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The Importance of qPCR Diagnosis in Chronic Canine Babesiosis: A Case Study

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Abstract: Canine babesiosis is a tick-borne vector disease caused by hemoparasites belonging to different species of Babesia. It is one of the most important tick-borne infections in dogs worldwide, with a global distribution and rapid expansion, mainly caused by Babesia canis and Babesia gibsoni. The chronic form of the disease represents a significant diagnostic challenge because parasitemia is low or absent on peripheral blood smear examination. We present the case of a 5-year-old stray female dog with four puppies approximately two months old, in which blood smear examination was negative for hemoparasites despite a clinical and hematological profile suggestive of infection: leukopenia (WBC 5.07 × 10^9/L), lymphopenia (LYM 0.75 × 10^9/L), normocytic normochromic anemia (RBC 5.29 × 10^{12}/L, HCT 35.78%), and severe thrombocytopenia (PLT = 0 × 10^9/L)
The diagnosis was established by quantitative real-time PCR (qPCR), which detected the DNA of both Babesia canis and Babesia gibsoni. The diagnosis of chronic babesiosis requires PCR because the low and intermittent parasitemia makes microscopy unreliable. Serological tests (IFAT, ELISA) complement PCR by identifying previous exposure and chronic carriers. Furthermore, species identification by molecular methods is essential because treatment protocols differ significantly. This case highlights the limitations of blood smear examination in the chronic form and supports the use of qPCR as the gold standard for the diagnosis of chronic canine babesiosis, with direct implications for therapeutic management and the risk of vertical transmission to the puppies.

Keywords: ***Babesia canis, Babesia gibsoni, thrombocytopenia, molecular diagnosis, negative blood smear***

Materials and Methods

2.1.Case presentation: The described case concerns a female mixed-breed dog, approximately 5 years old, stray, presented for consultation by an animal protection organization from Iaşi County. The animal was accompanied by four approximately 2-month-old nursing puppies. Vaccination and internal/external deworming had not been performed, and the medical history was unknown.

2.2. Clinical examination: At presentation, the dog showed a moderately altered general condition, mildly pale mucous membranes, vitreous-appearing eyes, apathy, partial anorexia, and reduced exercise tolerance. Rectal temperature was 38.9°C, heart rate was 104 bpm, with no audible cardiac murmur. No evident splenomegaly on abdominal palpation or icterus was detected. On inspection, several ticks were found and removed, particularly from the head and neck region. Blood samples were collected for complete blood count, biochemical analysis, blood smear examination, and PCR testing.

- **Extraction ADN →** PureLink Genomic DNA Mini Kit
- **Testing qPCR**

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- **CFX96 Real-Time**
- **Principle TaqMan**
- **Channels: FAM, HEX, Cy5**

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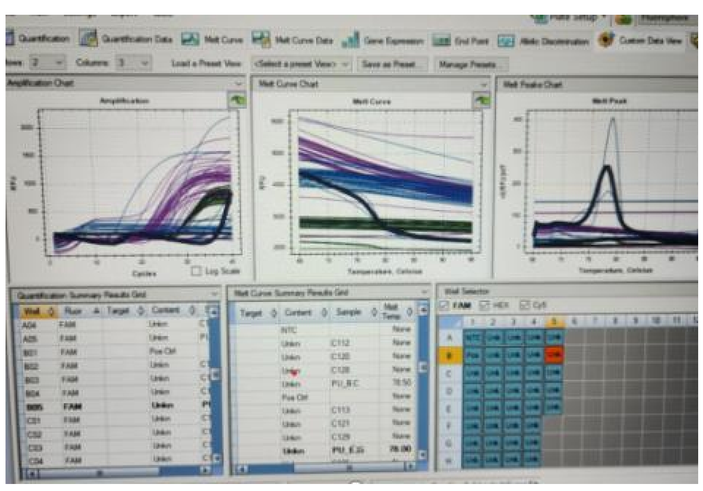
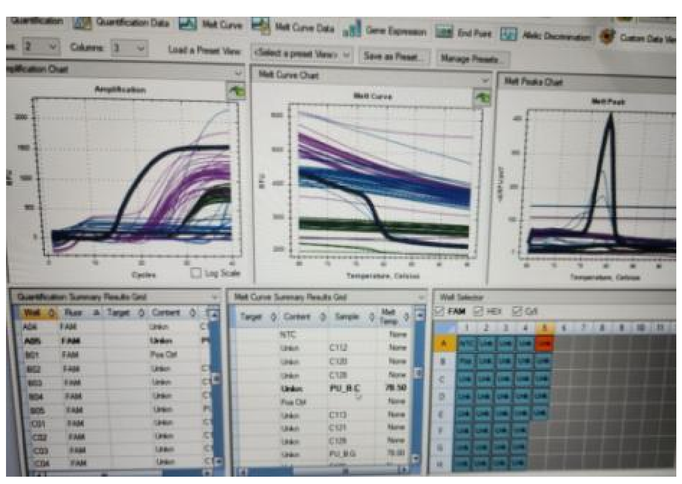
- ***Babesia canis***
- ***Babesia gibsoni***

CFX96 Real-Time



Positive sample for *Babesia gibsoni* with Tm 78°C



Positive sample for *Babesia canis* with Tm 78.5°C

Results

The May–Grünwald–Giemsa–stained blood smear, examined in multiple microscopic fields at 100× magnification (oil immersion), did not reveal intraerythrocytic structures suggestive of Babesia spp. or other hemoparasites.The major hematological alterations observed were: moderate leukopenia (WBC 5.07 × 10^9/L, RI: 6–17), lymphopenia (LYM 0.75 × 10^9/L, RI: 1–4.8), and mild normocytic normochromic anemia (RBC 5.29 × 10^{12}/L, HCT 35.78%).
The anemia was mild, normocytic, and normochromic. In acute babesiosis, severe hemolytic anemia is typically observed. In contrast, the chronic form produces a more subtle anemia, as parasitemia is low and hemolysis occurs gradually. The absence of reticulocytosis or spherocytes on the blood smear supports the chronic nature of the infection rather than an acute hemolytic process. Severe thrombocytopenia in the absence of clinical signs of active hemorrhage (no icterus, only mildly pale mucous membranes) suggests a chronic, partially compensated process rather than an acute fulminant condition. The qPCR assay detected the presence of *Babesia canis* and *Babesia gibsoni.*

Discussions

Thrombocytopenia, recognized as the most consistent hematological abnormality in canine babesiosis and reported in more than 90% of confirmed cases regardless of the infecting species. Because conventional microscopy and serology have limited sensitivity and specificity in chronic or subclinical infections, particularly in cases of low or intermittent parasitemia, qPCR was considered the diagnostic gold standard in this case, allowing simultaneous detection and differentiation of Babesia canis and Babesia gibsoni, a rare but clinically significant co-infection requiring distinct therapeutic protocols.

Conclusions

This case illustrates a typical form of chronic babesiosis, in which the clinical and hematological findings suggest infection, but the blood smear remains negative. The diagnosis was confirmed exclusively by multiplex qPCR, revealing a co-infection with Babesia canis and Babesia gibsoni. The result highlights the limitations of morphological diagnosis under conditions of low parasitemia and the importance of molecular methods. Species identification allowed the initiation of appropriate treatment, preventing therapeutic failure.