite population structure. Although the present study cannot determine the precise route of introduction, the finding emphasizes the importance of continuous molecular surveillance for detecting newly introduced or emerging parasite lineages.

Regional clustering among Malaysian isolates further suggests the coexistence of multiple transmission networks within the country. Most isolates from the Central and Southern regions clustered with reference strains from Africa and South America, whereas isolates from the Northern region of Peninsular Malaysia and Sabah grouped more closely with Asian isolates, particularly those from Thailand and China. Similar phylogenetic relationships have been reported in Indonesia, where *T. evansi* isolates exhibited high genetic similarity with Chinese strains (Setiawan *et al.*, 2021). Likewise, the clustering of Malaysian isolates with neighbouring Asian strains is consistent with previous evidence of transboundary dissemination of surra within Southeast Asia (Mohd Rajdi *et al.*, 2021). Collectively, these findings suggest that Malaysian *T. evansi* populations comprise multiple genetic lineages shaped by both regional transmission and international animal movement.

Overall, the present study demonstrates that *T. evansi* populations circulating in Malaysia are genetically diverse and influenced by both local epidemiological factors and transboundary dissemination. The predominance of infections in Peninsular Malaysia, together with the close phylogenetic relationships between Malaysian isolates and strains from multiple continents, underscores the importance of integrating molecular surveillance with strengthened quarantine measures, movement control, and vector management programmes to reduce the risk of disease introduction and limit the further spread of equine trypanosomiasis within Malaysia.

CONCLUSION

This study provides the first comprehensive molecular and phylogenetic characterization of *Trypanosoma evansi* infection among horses across Malaysia. Molecular detection demonstrated that *T. evansi* remains an important equine pathogen, with infections occurring predominantly in Peninsular Malaysia, indicating regional differences in transmission dynamics. Phylogenetic analysis revealed that Malaysian isolates are genetically diverse and closely related to strains circulating in Asia, the Middle East, Africa, and South America, highlighting the influence of transboundary animal movement on parasite dissemination. These findings provide updated baseline data on the molecular epidemiology of equine trypanosomiasis in Malaysia and underscore the importance of integrating molecular surveillance with strengthened biosecurity, quarantine, and vector control measures. Continued molecular monitoring is essential to support early detection of emerging lineages, improve disease surveillance, and safeguard the health and sustainability of Malaysia's equine industry.

This work was under the Fundamental Research Grant Scheme (FRGS) funded by the Ministry of Higher Education (KPT), Malaysia under project number FRGS/1/2022/WAB04/UMK/03/1. We are thankful to Ms. C.W Salma from Molecular Biology Laboratory, University Veterinary Diagnostic Centre (UVDC), UMK for technical help in the molecular work and Mr. Fakhrul Nizam from *Hospital Pengajar Veterinar* (HPV), UMK for his help in field investigation.

NOVELTY STATEMENT

This study provides the first nationwide molecular and phylogenetic characterization of *Trypanosoma evansi* in Malaysian horses, establishing updated baseline data on its prevalence and genetic diversity. The findings reveal close genetic relationships between Malaysian and globally circulating *T. evansi* strains, providing new insights into parasite diversity and supporting future molecular surveillance and control strategies.

AUTHOR'S CONTRIBUTION

Nur Zul Izzati Mohd Rajdi: Methodology, Investigation; Writing-original draft. **Mimi Armiladiana Mohamad:** Investigation; Resources. **Rumaizi Shaari:** Investigation; Writing-review and editing. **Mohd Farhan Hanif Reduan:** Formal analysis; Writing-original draft. **Mohammad Sabri Abdul Rahman:** Methodology; Formal analysis; Writing-review and editing. **Mohammad Dauda Goni:** Writing-review and editing. **Tan Li Peng:** Formal analysis; Writing-original draft; Methodology.

GENERATE AI AND AI ASSISTED TECHNOLOGY STATEMENT

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any potential conflict of interest.

REFERENCES

- Berlin D, Loeb E, Baneth G (2009). Disseminated central nervous system disease caused by *Trypanosoma evansi* in a horse. *Vet. Parasitol.*, 161(3–4): 316–319. <https://doi.org/10.1016/j.vetpar.2009.01.030>
- BERNAMA (2003). M'sia terima kargo pertama 1,420 lembu, 33 kuda dari Argentina. Perdana Leadership Foundation Digital Library.

- Birhanu H, Gebrehiwot T, Goddeeris BM, Büscher P, Van Reet N (2016). New *Trypanosoma evansi* type B isolates from Ethiopian dromedary camels. *PLoS Negl. Trop. Dis.*, 10(4): e0004556. <https://doi.org/10.1371/journal.pntd.0004556>
- Chandrawathani P, Yusof M, Slamah B, Rohaya MA (1998). A survey on equine piroplasmosis in Peninsular Malaysia. *J. Vet. Malays.*, 10(1): 25–26.
- Desquesnes M, Dávila AMR (2002). Applications of PCR-based tools for detection and identification of animal trypanosomes: A review and perspectives. *Vet. Parasitol.*, 109(3–4): 213–231. [https://doi.org/10.1016/S0304-4017\(02\)00270-4](https://doi.org/10.1016/S0304-4017(02)00270-4)
- Desquesnes M, Holzmüller P, Lai DH, Dargantes A, Lun ZR, Jittaplapong S (2013). *Trypanosoma evansi* and surra: A review and perspectives. *BioMed Res. Int.*, 2013: 194176. <https://doi.org/10.1155/2013/194176>
- Elobaid NI, Daffalla OM, Noureldin EM, Abdalla MA (2021). Phylogenetic analysis of *Trypanosoma evansi* isolates in naturally infected camels from the Kingdom of Saudi Arabia. *Int. J. Curr. Microbiol. Appl. Sci.*, 10(4): 532–543. <https://doi.org/10.20546/ijcmas.2021.1004.052>
- Elshafie EI, Sani RA, Hassan L, Sharma R, Bashir A, Abubakar IA (2013a). Active infection and morphometric study of *Trypanosoma evansi* among horses in Peninsular Malaysia. *Trop. Biomed.*, 30(3): 444–450.
- Elshafie EI, Sani RA, Hassan L, Sharma R, Bashir A, Abubakar IA (2013b). Seroprevalence and risk factors of *Trypanosoma evansi* infection in horses in Peninsular Malaysia. *Res. Vet. Sci.*, 94(2): 285–289. <https://doi.org/10.1016/j.rvsc.2012.09.004>
- Eyob E, Matios L (2013). Review on camel trypanosomosis (surra) due to *Trypanosoma evansi*: Epidemiology and host response. *J. Vet. Med. Anim. Health*, 5(12): 334–343.
- Gaithuma AK, Yamagishi J, Martinelli A, Hayashida K, Kawai N, Marsela M, Sugimoto C (2019). A single test approach for accurate and sensitive detection and taxonomic characterization of trypanosomes by comprehensive analysis of internal transcribed spacer 1 amplicons. *PLoS Negl. Trop. Dis.*, 13(2): e0006842. <https://doi.org/10.1371/journal.pntd.0006842>
- Herrera HM, Dávila AMR, Norek A, Abreu UG, Souza SS, D'Andrea PS, Jansen AM (2004). Enzootiology of *Trypanosoma evansi* in Pantanal, Brazil. *Vet. Parasitol.*, 125(3–4): 263–275. <https://doi.org/10.1016/j.vetpar.2004.07.013>
- Hoare CA (1964). Morphological and taxonomic studies on mammalian trypanosomes. X. Revision of systematics. *J. Protozool.*, 11(2): 200–207. <https://doi.org/10.1111/j.1550-7408.1964.tb01741.x>
- Jaimes-Duenez J, Jimenez-Leano A, Enrique-Nino S, Arias-Landazábal N, Bedoya-Ríos M, Rangel-Pachón D (2023). Clinical and epidemiological aspects of infection by *Babesia*, *Theileria* and *Trypanosoma* species in horses from northeastern Colombia. *Ticks Tick Borne Dis.*, 14(6): 102208. <https://doi.org/10.1016/j.ttbdis.2023.102208>
- Javanshir A, Tavassoli M, Esmailnejad B (2023). Morphological, serological, molecular detection, and phylogenetic analysis of *Trypanosoma evansi* in horses of different regions in Iran. *Parasitol. Res.*, 122(8): 1873–1881. <https://doi.org/10.1007/s00436-023-07888-2>
- Kamidi CM, Saarman NP, Dion K, Mireji PO, Ouma C, Murilla G, Aksoy S, Schnauffer A, Caccone A (2017). Multiple evolutionary origins of *Trypanosoma evansi* in Kenya. *PLoS*

- Negl. Trop. Dis., 11(9): e0005895. <https://doi.org/10.1371/journal.pntd.0005895>
- Kamyngkird K, Chalermwong P, Saechan V, Kaewnoi D, Desquesnes M, Ngasaman R (2020). Investigation of *Trypanosoma evansi* infection in bullfighting cattle in southern Thailand. Vet. World, 13(8): 1674–1680. <https://doi.org/10.14202/vetworld.2020.1674-1678>
- Kazim AR, Houssaini J, Tappe D, Heo CC (2022). A checklist of the ticks of Malaysia (Acari: Argasidae, Ixodidae), with associated hosts, geographical distribution, and pathogens. Zootaxa, 5190(4): 485–530. <https://doi.org/10.11646/zootaxa.5190.4.2>
- Khuchareontaworn S, Singhapphan P, Viseshakul N, Chansiri K (2007). Genetic diversity of *Trypanosoma evansi* in buffalo based on internal transcribed spacers (ITS) regions. J. Vet. Med. Sci., 69(5): 487–493. <https://doi.org/10.1292/jvms.69.487>
- Lai DH, Hashimi H, Lun ZR, Ayala FJ, Lukeš J (2008). Adaptations of *Trypanosoma brucei* to gradual loss of kinetoplast DNA: *Trypanosoma equiperdum* and *Trypanosoma evansi* are petite mutants of *T. brucei*. Proc. Natl. Acad. Sci. USA, 105(6): 1999–2004. <https://doi.org/10.1073/pnas.0711799105>
- Mohammed K, Tukur SM, Watanabe M, Abd Rani PA, Lau SF, Shettima YM, Watanabe M (2016). Factors influencing the prevalence and distribution of ticks and tick-borne pathogens among domestic animals in Malaysia. Pertanika J. Scholarly Res. Rev., 2(2).
- Mohd Rajdi NZI, Mohamad MA, Tan LP, Choong SS, Reduan MFH, Hamdan RH, Zalati CWS (2021). First case report on the occurrence of *Trypanosoma evansi* in a Siam B mare in Kelantan, Malaysia. Vet. Med. Sci., 7(2): 303–309. <https://doi.org/10.1002/vms3.379>
- Naama TA, Rohaya MA, Tuba Thabitah ATT, Nadiah H, Khor SK, Nurul Faizah Z, Wan Norulhuda WAW, Faizah Hanim MS, Masrin A, Chandrawathani P (2021). Common equine parasites diagnosed in Peninsular Malaysia from the year 2014 to 2018. Malays. J. Vet. Res., 12(2): 26–36.
- Njiru ZK, Constantine CC, Guya S, Crowther J, Kiragu JM, Thompson RCA, Dávila AMR (2005). The use of ITS1 rDNA PCR in detecting pathogenic African trypanosomes. Parasitol. Res., 95(3): 186–192. <https://doi.org/10.1007/s00436-004-1267-5>
- Ola-Fadunsin SD, Gimba FI, Abdullah DA, Jesse Abdullah Ff, Sani RA (2020). Dataset on the diversity and distribution of biting flies collected from cattle farms in Peninsular Malaysia. Data Brief, 29: 105177. <https://doi.org/10.1016/j.dib.2020.105315>
- Setiawan A, Nurcahyo W, Priyowidodo D, Budiati RT, Susanti DSR (2021). Genetic and parasitological identification of *Trypanosoma evansi* infecting cattle in South Sulawesi, Indonesia. Vet. World, 14(1): 113–119. <https://doi.org/10.14202/vetworld.2021.113-119>
- Shorba M, Shoulah SA, Arnaout F, Selim A (2024). Seroprevalence and associated risk factors for *Trypanosoma evansi* infection in equine in North of Egypt. J. Hellenic Vet. Med. Soc., 75(3): 8065–8072. <https://doi.org/10.12681/jhvms.36449>
- Stevens J, Rambaut A (2001). Evolutionary rate differences in trypanosomes. Infect. Genet. Evol., 1(2): 143–150. [https://doi.org/10.1016/S1567-1348\(01\)00018-1](https://doi.org/10.1016/S1567-1348(01)00018-1)
- Suprihati E, Suwanti LT, Yudhana A, Kusumaningrum AI (2022). Comparison of ITS-1 and TBR-1/2 primer sensitivity for the detection of *Trypanosoma evansi* local isolates in experimental rats using a polymerase chain reaction. Vet. World, 15(7): 1772–1778. <https://doi.org/10.14202/vetworld.2022.1772-1778>
- Thi Nguyen G, Jhaiaun P, Mutchimadilok T, Chimnoi W, Nimsuphan B, Ngasaman R, Arunvipas P, Phasuk J, Inpankaew T, Kamyngkird K (2025). Serological detection of *Trypanosoma evansi* infection in cattle using an in vitro cultivated antigen for indirect ELISA in Thailand. Vet. Parasitol., 337: 110483. <https://doi.org/10.1016/j.vetpar.2025.110483>
- Verma R, Punia V, Das G, Kumar S, Nath S, Swamy M (2024). Assessment of genetic diversity of *Trypanosoma evansi* in domestic animal populations through ITS-1 gene sequence analysis. Parasitol. Res., 123(1): 2. <https://doi.org/10.1007/s00436-023-08024-w>
- Zangoie F, Ganjali M, Keighobadi M, Nabavi R (2018). Molecular detection of *Trypanosoma evansi* in camels. Trop. Biomed., 35(4): 1140–1147.
- Zawida Z, Chandrawathani P, Nurulaini R, Khor SK, Adnan M, Premaalatha B, Zaini CM, Jamnah O, Azizah D, Norlida A, Noor Suhaila S (2010). Prevalence of protozoan diseases in local horses in Peninsular Malaysia. Indian J. Comp. Microbiol. Immunol. Infect. Dis., 31(1–2): 48–50.