Case Report

Trypanosoma evansi in Dogs from Vietnam's Mekong Delta: A Case Series of Four Clinically Affected Dogs

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Abstract | *Trypanosoma evansi*, the causative agent of surra, is a hemoflagellate protozoan widely distributed in Asia, Africa, and Latin America. While horses, camels, and cattle are primary hosts, canine infections have been increasingly reported in endemic regions. In Vietnam, knowledge of canine trypanosomiasis remains limited, despite its potential epidemiological and zoonotic significance. Four domestic dogs from the Mekong Delta, Vietnam, presenting with clinical signs suggestive of trypanosomiasis, were examined. Clinical assessment, complete blood count, and serum biochemical profiling were conducted. Thin blood smears were prepared and stained with Giemsa for parasitological evaluation, and parasitemia was graded semi-quantitatively. Molecular detection targeting the RoTat 1.2 VSG gene was performed by PCR, followed by sequencing and phylogenetic analysis using the Maximum Likelihood method. All dogs exhibited clinical signs including fever, lethargy, pale mucous membranes, anorexia, and chronic weight loss. Trypomastigotes were detected in all blood smears, with parasitemia graded as low (+) in one dog, moderate (++) in two dogs, and high (+++) in one dog. PCR targeting the RoTat 1.2 VSG gene confirmed the presence of *T. evansi* in all four cases, and sequencing revealed 99–100% identity with reference strains. Hematological alterations observed in the infected dogs included normocytic–normochromic anemia (RBC 3.1–5.2 $\times 10^{12}$/L; HGB 5.3–10.5 g/dL; HCT 17.6–30.4%) and severe thrombocytopenia (PLT 9–43 $\times 10^{9}$/L). Biochemical disturbances included marked elevation of AST (2553.7 U/L) and ALT (2372.6 U/L) in one dog, increased creatinine (545 µmol/L) in another, and hypoglycemia in two cases. Phylogenetic analysis demonstrated that Vietnamese isolates clustered within the *T. evansi* Asian lineage, closely related to strains from Malaysia, India, and Indonesia, with high bootstrap support. This case series integrates clinical, hematological, biochemical, parasitological, and molecular findings from four dogs in the Mekong Delta. While limited in scale, these data illustrate the spectrum of severe clinical presentations and underscore the diagnostic value of combining smear microscopy with PCR.

Keywords | *Trypanosoma evansi*, Canine trypanosomiasis, Giemsa-stained smear, Phylogenetic analysis, Mekong delta, Vietnam

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INTRODUCTION

Trypanosoma evansi, the causative agent of surra, is a hemoflagellate protozoan widely distributed in Asia, Africa, and Latin America, where it affects a broad range of domestic and wild animals (Desquesnes *et al.*, 2013). The parasite is mechanically transmitted by hematophagous flies, particularly *Tabanus* and *Stomoxys* spp., and causes

significant morbidity and mortality in livestock, leading to substantial economic losses (Giordani *et al*., 2016). Although horses, camels, and cattle are considered the primary hosts, canine infections have also been increasingly documented in endemic regions, highlighting the parasite's broad host adaptability and zoonotic potential (Saminathan *et al*., 2016; Aregawi *et al*., 2019).

In dogs, *T. evansi* infection often manifests as acute or chronic disease, characterized by pyrexia, anemia, weight loss, lymphadenopathy, and neurological disorders in severe cases (Al-Abedi *et al*., 2018). Hematological alterations such as anemia, leukocyte changes, and thrombocytopenia, along with biochemical disturbances including liver and kidney dysfunction, have been reported in naturally infected dogs (Lisulo *et al*., 2024; Ahmadi-hamedani *et al*., 2014). Nevertheless, clinical signs are nonspecific and may overlap with other hemoparasitic infections, making accurate diagnosis challenging. Traditional microscopic examination of Giemsa-stained blood smears remains the most commonly used diagnostic method due to its simplicity and low cost; however, its sensitivity decreases in chronic infections with low parasitemia (Desquesnes *et al*., 2013). In recent years, molecular assays such as PCR targeting the RoTat 1.2 variable surface glycoprotein (VSG) gene have been developed, providing higher sensitivity and specificity for *T. evansi* detection (Claes *et al*., 2004; Njiru *et al*., 2004). Sequencing and phylogenetic analysis of PCR amplicons have further enabled the characterization of genetic diversity and evolutionary relationships among field isolates (Birhanu *et al*., 2015).

Despite the endemic presence of *T. evansi* in Southeast Asia, including Vietnam, only limited information is available regarding its occurrence and clinical impact in dogs. Previous studies in the region have focused primarily on livestock, while canine infections remain underexplored. This knowledge gap hinders the understanding of the epidemiological role of dogs as potential reservoirs and their contribution to disease transmission cycles. Therefore, the present study aimed to investigate *T. evansi* infection in domestic dogs in the Mekong Delta region of Vietnam by integrating clinical assessment, hematological and biochemical profiling, microscopic examination, and molecular confirmation through PCR and sequencing. This comprehensive approach not only provides diagnostic confirmation but also contributes case-based insights into clinicopathological patterns and genetic identity of canine trypanosomiasis in Vietnam.

MATERIALS AND METHODS

Animals and clinical examination

This study was conducted from June 2023 to June 2025 on four domestic dogs (*Canis lupus familiaris*) of different ages and sexes that were presented to the Veterinary Teaching Clinic, Faculty of Veterinary Medicine, Can Tho University, Vietnam, with clinical signs suggestive of trypanosomiasis, such as fever, anorexia, pale mucous membranes, and lethargy. A complete physical examination was performed following the standard clinical protocol of the Veterinary Teaching Clinic. All diagnostic and sampling procedures complied with institutional and national guidelines for the ethical care and use of animals in research, including the Vietnam National Technical Regulation on Animal Diseases–General requirements for sample collection, storage, and shipment (QCVN 01-83:2011/ BNNPTNT) issued by the Ministry of Agriculture and Rural Development (2011). These four dogs were the only clinically suspected cases presented to the Veterinary Teaching Clinic during the study period. No active community surveillance was undertaken, these represent all clinic-suspected cases during the study period.

Sample collection

Approximately 3 mL of whole blood was aseptically collected from the cephalic vein of each dog using EDTA-coated vacutainer tubes for hematological analysis. An additional 3 mL of blood was collected into plain tubes, allowed to clot, and centrifuged at 3,000 rpm for 10 min to obtain serum for biochemical analysis. All samples were processed within two hours after collection.

Hematological analysis

Complete blood count (CBC) was performed using an automated hematology analyzer (DREW SCIENTIFIC Excell 2280, USA). Parameters measured included total white blood cell (WBC) count, differential leukocyte count (neutrophils, lymphocytes, monocytes, eosinophils, basophils), red blood cell (RBC) count, hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet (PLT) count, mean platelet volume (MPV), platelet distribution width (PDW), and plateletcrit (PCT). Absolute values of leukocyte subtypes were calculated by multiplying total WBC by the respective differential percentage.

Microscopic examination (Giemsa-stained smears)

Thin blood smears were prepared immediately after sample collection, air-dried, and fixed with absolute methanol for 2–3 minutes. The slides were then stained with 10% Giemsa solution (Merck, Germany) for 30 minutes, rinsed gently with distilled water, and air-dried. Smears were examined under a light microscope (Olympus CX43, Japan) at 1000× magnification using immersion oil. The presence of *Trypanosoma evansi* trypomastigotes was identified based

on their characteristic morphology, including elongated body, central nucleus, posterior kinetoplast, and undulating membrane. For each sample, at least 20 oil-immersion fields were examined to determine parasitemia grade. Parasitemia was semi-quantitatively graded as (+) low: 1–5 parasites per microscopic field at 1000×; (++) moderate: 6–20 parasites per field; (+++) high: >20 parasites per field (Njiru *et al.*, 2004) according to the number of parasites observed per microscopic field. In addition to identifying *Trypanosoma evansi*, all Giemsa-stained blood smears were examined for other hemoparasites commonly found in the region, including *Babesia* spp., *Ehrlichia* spp., *Anaplasma* spp., and *Hepatozoon* spp. No other blood parasites were detected in any of the samples.

Serum biochemical analysis

Serum biochemical parameters, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), urea, creatinine, and glucose, were determined using a semi-automated biochemistry analyzer (BSI 3000 Evolution, Italy).

Treatment protocol

Following the clinical and laboratory confirmation of *Trypanosoma evansi* infection, all affected dogs were treated with diminazene aceturate (3.5 mg/kg body weight, intramuscularly, single dose). Supportive therapy consisting of fluid supplementation, multivitamin B complex, and iron tonics was also administered to correct anemia and dehydration. Clinical progress was monitored daily until recovery or death. The treatment protocol followed the standard recommendations of the Veterinary Teaching Clinic, Faculty of Veterinary Medicine, Can Tho University.

Molecular detection and sequencing

Genomic DNA was extracted from 200 µL of EDTA-anticoagulated blood using the TopPURE® DNA Extraction Kit (ABT, Vietnam) according to the manufacturer's instructions. Molecular detection of *T. evansi* was performed by PCR amplification targeting the RoTat 1.2 VSG gene, a species-specific marker for *T. evansi*. Primer sequences (5′–3′) were: RoTat 1.2 Forward: GCG GGG TGT TTA AAG CAA TA (annealing temperature 59 °C) and RoTat 1.2 Reverse: ATT AGT GCT GCG TGT GTT CG (annealing temperature 59 °C) (Claes *et al.*, 2004). The PCR reaction mixture (25 µL total volume) contained 12.5 µL of GoTaq® Green Master Mix (Promega, USA), 1 µL each of forward and reverse primers (10 µM), 2 µL of DNA template, and nuclease-free water. The amplification protocol was: Initial denaturation at 94 °C for 4 min; 35 cycles of denaturation at 94 °C for 60 s, annealing at 59 °C for 60 s, extension at 72 °C for 60 s; and a final extension at 72 °C for 5 min. Positive amplicons were confirmed by electrophoresis on a 1.5% agarose gel stained with GelGreen® (ABT, Vietnam). A 100 bp DNA ladder (Bioline, UK) was used as a size marker, and gels were run at 100 V for 35 min. PCR products of the expected size (~205 bp) were purified using the TopPURE® PCR/Gel Purification Kit (ABT, Vietnam) and subjected to bidirectional Sanger sequencing (NamKhoa Biotech, Vietnam).

Raw chromatograms were inspected and manually edited using BioEdit v7.5.2. Consensus sequences were compared with reference sequences in GenBank using BLASTn (NCBI). Multiple sequence alignment was conducted with ClustalW implemented in BioEdit, and phylogenetic analysis was performed using the Maximum Likelihood method in MEGA v12 (Kumar *et al.*, 2024), with 1,000 bootstrap replicates. The resulting phylogeny was used to infer genetic relationships of Vietnamese isolates with global *T. evansi* strains. The nucleotide sequences obtained in this study were deposited in GenBank under accession numbers PX418577–PX418580.

RESULTS

Clinical findings and parasitological confirmation

Four domestic dogs from different provinces in the Mekong Delta, Vietnam, were examined (Table 1). The animals were between 2–5 years old, of local or mixed breeds, and included two males and two females. Management systems varied from free-roaming to household pet. The main clinical signs observed were fever, lethargy, pale mucous membranes, anorexia, chronic weight loss, lymphadenopathy, and neurological disorders such as hind limb weakness.

Microscopic examination of Giemsa-stained smears revealed the presence of *Trypanosoma evansi* trypomastigotes in all dogs (Figure 1). Parasitemia was graded as low (+) in Dog 1, moderate (++) in Dog 2 and Dog 4, and high (+++) in Dog 3. PCR targeting the RoTat 1.2 VSG gene confirmed the presence of *T. evansi* in all cases, with amplification of the expected ~205 bp fragment (Figure 2).

Hematological alterations

All infected dogs exhibited anemia (Table 2), reflected by decreased RBC counts (3.1–5.2 $\times 10^{12}$/L), hemoglobin (5.3–10.5 g/dL), and hematocrit (17.6–30.4%), compared with reference ranges (5.5–8.5 $\times 10^{12}$/L; 12–18 g/dL; 37–55%). Anemia was most severe in Dog 3 and Dog 4. Mean corpuscular indices (MCV, MCH, MCHC) indicated a predominantly normocytic–normochromic anemia, though Dog 4 exhibited a lower MCHC (30.1 g/dL), compatible with chronic disease or iron-restricted erythropoiesis, potentially co-existing with infection.

Table 1: Signalment, management, clinical signs, and parasitological results of four dogs examined for *Trypanosoma evansi* infection.

Dog ID	Location (Province, Vietnam)	Breed	Age (years)	Sex	Management system	Clinical signs	Parasitemia	PCR Result
Dog 1	Can Tho city	Local	2	Male	Household pet	Fever (39.8 °C), pale mucous membranes, lethargy	+	+
Dog 2	Can Tho city	Mixed breed	3	Female	Free-roaming	Anorexia, pale mucous membranes, enlarged lymph nodes	++	+
Dog 3	Hau Giang province	Local	4	Male	Free-roaming	High fever (40.2 °C), emaciation, anemia, corneal opacity	+++	+
Dog 4	Kien Giang province	Mixed breed	5	Female	Household pet	Chronic weight loss, lethargy, jaundice, hind limb weakness	++	+

Note: Parasitemia was graded as (+) low (1–5 parasites per microscopic field at 1000×), (++) moderate (6–20 parasites per field), and (+++) high (>20 parasites per field). PCR targeting the RoTat 1.2 VSG gene was positive. The sequences were deposited in GenBank under accession numbers PX418577–PX418580..

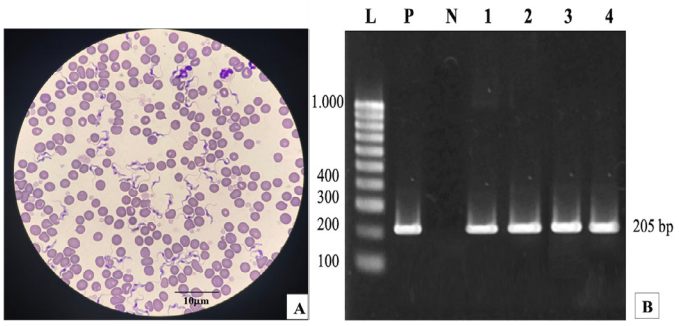

Figure 1: (A) Giemsa-stained blood smear showing *Trypanosoma evansi* trypomastigotes (×1000 magnification). Arrows indicate typical forms with a central nucleus (n), undulating membrane (um), and free flagellum (ff). (B) Agarose gel electrophoresis of PCR amplification of the RoTat 1.2 VSG gene. Lanes: L = 100 bp DNA ladder; P = Positive control; N = Negative control; 1–4 = Dog samples (D1CT, D2CT, D3HG, D4KG).

White blood cell alterations varied among dogs. Dogs 1 and 2 presented leukopenia (1.75 $\times 10^9$/L), while Dogs 3 and 4 showed WBC counts within reference intervals but with a marked neutrophilia (Gra% >85%) and lymphopenia (0.3–0.8 $\times 10^9$/L, below the reference range of 1.0–4.8). Monocytes were slightly elevated in Dogs 1–2. Importantly, thrombocytopenia was observed in these cases across all animals (PLT 9–43 $\times 10^9$/L versus 200–900), indicating severe platelet depletion.

Biochemical changes

Biochemical analysis revealed marked hepatic and renal dysfunction in infected dogs (Table 3). Dog 3 showed extremely elevated AST (2553.7 U/L) and ALT (2372.6 U/L), consistent with severe hepatocellular damage. Dog 4 also had elevated ALT (400 U/L). Creatinine was markedly increased in Dog 4 (545 µmol/L), reflecting impaired renal function. Serum glucose was reduced in Dog 3 (0.68 mmol/L) and Dog 4 (2.8 mmol/L), indicative of hypoglycemia.

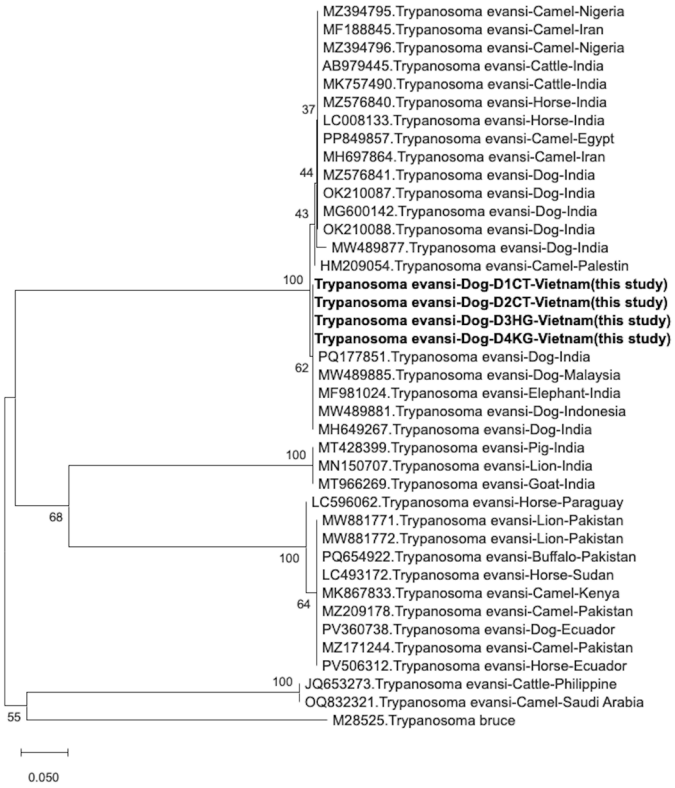

Figure 2: Maximum likelihood phylogenetic tree based on partial RoTat 1.2 VSG gene sequences (~205 bp) of Trypanosoma evansi isolates obtained from dogs in Vietnam (bold font). Bootstrap values (1000 replicates) are shown at major nodes. Scale bar indicates nucleotide substitutions per site. High bootstrap values reflect stability for a conserved marker but do not imply fine-scale evolutionary resolution.

Treatment and outcome

All infected dogs received symptomatic treatment immediately after diagnosis. Diminazene aceturate (3.5 mg/kg, intramuscularly) was administered once, followed by supportive therapy including fluid replacement and vitamin B complex supplementation. Two dogs (Dog 1 and Dog 2) showed gradual clinical improvement and

recovered within 10 days, while the other two (Dog 3 and Dog 4) exhibited severe anemia and neurological signs and eventually died despite treatment. The outcomes correlated with the severity of parasitemia and hematobiochemical alterations. Post-treatment parasitological cure was assessed clinically; follow-up PCR or smear microscopy was not performed on the survivors.

Table 2: Hematological parameters of dogs naturally infected with *Trypanosoma evansi* in the Mekong Delta, Vietnam.

Parameter	Unit	Reference range*	Dog 1	Dog 2	Dog 3	Dog 4
WBC	10^9/L	6.00–17.00	1.75	1.75	7.9	5.9
Neu%	%	60.0–70.0	55.3	61.9	55.0	63.2
Lym%	%	12.0–30.0	21.1	17.0	9.5	9.2
Mon%	%	3.0–10.0	21.1	18.1	3.1	3.2
Eos%	%	2.0–10.0	2.2	2.6	2.9	4.8
Bas%	%	0.0–1.30	0.3	0.4	0.5	0.2
Gra%	%	60.0–83.0	57.8	64.9	87.4	87.6
Neu	10^9/L	3.00–11.40	0.97	1.08	4.35	3.73
Lym	10^9/L	1.0–4.80	0.37	0.30	0.75	0.54
Mon	10^9/L	0.15–1.35	0.37	0.32	0.24	0.19
Eos	10^9/L	0.10–0.75	0.04	0.05	0.23	0.28
Bas	10^9/L	0.00–0.12	0.01	0.01	0.04	0.01
Gra	10^9/L	1.0–12.6	1.01	1.14	6.9	5.2
RBC	10^{12}/L	5.50–8.50	5.2	4.48	3.1	3.51
HGB	g/dL	12.0–18.0	10.5	9.0	6.7	5.3
HCT	%	37.0–55.0	30.4	26.2	19.4	17.6
MCV	fL	60.0–77.0	58.5	58.6	62.7	50.2
MCH	pg	19.5–24.5	20.1	20.1	21.6	15.0
MCHC	g/dL	32.0–36.0	34.3	34.3	34.5	30.1
RDW-CV	%	12.5–17.2	15.4	15.2	15.9	17.7
RDW-SD	fL	33.2–46.3	39.4	39.1	45.0	46.0
PLT	10^9/L	200-900	43	26	9	42
MPV	fL	8.0-14.10	10.6	9.9	11.2	8.6
PDW	%	1.0-30.00	7.4	6.4	12.4	15.3
PCT	%	0.090-0.580	0.046	0.026	0.010	0.036

Note: WBC= white blood cells; Neu= neutrophils; Lym= lymphocytes; Mon= monocytes; Eos= eosinophils; Bas= basophils; Gra= granulocytes; RBC= red blood cells; HGB= hemoglobin; HCT= hematocrit; MCV= mean corpuscular volume; MCH= mean corpuscular hemoglobin; MCHC= mean corpuscular hemoglobin concentration; RDW= red cell distribution width; PLT= platelets; MPV= mean platelet volume; PDW= platelet distribution width; PCT= plateletcrit. *Merck Veterinary Manual (2013).

Phylogenetic analysis

PCR amplification of the RoTat 1.2 VSG gene from the four Vietnamese canine isolates yielded products of the expected size (~205 bp). Sequencing and BLASTn analysis confirmed that all isolates shared 99–100% identity with *T. evansi* reference sequences in GenBank. The Maximum Likelihood phylogenetic tree constructed from partial RoTat 1.2 VSG gene sequences demonstrated that the Vietnamese isolates (Dog-D1CT, Dog-D2CT, Dog-D3HG, and Dog-D4KG) clustered within the *T. evansi* clade (Figure 2). These isolates grouped closely with dog-derived strains from India, Malaysia and Indonesia indicating their affiliation with the Asian lineage of *T. evansi*. The Vietnamese canine isolates were clearly separated from *T. brucei* which were used as outgroups. The overall topology of the tree highlighted low genetic variability among Asian *T. evansi* isolates, supporting the hypothesis of a genetically conserved lineage circulating across Southeast Asia. Notably, the clustering of Dog-D1CT, Dog-D2CT, Dog-D3HG, and Dog-D4KG reflected near-identical sequences, consistent with circulation of a conserved Asian lineage; higher-resolution markers are required to resolve local transmission dynamics.

Table 3: Serum biochemical parameters of dogs naturally infected with *Trypanosoma evansi* in the Mekong Delta, Vietnam

Parameter	Unit	Reference range*	Dog 1	Dog 2	Dog 3	Dog 4
AST	U/L	15.00–80.00	75.0	82.0	2553.7	95.0
ALT	U/L	10.00–120.0	60.0	55.0	2372.6	400
GGT	U/L	1.00–12.00	6.00	5.00	2.30	7.00
URE	mmol/L	6.10–11.40	7.50	6.80	8.40	10.2
CREATININ	µmol/L	80.00–186.0	120	145	113.7	545
GLUCOSE	mmol/L	3.50–6.70	4.20	3.80	0.68	2.80

Note: AST= aspartate aminotransferase; ALT= alanine aminotransferase; GGT= gamma-glutamyl transferase; URE= urea; CREATININ= creatinine; GLUCOSE= blood glucose. *Merck Veterinary Manual (2013).

DISCUSSION

The present study demonstrated that *Trypanosoma evansi* infection in domestic dogs from the Mekong Delta region of Vietnam is associated with a constellation of hematological alterations, biochemical disturbances, and molecular confirmation. All four dogs were positive for trypomastigotes in Giemsa-stained smears, with parasitemia ranging from low (+) to high (+++), and PCR targeting the RoTat 1.2 VSG gene confirmed the infections. Sequencing further verified identity with *T. evansi* reference strains, reinforcing the reliability of molecular assays for diagnosis. It should be emphasized that the present findings are based on only four cases, and therefore broader generalizations about canine surra in Vietnam should be made cautiously. The clinic-based passive case finding likely underestimates the true

spectrum of disease, particularly subclinical infections. Causal attribution is limited by the absence of a control group and the clinic-based, small sample; unmeasured comorbidities (e.g., nutritional deficiencies or concurrent infections) could contribute to abnormalities. Future case–control designs comparing infected dogs to clinic-matched *T. evansi*-negative dogs from the same localities are required to ascribe specificity of hematobiochemical changes

Among the four dogs, anemia was commonly observed, reflected by reduced RBC counts, hemoglobin, and hematocrit values. This aligns with previous reports indicating that anemia is the hallmark of canine trypanosomiasis (Lisulo *et al*., 2024; Ahmadi-hamedani *et al*., 2014). The anemia in our study was largely normocytic–normochromic, suggesting hemolysis and bone marrow suppression rather than nutritional deficiency. Thrombocytopenia was also pronounced across all cases, in agreement with Losos (1986) and Al-Abedi *et al*. (2018), who described platelet destruction mediated by immune mechanisms during *T. evansi* infection.

Leukocyte responses varied among dogs. Dog-D1CT and Dog-D2CT had leukopenia, whereas Dog-D3HG/Dog-D4KG had neutrophilia with lymphopenia. Without longitudinal timing of infection, stage-based interpretations remain speculative and individual variation or unmeasured cofactors may explain these patterns. Such variability has been highlighted in previous canine studies (Saminathan *et al*., 2016), underscoring that leukogram changes may depend on parasitemia and disease chronicity. However, this interpretation remains speculative given the absence of longitudinal data.

The therapeutic management in this study was based on the administration of diminazene aceturate (3.5 mg/kg, IM), which has been commonly used against *T. evansi* infections in domestic animals. Two dogs (Dog-D1CT and Dog-D2CT) responded positively to treatment and fully recovered, whereas Dog-D3HG and Dog-D4KG, which presented with severe anemia, hepatic dysfunction, and neurological signs, did not survive despite supportive care. These outcomes are consistent with previous reports indicating that prognosis depends largely on the stage of infection, parasitemia level, and degree of organ involvement (Nguyen *et al*., 2013; Aregawi *et al*., 2019). The findings highlight the importance of early diagnosis and timely trypanocidal therapy to improve survival in canine surra.

Biochemical findings indicated significant organ dysfunction. Dog-D3HG exhibited extremely elevated AST and ALT levels (>2000 U/L), reflecting severe hepatocellular injury, while Dog-D4KG showed elevated creatinine (545 µmol/L), suggestive of renal impairment. These results are consistent with reports in cattle, buffaloes, and horses, where *T. evansi* causes hepatic and renal pathology (Desquesnes *et al*., 2013; Nguyen *et al*., 2013; Giordani *et al*., 2016). Moreover, hypoglycemia observed in Dog-D3HG and Dog-D4KG may reflect parasite-induced glucose consumption and impaired hepatic gluconeogenesis, as previously suggested in bovine and canine infections (Aregawi *et al*., 2019).

Our findings align closely with recent case reports from Vietnam. Khanh *et al*. (2021) described the first confirmed case of canine trypanosomiasis in Hanoi, where the dog presented with anemia, thrombocytopenia, elevated ALT, and mild hypoglycemia, confirmed by both Giemsa smear and PCR. Similarly, Nguyen *et al*. (2021) reported circulation of *T. evansi* among dogs in endemic areas, highlighting the epidemiological importance of canine reservoirs. Furthermore, Nguyen *et al*. (2016) documented a human case of surra in Vietnam, emphasizing the zoonotic potential of the parasite. Compared to these studies, our work expands the knowledge base by integrating clinical, hematological, biochemical, parasitological, and molecular sequencing data from naturally infected dogs, thereby providing a more comprehensive picture of the disease in Vietnam.

The phylogenetic analysis of partial RoTat 1.2 VSG sequences demonstrated that all Vietnamese canine isolates clustered within the *T. evansi* lineage with strong bootstrap support, clearly separated from *T. brucei* spp. isolates used as outgroups. The isolates showed 99–100% identity with strains from Malaysia, India, and Indonesia, confirming the circulation of a genetically conserved Asian lineage. This finding is consistent with previous molecular studies indicating limited sequence variability among Asian *T. evansi* isolates (Claes *et al*., 2004; Birhanu *et al*., 2015; Nguyen *et al*., 2021). Because the RoTat 1.2 fragment is short and highly conserved, it mainly confirms species identity and broad Asian clustering rather than fine-scale transmission. The near-identical sequences of Dog-D3HG and Dog-D4KG are consistent with a conserved Asian lineage; higher-resolution markers (e.g., minicircle kDNA, cathepsin-L–like, satellite DNA, or genome-wide SNPs) are required to resolve local transmission. From an epidemiological perspective, these results highlight the potential role of dogs as reservoir hosts facilitating parasite transmission in endemic areas.

Although Giemsa smears were screened for *Babesia*, *Ehrlichia*, *Anaplasma*, and *Hepatozoon* with negative results, we did not perform a multiplex PCR panel; therefore, low-level co-infections cannot be excluded and could partly explain clinicopathological variability. In this clinic-based series, all cases were smear-positive, and PCR mainly added

species-level confirmation and sequence data. In settings with low parasitemia or chronic infections, however, PCR can detect infections missed by microscopy; thus, combining smear and PCR provides complementary sensitivity and specificity. Similar mixed infections have been reported in dogs from endemic areas (Azhahianambi *et al*., 2018; Nguyen *et al*., 2021). Accordingly, subclinical or low-level co-infections cannot be excluded. Because microscopy may miss low-grade co-infections, future studies will employ multiplex PCR panels (e.g., *Babesia*, *Ehrlichia/Anaplasma*, *Hepatozoon*) alongside *T. evansi* assays. This limitation has been acknowledged and should be addressed in future molecular epidemiological studies.

Taken together, the present findings support that canine trypanosomiasis can involve multiple organ systems, though manifestations may vary widely across individuals involving the hematopoietic, hepatic, and renal systems. Potential co-infections undetected by microscopy and not excluded by PCR could modulate organ-specific injury and confound clinicopathological attribution. The integration of parasitological, hematobiochemical, and molecular data provides a robust diagnostic framework with both sensitivity and specificity. Importantly, the phylogenetic clustering of Vietnamese isolates with Asian strains underscores the regional epidemiological connection and the underdiagnosed role of dogs in sustaining transmission cycles of surra. These findings call for increased clinical awareness, surveillance, and molecular epidemiology to better control *T. evansi* in Vietnam and beyond.

CONCLUSION

This clinic-based case series integrates clinical, hematobiochemical, and molecular data on canine *Trypanosoma evansi* in the Mekong Delta. The findings show that clinicals presentations can be severe yet heterogeneous. In a smear-positive context, PCR primarily provided species-level confirmation and sequence information; nonetheless, smear–PCR complementarity is critical when parasitemia is low. Phylogenetic clustering using the short, conserved RoTat 1.2 fragment supports species identity and broad Asian affinity but has limited value for fine-scale transmission inference. Major limitations include the very small, clinically selected sample and absence of controls, precluding population-level generalization. Dogs should be included in active surveillance programs where feasible to generate stronger evidence of their role as reservoirs. Priorities are a community-based cross-sectional survey with multiplex diagnostics (capturing subclinical infections), a matched case–control study to define disease markers, and higher-resolution parasite genomics.

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NOVELTY STATEMENT

This is the first report providing an integrated clinical, hematobiochemical, and molecular characterization of *Trypanosoma evansi* infection in dogs from the Mekong Delta region of Vietnam, revealing their close genetic relationship with other Asian isolates.

AUTHOR'S CONTRIBUTION

All authors contributed to the conception and design of the study.
TTT and DTT performed sample collection and clinical examinations.
NTPC supervised the laboratory analyses and data interpretation.
NDC and LDG assisted with sample collection.
TNB carried out the molecular analyses and sequencing.
All authors participated in drafting, revising, and approving the final version of the manuscript for submission.

Generative AI or AI-Assisted technology ST atement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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Ethics approval and consent to participate

Informed consent and agreement were obtained from all dog owners prior to sample collection. Animal examinations were conducted with strict regard for welfare considerations. This study was performed exclusively on naturally infected animals. Diagnostic samples were used,

and no experimental infections were carried out.

Conflict of interest

The authors have declared no conflict of interest.

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