

## Research Article



# Clinical and Molecular Features of *Babesia gibsoni* Infection in Dogs from Can Tho, Vietnam

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**Abstract** | *Babesia gibsoni* is a tick-borne protozoan parasite responsible for canine babesiosis, a disease associated with anemia, thrombocytopenia, and systemic complications. Although widely reported in Asia, clinical and molecular data from the Mekong Delta of Vietnam remain scarce. This study aimed to characterize the clinical features, hematological and biochemical alterations, and molecular identity of naturally occurring *B. gibsoni* infections in dogs from Can Tho, Vietnam. Between February and December 2023, twenty-one dogs with suspected babesiosis were examined at the Can Tho University Veterinary Teaching Clinic. Infection was confirmed by PCR targeting the 18S rRNA gene. Clinical signs, hematological profiles, and serum biochemistry were recorded. Three representative isolates were sequenced and analyzed phylogenetically using the maximum likelihood method. Most affected dogs were under 24 months of age, predominantly mixed-breed and Poodle types. Clinical signs included pale mucous membranes, lethargy, fever, and heavy tick infestation. Hematological findings showed normocytic hypochromic anemia (52.4%), severe thrombocytopenia (100%), lymphocytopenia (80.9%), and neutropenia (57.1%). Biochemical changes included elevated ALT (38.1%), AST (14.3%), and BUN (19.1%), consistent with hepatic dysfunction and prerenal azotemia. Phylogenetic analysis of three isolates (GenBank: PP716410–PP716412) revealed 100% identity with reference strains from Asia, Europe, and the United States, clustering within the global *B. gibsoni* clade. This is the first integrated clinical, hematological, biochemical, and molecular characterization of *B. gibsoni* in southern Vietnam. The findings confirm endemic circulation of a globally conserved lineage and highlight the parasite's significant hematopathological impact, providing essential data for disease surveillance and control strategies in the region.

**Keywords** | *Babesia gibsoni*, Protozoan parasite, Hematology, Biochemical indices, Phylogenetic analysis, Vietnam

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## INTRODUCTION

*Babesia gibsoni* is a tick-borne intraerythrocytic protozoan parasite that causes canine babesiosis, a disease of global veterinary importance. Infected dogs commonly present with anemia, thrombocytopenia, and systemic

complications, which can lead to severe illness or death if untreated. Transmission occurs primarily via competent tick vectors such as *Haemaphysalis* and *Rhipicephalus* spp., although direct dog-to-dog transmission through bites and vertical transmission have also been reported (Inokuma *et al.*, 2004; Liu *et al.*, 2022).

Clinically, canine babesiosis manifests with nonspecific signs including fever, lethargy, pallor, and splenomegaly, making diagnosis based on clinical examination alone unreliable. While microscopic examination of Giemsa-stained blood smears remains a useful screening tool, its sensitivity is limited in cases of low parasitemia (Teodorowski *et al.*, 2022). Molecular methods, particularly PCR targeting the 18S rRNA gene, provide higher sensitivity and specificity and are widely applied for definitive diagnosis and species identification (Inokuma *et al.*, 2004; Yin *et al.*, 2023). Sequencing of this gene also facilitates phylogenetic analysis, offering insights into strain distribution and genetic relatedness among global isolates (Schäfer *et al.*, 2023).

Despite increasing reports of *B. gibsoni* infection in Asia, Europe, and the Americas, information on its occurrence in Vietnam remains limited, particularly in the Mekong Delta where tick exposure is high and veterinary molecular surveillance is scarce. Existing studies often lack integrated clinical, hematological, biochemical, and molecular characterization, leaving important epidemiological gaps. Therefore, this study aimed to characterize the clinical manifestations, hematological and biochemical alterations, and molecular features of naturally occurring *B. gibsoni* infections in dogs from Can Tho, Vietnam. In addition, phylogenetic analysis was performed to determine the genetic relationship of local isolates with strains from other regions, providing baseline data for disease surveillance and control strategies.

## MATERIALS AND METHODS

### STUDY DESIGN

This retrospective study was conducted at the Veterinary Teaching Clinic, Can Tho University (Vietnam), between February and December 2023. 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).

The study area is located in the Mekong Delta region, characterized by a tropical monsoon climate and a high prevalence of vector-borne diseases in domestic animals, including canine babesiosis.

### CASE SELECTION AND INCLUSION CRITERIA

Medical records of dogs presented to the clinic during the study period were screened. Inclusion criteria were: (1) confirmed infection with *Babesia gibsoni* by PCR, and

(2) complete clinical, hematological, and biochemical data available at the time of diagnosis. Dogs were excluded if they showed evidence of co-infection with *Ehrlichia* spp., *Anaplasma* spp., or *Hepatozoon* spp. based on microscopic examination of Giemsa-stained blood smears or if they had atypical hematologic profiles inconsistent with babesiosis.

Signalment data, including age, sex, breed, body weight, and geographic origin, were recorded. Clinical signs at presentation were extracted from case files and analyzed for frequency and distribution.

### HEMATOLOGICAL AND BIOCHEMICAL ANALYSIS

Peripheral blood samples were collected via cephalic or jugular venipuncture into EDTA tubes. Complete blood counts (CBCs) were performed using an Excell 2280 hematology analyzer (Drew Scientific Inc., USA), including RBC count, HGB, HCT, MCV, MCH, MCHC, WBC count, differential leukocyte count, and platelet count. Thin blood smears were prepared for each case, stained with 10% Giemsa, and examined under light microscopy at 400× and 1000× magnification (Nikon Eclipse E100WLED, Japan).

Serum biochemical parameters, including ALT, AST, BUN, and creatinine, were measured using a 3000 Evolution semi-automated chemistry analyzer (Biochemical Systems International, Italy). All values were interpreted against species-specific reference ranges (Merck Veterinary Manual, 2013). Results were categorized as decreased, normal, or increased relative to reference values.

### MOLECULAR DETECTION OF *BABESIA GIBSONI*

Genomic DNA was extracted from 200 µL of EDTA-anticoagulated blood using the TopPURE® Viral DNA/RNA Extraction Kit (ABT, Vietnam), following the manufacturer's instructions. The quality and concentration of the extracted DNA were assessed spectrophotometrically, and aliquots were stored at -20 °C until subsequent molecular analysis.

Detection of *B. gibsoni* was conducted by PCR targeting the 18S rRNA gene using species-specific primers as described by Inokuma *et al.* (2004). Genomic DNA was extracted from whole blood samples using a commercial extraction kit following the manufacturer's protocol. PCR amplification was performed using the primer pair Gib599F (5'-CTCGGCTACTTGCCTTGTC-3') and Gib1270R (5'-GCCGAAACTGAAATAACGGC-3'), which amplifies a 671 bp fragment. The thermal cycling protocol consisted of an initial denaturation at 94°C for 5 min; followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 45 s; with a final extension at 72°C for 7 min. PCR products were resolved by electrophoresis on a 1.5% agarose gel

stained with SYBR™ Safe and visualized under UV transillumination.

To confirm species identity and exclude mixed infections, all dogs were screened for *Ehrlichia/Anaplasma* (intracytoplasmic morulae in platelets or leukocytes) and *Hepatozoon* spp. (gamonts in leukocytes) by examination of Giemsa-stained thin blood smears under high-power microscopy. Dogs with any evidence of co-infection were excluded from the study, in accordance with the diagnostic criteria established by Inokuma *et al.* (2002) and O'Dwyer (2011). Only PCR-positive samples for *Babesia gibsoni* were included in the final dataset. Species identity was further confirmed by PCR using *B. gibsoni*-specific primers, and cases with ambiguous amplicons or mixed *Babesia* spp. signals were excluded.

### DNA SEQUENCING AND PHYLOGENETIC ANALYSIS

Positive PCR products were purified and sequenced using an ABI 3730XL platform (NamKhoa Biotek, Vietnam). Raw sequences were trimmed and aligned using Geneious Prime v2024. BLASTn analysis was conducted to confirm species identity. Multiple sequence alignment was performed using ClustalW in MEGA v12 (Kumar *et al.*, 2024), and a phylogenetic tree was constructed using the Maximum Likelihood method with 1,000 bootstrap replicates, based on the Kimura 2-parameter model. *Babesia gibsoni* sequences from this study were compared with global reference sequences available in GenBank.

Three PCR-positive samples (CT01, CT02, and CT03) were selected for sequencing based on clinical severity, hematological diversity, and geographical representation. These samples were chosen to ensure that the phylogenetic analysis reflected the broader characteristics of the infected population.

### STATISTICAL ANALYSIS

All data were recorded in Microsoft Excel 2019 and analyzed using R Studio (version 2023.06). Normality was assessed using skewness. Continuous variables are reported as mean  $\pm$  standard error (SE), with minimum–maximum and coefficient of variation (CV%) values. Categorical variables are summarized as frequencies and percentages. Differences between groups were not statistically tested due to the small sample size and descriptive nature of the study.

## RESULTS

Epidemiological and clinical characteristics

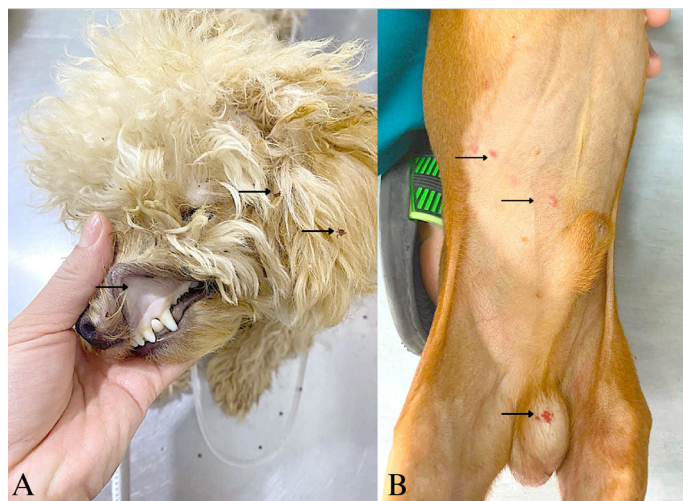
A total of 21 dogs tested positive for *Babesia gibsoni* by PCR targeting the 18S rRNA gene (Table 1). All diagnoses were confirmed by molecular methods without reliance on

microscopy.

The infected dogs ranged in age from 6 to 48 months, with 66.7% (14/21) under 24 months of age. Sex distribution was nearly equal, with 11 males and 10 females.

Mixed-breed dogs accounted for the highest proportion of cases (9/21; 42.9%), followed by Poodles (5/21), Phu Quoc dogs (3/21), and other breeds including Chihuahua, Husky, Pug, Shih Tzu, and Golden Retriever.

Clinically, the most frequently reported signs included pale mucous membranes (7/21), lethargy (6/21), fever (6/21), tick infestation (5/21), anemia (5/21), and weakness (5/21) (Figure 1). Additional findings comprised splenomegaly, icterus, hematuria, lymphadenopathy, emaciation, and anorexia, with many dogs presenting with multiple concurrent signs consistent with hemolytic and systemic inflammatory processes. All 21 dogs were positive on both blood smear and PCR.



**Figure 1:** Clinical manifestations in dogs naturally infected with *Babesia gibsoni*. (A) Pale oral mucous membranes, consistent with anemia and hemolysis, with evidence of tick infestation. (B) Multiple petechial hemorrhages on the ventral abdomen, indicative of thrombocytopenia associated with babesiosis.

Geographically, cases were distributed across seven districts of Can Tho city. The highest numbers were recorded in Ninh Kieu (5/21), Binh Thuy (4/21), and Cai Rang (4/21), with additional cases in Phong Dien, O Mon, Thot Not, and Co Do districts.

### HEMATOLOGICAL FINDINGS IN DOGS INFECTED WITH *BABESIA GIBSONI*

Hematological parameters of the 21 PCR-confirmed *Babesia gibsoni*-infected dogs are summarized in Table 2. Red blood cell (RBC) counts were decreased in 57.14% (12/21) of cases, with values ranging from 2.19 to

**Table 1:** Summary of epidemiological, clinical and diagnostic characteristics of 21 dogs PCR-positive for *Babesia gibsoni*.

| Sample ID | Age (months) | Sex | Breed            | Geographic origin   | Clinical signs                     | Smear | PCR result |
|-----------|--------------|-----|------------------|---------------------|------------------------------------|-------|------------|
| CT01      | 9            | M   | Mixed-breed      | Phong Dien District | Pale mucous membrane, lethargy     | +     | +          |
| CT02      | 12           | F   | Poodle           | Ninh Kieu District  | Fever, anorexia, tick infestation  | +     | +          |
| CT03      | 24           | M   | Husky            | Binh Thuy District  | Weakness, dark urine               | +     | +          |
| CT04      | 36           | M   | Mixed-breed      | Cai Rang District   | Anemia, emaciation                 | +     | +          |
| CT05      | 10           | F   | Phu Quoc         | Co Do District      | Fever, mucosal pallor              | +     | +          |
| CT06      | 14           | M   | Poodle           | Thot Not District   | Lethargy, enlarged lymph nodes     | +     | +          |
| CT07      | 18           | F   | Shih Tzu         | O Mon District      | Anemia, splenomegaly               | +     | +          |
| CT08      | 6            | M   | Chihuahua        | Ninh Kieu District  | Tick infestation, fever            | +     | +          |
| CT09      | 48           | F   | Mixed-breed      | Binh Thuy District  | Chronic weight loss, anemia        | +     | +          |
| CT10      | 22           | M   | Pug              | Phong Dien District | Lethargy, icterus                  | +     | +          |
| CT11      | 16           | M   | Golden Retriever | Ninh Kieu District  | Fever, hematuria                   | +     | +          |
| CT12      | 30           | F   | Phu Quoc         | Cai Rang District   | Weakness, tick infestation         | +     | +          |
| CT13      | 11           | F   | Mixed-breed      | O Mon District      | Splenomegaly, anorexia             | +     | +          |
| CT14      | 15           | M   | Poodle           | Ninh Kieu District  | Lethargy, mild jaundice            | +     | +          |
| CT15      | 8            | F   | Chihuahua        | Binh Thuy District  | Pale gums, fever                   | +     | +          |
| CT16      | 19           | M   | Mixed-breed      | Binh Thuy District  | Anorexia, lymphadenopathy          | +     | +          |
| CT17      | 21           | F   | Poodle           | Thot Not District   | Weakness, mucosal pallor           | +     | +          |
| CT18      | 25           | M   | Mixed-breed      | Ninh Kieu District  | Emaciation, chronic anemia         | +     | +          |
| CT19      | 32           | M   | Phu Quoc         | Cai Rang District   | Dark urine, weakness               | +     | +          |
| CT20      | 13           | F   | Mixed-breed      | Cai Rang District   | Tick infestation, depression       | +     | +          |
| CT21      | 7            | M   | Poodle           | Phong Dien District | Pale mucous membrane, splenomegaly | +     | +          |

**Table 2:** Hematological parameters in dogs naturally infected with *Babesia gibsoni*.

| Parameter   | Reference range* | Unit                 | Category  | Min-max     | Mean ± SE   | CV%   | Number of cases (n=21) | Percentage (%) |
|-------------|------------------|----------------------|-----------|-------------|-------------|-------|------------------------|----------------|
| RBC         | 5.5–8.5          | x10 <sup>6</sup> /μL | Decreased | 2.19–5.12   | 3.41±0.60   | 58.87 | 12                     | 57.14          |
|             |                  |                      | Normal    | 5.58–8.50   | 6.78±0.26   | 11.47 | 9                      | 42.86          |
| Hematocrit  | 32.5–58.0        | %                    | Decreased | 8.30–30.70  | 18.84±3.72  | 59.21 | 10                     | 47.62          |
|             |                  |                      | Normal    | 34.10–44.60 | 38.58±1.10  | 9.48  | 11                     | 52.38          |
| MCV         | 60.0–76.0        | fL                   | Normal    | 65.10–75.20 | 70.35±0.74  | 4.36  | 17                     | 80.95          |
|             |                  |                      | Increased | 76.20–84.20 | 79.57±4.15  | 5.21  | 4                      | 19.05          |
| Hemoglobin  | 110–195          | g/L                  | Decreased | 40.00–98.00 | 62.10±12.90 | 65.69 | 11                     | 52.38          |
|             |                  |                      | Normal    | 111.0–161.0 | 133.30±4.53 | 10.74 | 10                     | 47.62          |
| MCH         | 20.0–27.0        | pg                   | Decreased | 5.00–19.90  | 17.66±1.02  | 20.76 | 14                     | 66.67          |
|             |                  |                      | Normal    | 20.90–22.00 | 21.57±0.16  | 1.94  | 7                      | 33.33          |
| MCHC        | 32.0–36.0        | g/dL                 | Decreased | 25.40–30.10 | 27.68±2.51  | 4.44  | 19                     | 90.48          |
|             |                  |                      | Increased | 44.00–73.00 | 58.50±20.51 | 35.05 | 2                      | 9.52           |
| WBC         | 6.0–17.0         | x10 <sup>3</sup> /μL | Decreased | 4.73–5.90   | 5.40±2.06   | 9.15  | 4                      | 19.05          |
|             |                  |                      | Normal    | 6.80–16.82  | 10.72±0.90  | 32.38 | 15                     | 71.43          |
|             |                  |                      | Increased | 17.82–17.89 | 17.86±0.05  | 0.28  | 2                      | 9.52           |
| Neutrophils | 45.0–75.0        | %                    | Decreased | 10.70–42.00 | 21.26±7.77  | 121.2 | 12                     | 57.14          |
|             |                  |                      | Normal    | 47.00–74.00 | 61.58±4.21  | 16.73 | 6                      | 28.57          |
|             |                  |                      | Increased | 77.00–79.00 | 78.33±1.15  | 1.47  | 3                      | 14.29          |
| Lymphocytes | 25.0–45.0        | %                    | Decreased | 5.30–20.21  | 11.89±2.05  | 69.13 | 17                     | 80.95          |
|             |                  |                      | Normal    | 25.80–32.10 | 28.05±1.42  | 10.13 | 4                      | 19.05          |
| Monocytes   | 3.0–10.0         | %                    | Normal    | 3.76–6.58   | 4.44±0.15   | 15.48 | 21                     | 100.0          |
| Eosinophils | 2.0–10.0         | %                    | Normal    | 7.20–9.90   | 8.82±0.15   | 7.63  | 21                     | 100.0          |
| Basophils   | 0.1–2.5          | %                    | Decreased | 0.065–0.096 | 0.08±0.01   | 19.54 | 3                      | 14.29          |
|             |                  |                      | Normal    | 0.11–0.632  | 0.28±0.04   | 57.20 | 18                     | 85.71          |
| PLT         | 117–490          | x10 <sup>3</sup> /μL | Normal    | 13.00–115.0 | 68.90±6.34  | 41.16 | 21                     | 100.0          |

**Abbreviations:** RBC= Red Blood Cell Count; MCV= Mean Corpuscular Volume; MCH= Mean Corpuscular Hemoglobin; MCHC= Mean Corpuscular Hemoglobin Concentration; WBC= White Blood Cell Count; PLT= Platelet count; SE= Standard error; CV%=Coefficients of variation [CV% = (SD/Mean)×100]. \*Merck Veterinary Manual (2013)

**Table 3:** Serum biochemical parameters in dogs naturally infected with *Babesia gibsoni*.

| Parameter  | Reference range* | Unit  | Category  | Min-Max     | Mean±SE      | CV%   | Number of cases (n=21) | Percentage (%) |
|------------|------------------|-------|-----------|-------------|--------------|-------|------------------------|----------------|
| AST        | 8.9–48.5         | U/L   | Normal    | 22.00–46.00 | 32.24±1.558  | 20.27 | 18                     | 85.71          |
|            |                  |       | Increased | 107.0–134.0 | 119.67±13.58 | 11.35 | 3                      | 14.29          |
| ALT        | 8.2–57.3         | U/L   | Normal    | 48.00–56.00 | 52.17±0.86   | 5.71  | 12                     | 57.14          |
|            |                  |       | Increased | 60.00–92.00 | 71.00±10.81  | 15.23 | 8                      | 38.10          |
| BUN        | 7.0–28.0         | mg/dL | Normal    | 9.50–26.90  | 22.93±1.07   | 18.63 | 17                     | 80.95          |
|            |                  |       | Increased | 27.30–29.20 | 28.28±0.84   | 2.98  | 4                      | 19.05          |
| Creatinine | 0.5–1.7          | mg/dL | Normal    | 0.60–1.60   | 1.02±0.06    | 26.42 | 18                     | 85.71          |
|            |                  |       | Increased | 1.70–1.90   | 1.80±0.14    | 7.86  | 2                      | 9.52           |

AST=Aspartate aminotransferase; ALT= Alanine aminotransferase; BUN= Blood urea nitrogen; SE= Standard error; CV%= Coefficients of variation [CV% = (SD/Mean)×100]; \*Merck Veterinary Manual (2013).

5.12 ×10<sup>6</sup>/μL (mean ± SE: 3.41 ± 0.60). Hematocrit (HCT) was below the reference range in 47.62% (10/21), with a mean of 18.84 ± 3.72%. Hemoglobin concentration was reduced in 52.38% (11/21) of dogs, ranging from 40.0 to 98.0 g/L (mean: 62.10 ± 12.90).

Macrocytosis, defined by elevated mean corpuscular volume (MCV), was observed in 19.05% (4/21) of dogs, while 80.95% had MCV values within normal limits. Mean corpuscular hemoglobin (MCH) was decreased in 66.67% (14/21) of cases, with a mean of 17.66 ± 1.02 pg. Decreased mean corpuscular hemoglobin concentration (MCHC) was present in 90.48% (19/21) of dogs, while 2 dogs (9.52%) exhibited increased MCHC values.

White blood cell (WBC) counts remained within normal limits in 71.43% (15/21) of dogs. Leukopenia was detected in 4 dogs (19.05%), and leukocytosis in 2 cases (9.52%). Neutropenia was present in 57.14% (12/21), whereas 14.29% (3/21) showed neutrophilia. Lymphopenia was observed in 80.95% (17/21) of dogs, with a mean lymphocyte percentage of 11.89 ± 2.05%. All dogs had normal monocyte and eosinophil levels.

Basophil counts were decreased in 14.29% (3/21), while 85.71% remained within normal range. Despite being reported as “normal,” platelet counts were markedly reduced in all dogs, ranging from 13.0 to 115.0 ×10<sup>3</sup>/μL (mean: 68.90 ± 6.34), consistent with thrombocytopenia.

The coefficients of variation (CV%) were notably high for several parameters, including neutrophils (121.2%), hemoglobin (65.69%), and lymphocytes (69.13%), indicating substantial inter-individual variability in hematologic responses to infection.

#### SERUM BIOCHEMICAL PARAMETERS IN DOGS INFECTED WITH *BABESIA GIBSONI*

Serum biochemical profiles of 21 PCR-confirmed *Babesia*

*gibsoni*-infected dogs are summarized in Table 3. Aspartate aminotransferase (AST) levels remained within the reference range in 85.71% (18/21) of dogs, with a mean value of 32.24 ± 1.56 U/L. Elevated AST was observed in 14.29% (3/21), with values ranging from 107.0 to 134.0 U/L.

Alanine aminotransferase (ALT) was within normal limits in 57.14% (12/21) of cases (mean: 52.17 ± 0.86 U/L). ALT elevation was recorded in 38.10% (8/21) of dogs, ranging from 60.0 to 92.0 U/L, indicating mild to moderate hepatocellular injury.

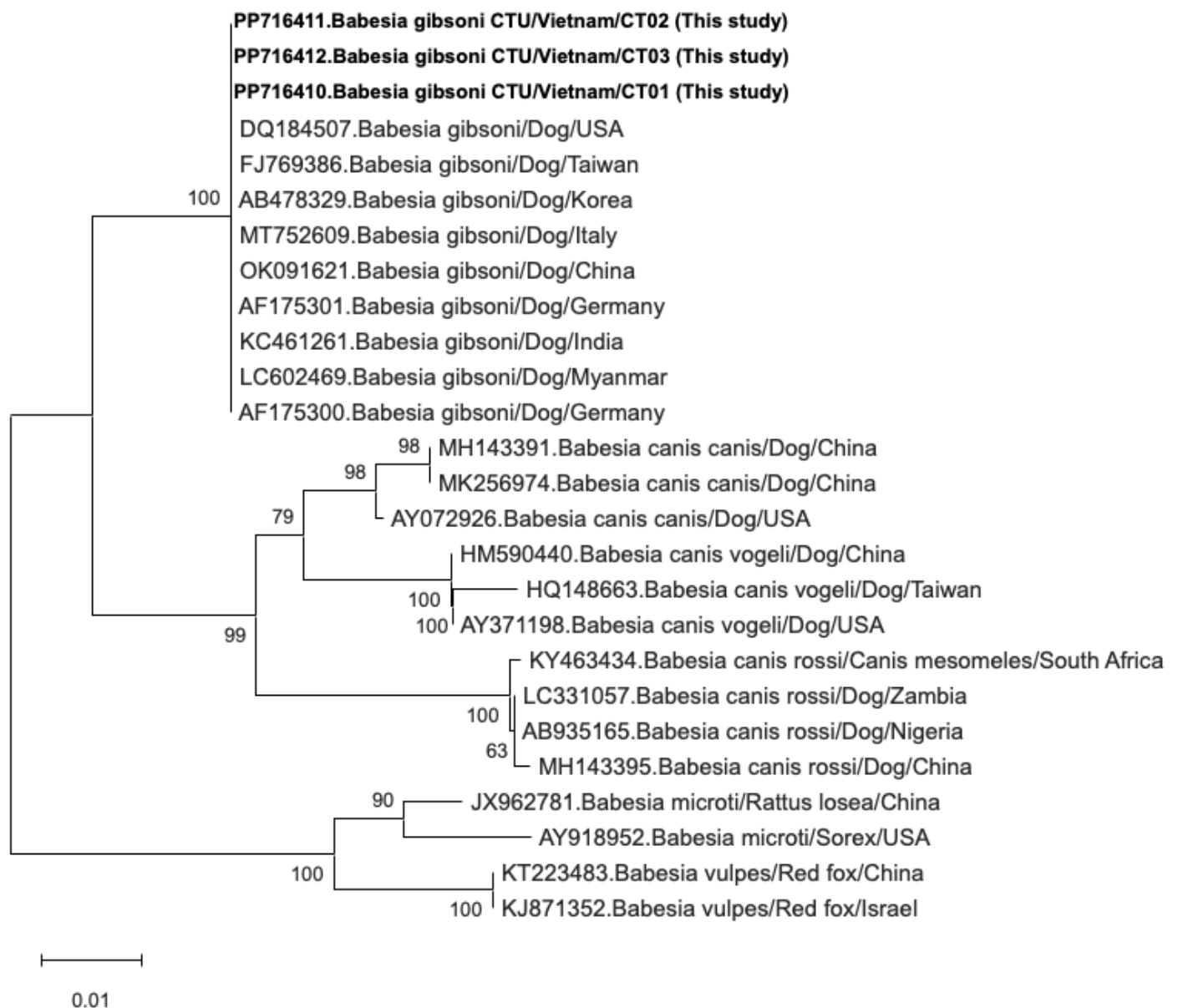
Blood urea nitrogen (BUN) concentrations were normal in 80.95% (17/21), with a mean of 22.93 ± 1.07 mg/dL. Four dogs (19.05%) exhibited mildly elevated BUN levels (mean: 28.28 ± 0.84 mg/dL), suggesting early or subclinical renal involvement.

Serum creatinine levels were within the normal range in 85.71% (18/21) of dogs (mean: 1.02 ± 0.06 mg/dL). Mild elevation was seen in 9.52% (2/21), with values between 1.70 and 1.90 mg/dL, not exceeding the upper reference threshold significantly.

Overall, serum biochemistry results indicated that most infected dogs maintained near-normal hepatic and renal function. However, mild increases in liver enzymes and nitrogenous waste products were observed in a subset of animals, reflecting possible hepatic stress and early renal impairment.

#### PHYLOGENETIC ANALYSIS

A phylogenetic tree was constructed based on partial 18S rRNA gene sequences to determine the genetic relationships of the *Babesia* isolates obtained from infected dogs in this study. The Maximum Likelihood method was used, with bootstrap analysis based on 1,000 replicates to assess nodal support (Figure 2).



**Figure 2:** Maximum likelihood phylogenetic tree based on partial 18S rRNA gene sequences of *Babesia gibsoni* isolates from Can Tho city, Vietnam and reference strains.

The three *Babesia gibsoni* isolates obtained from Can Tho, Vietnam (CT01, CT02, CT03) clustered tightly within the *B. gibsoni* clade and showed the highest sequence similarity to reference strains from the USA (DQ184507), Taiwan (FJ769386), Korea (AB478329), and Italy (MT752609). These Vietnamese isolates formed a strongly supported monophyletic group (bootstrap value = 100), confirming their classification as *B. gibsoni* and indicating high genetic conservation with globally circulating strains.

The *B. gibsoni* clade was clearly separated from other canine *Babesia* species, including *B. canis canis*, *B. canis rossi*, and *B. canis vogeli*, with strong bootstrap support (98–100), further validating the species-level identification. Outgroup taxa included *Babesia microti* and *Babesia vulpes*, which were placed distantly from the canine-associated *Babesia* clusters.

These results confirm that the Vietnamese isolates belong to *B. gibsoni* and share close evolutionary relationships with strains circulating in Asia, Europe, and North America, suggesting low genetic divergence across geographic regions.

## DISCUSSION

The present study confirms that *Babesia gibsoni* is an endemic pathogen in dogs in the Mekong Delta, with epidemiological, clinical, and hematological characteristics comparable to international reports. The higher prevalence in dogs younger than 24 months suggests increased susceptibility due to immature immunity or greater tick exposure, consistent with observations from South Korea and India (Lee *et al.*, 2009; Selvaraj *et al.*, 2010). The absence of sex predisposition aligns with studies from

Japan and Hong Kong (Inokuma *et al.*, 2005; Chan *et al.*, 2025).

Clinically, common signs such as pale mucous membranes, fever, lethargy, and splenomegaly represent classic indicators of babesiosis and are in agreement with descriptions from India and Europe (Bilwal *et al.*, 2017; Furlanello *et al.*, 2005). The occurrence of petechiae reflects severe thrombocytopenia and has been frequently reported in advanced cases (Vishnurahav *et al.*, 2014; Karasová *et al.*, 2022). Several deaths due to progressive anemia were reported by owners, highlighting the clinical severity of the disease in this region. However, the lack of systematic post-mortem (PM) and histopathological data limited detailed evaluation of organ lesions.

Hematological analysis demonstrated predominantly normocytic-hypochromic anemia, together with severe thrombocytopenia in all cases, reflecting hemolysis and immune-mediated destruction of parasitized erythrocytes (Ganesan *et al.*, 2025). These findings are consistent with reports from Zambia and China (Nalubamba *et al.*, 2015; Liu *et al.*, 2022). High rates of lymphopenia and neutropenia were also recorded, in contrast to other studies that reported neutrophilia (Selvaraj *et al.*, 2010), possibly due to immune suppression or sequestration of leukocytes in inflamed tissues.

Biochemical alterations included elevated ALT, AST, and BUN, indicating hepatocellular damage and systemic metabolic stress. Similar changes have been documented in India and Thailand (Reddy *et al.*, 2014; Niwetpathomwat *et al.*, 2006). Although creatinine levels remained largely within reference intervals, increased BUN may be associated with dehydration, hemolysis, or gastrointestinal bleeding rather than intrinsic renal failure. The lack of bilirubin and ALP data restricted further assessment of hepatic dysfunction.

Phylogenetic analysis demonstrated 100% sequence identity of Vietnamese isolates with global *B. gibsoni* strains from Asia, Europe, and the United States. This highlights the highly conserved nature of the 18S rRNA gene, consistent with findings from India, China, and Germany (Singh *et al.*, 2016; Schäfer *et al.*, 2023; Zygner *et al.*, 2023). However, because this locus is too conserved, it cannot reveal fine-scale genetic diversity within local populations. Future studies should employ more variable markers such as ITS or Cox1 to better assess regional diversity and the potential emergence of novel variants.

From an epidemiological perspective, detection of *B. gibsoni* in multiple districts of Can Tho, both urban and peri-urban, suggests a wide risk of transmission. The

presence of *Rhipicephalus sanguineus* ticks on infected dogs reinforces its role as the principal vector in Vietnam, consistent with reports of tick distribution in northern regions (Nguyen *et al.*, 2019). This underlines the importance of comprehensive tick control strategies, including systemic acaricides, environmental management, and improved owner awareness.

In summary, this study provides the first integrated description of clinical, hematological, biochemical, and genetic features of *B. gibsoni* in southern Vietnam, demonstrating its significant pathological impact on dogs. The findings are consistent with international reports and emphasize the urgent need for epidemiological.

## CONCLUSIONS

This study confirms the circulation of *Babesia gibsoni* among domestic dogs in Can Tho city using PCR-based detection targeting the 18S rRNA gene. Infected dogs exhibited typical clinical signs of babesiosis, including anemia, lethargy, and thrombocytopenia. Hematological findings revealed normocytic, hypochromic anemia and marked thrombocytopenia, while biochemical profiles indicated hepatocellular enzyme elevations and pre-renal azotemia. Phylogenetic analysis showed that Vietnamese isolates clustered within the global *B. gibsoni* clade, with no evidence of novel variants. These results underscore the clinical relevance, molecular stability, and endemic transmission of *B. gibsoni* in southern Vietnam.

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## NOVELTY STATEMENT

This study provides the first integrated characterization of *Babesia gibsoni* infection in dogs from southern Vietnam, combining clinical observations, hematological and biochemical alterations, and molecular confirmation. The findings highlight the endemic presence of *B. gibsoni* in the Mekong Delta, demonstrate its significant hematopathological impact, and confirm genetic stability of local isolates within the global clade. These results generate essential baseline data for epidemiological surveillance and control strategies against canine babesiosis in Vietnam.

TTAD: Conceived and designed the study, curated data, and drafted the manuscript.

NTPC: Conducted investigations and analyzed data.

TTT: Collected samples and performed laboratory analyses.

TNB: Supervised the study, validated findings, and critically revised the manuscript

All authors read and approved the final version.

## GENERATIVE AI OR AI-ASSISTED TECHNOLOGY STATEMENT

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

## FUNDING

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## ETHICS STATEMENT

This study involved naturally infected animals and used only diagnostic samples; no experimental procedures were performed. Informed consent was obtained from all dog owners.

## CONFLICT OF INTEREST

The authors have declared no conflict of interests.

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