

A rare case report of active *Babesia caballi* infection in a mare from Gonbad-e-Kavus, Iran

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Article Info	Abstract
Article history: Received: 08 September 2025 Accepted: 07 January 2026 Available online: 15 July 2026	<i>Babesia caballi</i> is a tick-borne protozoan parasite responsible for causing equine babesiosis, a disease with considerable variation in prevalence across different regions of the world. This report presented a confirmed case of <i>B. caballi</i> infection in a three-year-old mare originating from Gonbad-e-Kavus, Iran, highlighting the clinical, laboratory and diagnostic aspects of the disease. The affected mare showed classical clinical signs including persistent fever, generalized weakness, tachycardia and pale mucous membranes, which are indicative of systemic infection and anemia. Laboratory evaluations revealed evidence of hepatic and renal compromise as well as hematological parameters consistent with active inflammatory processes. Microscopic examination of blood smears and molecular techniques such as PCR were employed to confirm the presence of <i>B. caballi</i> , while simultaneously excluding co-infections with other common equine pathogens including <i>Theileria equi</i> and <i>Anaplasma phagocytophilum</i> . This case underscored the critical need to combine conventional diagnostic approaches with molecular methods to achieve accurate detection, effective treatment and appropriate management strategies for equine babesiosis in endemic regions.
Keywords: <i>Babesia caballi</i> Equine Mare	

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Introduction

Equine babesiosis is a tick-borne intraerythrocytic disease caused by protozoan parasites of the genus *Babesia* and *Theileria*.¹ The disease is characterized by fever (39.00 - 42.00 °C), icterus, hemoglobinuria, hemolytic anemia and in severe cases death.

Babesia caballi is a large *Babesia* characteristically appearing within mature erythrocytes as nonpigmented, paired, pear-shaped bodies joined at an acute angle.¹

Among the *Babesia* species, *B. caballi* is of particular concern as it is pathogenic to horses and has a wide geographical distribution throughout the tropics and subtropics and to a lesser extent in temperate regions.¹ It is transmitted by *Dermacentor*, *Hyalomma*, and *Rhipicephalus* spp. ticks and can also be passed transovarially between tick generations.¹ Diagnosis relies on clinical signs, detection of parasites in Giemsa-stained blood smears, cellular enzyme-linked immunospecific assay testing and molecular assays such as PCR.¹ Although serological studies indicate the widespread presence of *B. caballi* in Iran, clinically confirmed cases have been

rarely reported with only two previous reports of *B. caballi* active case.^{2,3} Two active cases were microscopically documented in 1999 and 2000.^{2,3} Since then, despite multiple prevalence studies, no active clinical cases have been reported. Epidemiological investigations demonstrated considerable variability in prevalence in Iran and worldwide. In Iran, systematic reviews have confirmed its presence among domestic herbivores, with prevalence ranging from 8.80% in Mazandaran to 18.60% in Khorasan Razavi Province.⁴ Molecular and serological studies in North Khorasan and West Azerbaijan have also detected *B. caballi* at low levels.^{5,6}

In France, prevalence ranged from 0.00 to 26.50% depending on region, with 3.00% of ticks carrying *B. caballi* or *Theileria equi*.⁷ In Turkey, *B. caballi* was isolated from *Hyalomma* ticks collected in Kars Province.⁸ In Spain, seroprevalence in equids was 13.20%, with 10.80% showing co-infection with *T. equi*.⁹ Given the scarcity of molecularly confirmed active cases, reporting new clinical cases remains important for understanding disease epidemiology and improving equine health in the region.

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Case Description

A 3-year-old mare was presented in March 2025 with weakness, poor appetite and high fever from Gonbad-e Kavus, Golestan province. Clinical examination revealed a rectal temperature of 39.10 °C, tachypnea (26 breaths *per min*), tachycardia (45 beats *per min*), pale mucous membranes and delayed capillary refill time (> 2 sec). Biochemical analysis showed elevated urea, creatinine, alanine transaminase, albumin and bilirubin levels indicating renal and hepatic dysfunction and hemolysis. Hematological findings demonstrated a left shift, with an increased proportion of band neutrophils, consistent with active inflammation or infection. Microscopic examination of Giemsa-stained blood smears confirmed the presence of *B. caballi* (Fig. 1). The PCR finding was also positive for *B. caballi* DNA and negative for *T. equi* and *Anaplasma phagocytophilum* (Fig. 2).



Fig. 1. Arrow indicate *Babesia caballi* with a large, pear-shaped with acute angles (Giemsa staining, 100×).

The PCR targeting the rhoptry-associated *protein-1* (*rap-1*) gene of *B. caballi* was used for molecular detection. Genomic DNA was extracted from whole blood. A pair of species-specific primers amplifying a ~300 bp fragment of the *rap-1* gene were used: Forward primer (Bc-rap1-F): 5'-GTTATAGTTTCTTTGGCGTTGC-3' and Reverse primer (Bc-rap1-R): 5'-AGCTTGAGAACTCTCTCTCG-3'. These primers target a conserved region of the *B. caballi rap-1* gene previously validated for diagnostic assays.

The PCR amplification was performed in a 25.00 µL reaction mixture containing: 12.50 µL of 2.00 X PCR Master Mix (containing Taq DNA polymerase, dNTPs, MgCl₂, and reaction buffer; Sinaclon, Tehran, Iran), 1.00 µL

of each primer (10.00 pmol µL⁻¹), 3.00 µL of DNA template and nuclease-free water to volume. Amplification was performed using a programmable thermal cycler in a reaction volume containing 3.00 µm µL⁻¹ of DNA template and an appropriate amount of nuclease-free water. The thermal cycling conditions included an initial denaturation step at 95.00 °C for 5 minutes, followed by 35 cycles of denaturation at 95.00 °C for 30 seconds, annealing at 58.00 °C for 30 seconds, and extension at 72.00 °C for 45 seconds, with a final extension step at 72.00 °C for 5 min.

The PCR products were electrophoresed on a 2.00% agarose gel stained with ethidium bromide and visualized under ultraviolet illumination. A molecular size marker (250 - 1,000 bp) was used to determine amplicon length. Samples presenting a single band at approximately 300 bp were considered PCR-positive for *B. caballi*, while the negative control (no-template reaction) showed no amplification.

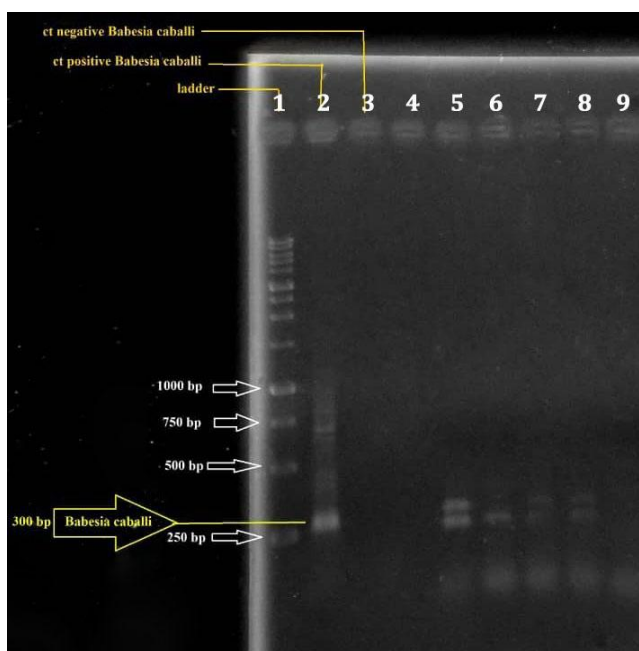


Fig. 2. Agarose electrophoresis gel. Lane 1: size marker. Lane 2: *Babesia caballi*-positive (300 bp), Lane3: negative control, Lanes 4 - 9: test samples (positive for *B. caballi*).

Discussion

This case report confirmed a rare active infection with *B. caballi* in a mare from Gonbad-e Kavus, Iran. While serological evidence indicated widespread exposure among equids. Molecularly studied reports confirmed active infection was rare in Iran with only two previous reports of *B. caballi* active case in Iran.^{2,3} The examined mare showed typical signs of equine babesiosis, with laboratory findings suggesting both hepatic and renal compromise. Confirmation by microscopy and PCR, together with the exclusion of co-infections, strengthened

the validity of this diagnosis. This report highlighted the integration of clinical, microscopic and molecular tools was necessary for accurate detection and surveillance. Improving awareness among veterinarians and enhancing diagnostic capacity are essential steps for effective management and control of equine babesiosis in endemic areas.

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Conflict of interest

The authors declare no known competing financial interests or personal relationships that could have influenced the work reported in this manuscript.

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