Original Article

Clinical and Diagnostic Study of Equine Hemomycoplasmosis in Basrah, Iraq

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ABSTRACT

Background: Equine hemomycoplasmosis, caused by *Mycoplasma haemofelis*, is an uncultivable parasite that infects the cell wall of erythrocytes and can be transmitted to humans, causing anemia and obvious clinical manifestations in diseased horses, which can end in severe emaciation and death.

Objectives: This study aimed to elucidate the clinical and diagnostic aspects of equine hemomycoplasmosis. A deeper understanding of the clinical variations observed in this disease is essential for tailoring effective control strategies to specific needs and challenges.

Methods: The study was conducted on 100 horses, aged 2-5 years, of both sexes. Diagnosis of the causative agent was based on cytological examinations (Giemsa-stained blood smears and fluorescent microscopical diagnosis using acridine orange staining). Moreover, the causative agent was confirmed using molecular diagnosis via quantitative real-time polymerase chain reaction (qPCR). However, hematological, biochemical, and gas analyses were also evaluated.

Results: The results indicated that the causative organism, a *M. haemofelis*-like organism, appears coccoid or rod-shaped. It may be observed alone or in a chain, invading the red blood cell (RBC) wall, as seen in Giemsa and acridine dye stains. Additionally, molecular diagnosis was confirmed using qPCR. Hematological analysis results indicated a macrocytic hypochromic anemia with significant differences in clotting factor indices affecting diseased horses. There was also a notable alteration in acid-base balance and anion gap values, reflecting systemic acidemia due to decreased pH in affected horses. Moreover, the biochemical profile of diseased horses showed significant differences compared to healthy controls, with increases in aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), total bilirubin, and troponin I. Conversely, levels of total protein, ferritin, and glucose were lower in diseased horses.

Conclusion: It was concluded that *Hemomycoplasma* spp. Infection in all domestic animals and horses remains a significant blood parasitic infection, causing severe and harmful pathological effects and resulting in substantial economic losses for infected animals. It can often be fatal. Therefore, to establish sustainable control programs and ultimately achieve eradication, a multifaceted approach is necessary, focusing on strengthening vaccination efforts, vector control, and improving disease surveillance and diagnostics.

Keywords: Biochemistry, Hematology, Horses, *Mycoplasma haemofelis*, Quantitative real-time polymerase chain reaction (qPCR)

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Introduction

Hemotropic mycoplasmas, also known as Hemomycoplasma or Hemoplasma, are small, gram-negative bacteria that lack cell membranes and are obligate erythrocyte parasites. These bacteria have been uncultivable to date, unlike mucosal mycoplasmas (Constable et al., 2017). Hemomycoplasma are pleomorphic, exhibiting various shapes, including rings, cocci, and rods, and possess small genomes. They usually adhere to the outside of red blood cells (RBCs) but can also be found individually or in chains (Messick, 2024). These organisms are classified within the Mycoplasma group, according to the 16S rRNA (Neimark et al., 2001; Urquhart et al., 2003). Transmission of Hemomycoplasma occurs through various arthropod vectors. Ticks, flies, fleas, and lice mechanically transmit these organisms to various livestock, including dogs, cats, sheep, goats, pigs, cows, and horses (Hornok et al., 2008; Peters et al., 2008; Saki, 2009; Willi et al., 2010). Hemomycoplasma is not definitively classified as a tick-borne disease; however, it may still influence the epidemiology of this bacterium, as certain species are occasionally identified in ticks (Barker et al., 2010; Akbari et al., 2024; Messick, 2024).

Mycoplasma haemofelis can cause sickness in horses. In 1978, light microscopy was used to identify an early sign of equine hemomycoplasma infection in Nigerian horses during a disease outbreak. In 2010, the first molecular confirmation of the organism in a horse was also published (Dieckmann et al., 2012). The disease affects horses of all ages and can lead to various pathological conditions, from severe anemia to long-term infection, sometimes with no clinical manifestation. However, clinical signs generally include anemia, pale mucus membranes, especially in the ocular and vaginal areas, poor performance, lethargy, partial to complete loss of body weight, lack of appetite, depression, and persistent fever, especially in the acute stages (Kalantari et al., 2020; Al Matwari et al., 2022; Ballados-González et al., 2025). Lymphadenopathy, including splenomegaly and enlarged internal lymph nodes, may also be observed. Jaundice, marked by a yellowish discoloration of the sclera caused by excessive destruction and hemolysis of RBCs, may be evident (Kahn et al., 2010; Dieckmann et al., 2012). The primary method for diagnosing Hemomycoplasma in animals has traditionally been cytology, using Giemsa-stained blood smears and acridine orange. However, the results may not correlate effectively with the polymerase chain reaction (PCR) technique, as identifying organisms on the erythrocyte surface in blood smear microscopy is considered nonspecific and relatively insensitive, often leading to discrepancies compared to PCR confirmation (Barker et al., 2010; Mandal, 2025).

Equine hemomycoplasmosis was suspected in horses from Basrah, Iraq. There is no study regarding the disease's occurrence in this area. Therefore, this study was designed to clarify clinical and diagnostic features. Gaining a better understanding of clinical variations is crucial for developing effective disease control strategies tailored to the specific needs and challenges.

Materials and Methods

Animals and study area

The study was conducted to examine 100 suspected male horses aged 2-5 years. Suspected animals showed signs of anemia, increased vital signs, loss of appetite, ticks were also found infesting some body regions. The study period was from March 2024 to August 2025. Suspected horses were mainly draft animals, although some were athletic, and they were housed in scattered enclosures throughout Basrah Governorate, Iraq. Twenty-five healthy, clinically normal horses were used as controls. All animals underwent a thorough clinical examination. Blood smears and fecal samples were also analyzed to rule out blood parasitic and gastrointestinal infections using standard laboratory methods (Mandal, 2025).

Hematology and collection of samples

Under sterile conditions, blood samples were drawn from each horse's jugular vein. Then, 2.5 mL of a tube containing ethylenediaminetetraacetic acid (EDTA) mixed with blood was used for a full blood count using a Hemoanalyzer (GENEX, USA). Additionally, a differential leukocyte count was performed on Giemsa-stained blood smears. Moreover, 2.5 mL of blood samples were collected with trisodium citrate to estimate prothrombin time, fibrinogen time, and activated partial thromboplastin time in plasma. Meanwhile, 2.5 mL of blood collected in plain tubes was used to extract serum for further biochemical analysis, including aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), total protein, total bilirubin, ferritin, troponin I, and glucose. Furthermore, 2 Furthermore, 2.5 mL of blood samples collected in EDTA tubes were used for DNA extraction and molecular diagnosis, which was performed using quantitative real-time PCR (qPCR).

Cytological diagnosis

Giemsa stain: The first step in diagnosing the causative agent involved ear smearing. A blood smear was prepared, air-dried, fixed in methanol for 3 to 5 minutes, and then stained with Giemsa. The Olympus BX50 microscope was used for examination at 1000x magnification with immersion oil (Stockham & Scott, 2025).

Acridine dye stain

Acridine orange dye was prepared according to Ciancaglini et al. (Ciancaglini et al., 2004) by dissolving 50 mg of acridine orange (BDH Chemicals Ltd, Poole, England) in 10 mL of distilled water (0.5%). The solution was stored in a dark bottle within the refrigerator for 4 weeks. To make the staining (working) solution, 1 mL of the stock dye solution was mixed with 0.5 mL of glacial acetic acid and added to 50 mL of distilled water. This solution can stain eight slides, with a final concentration of 0.01%. The pH of the dye solution was measured using a pH meter and found to be 3.

Evaluation of blood gases

According to Shiroshita et al. (1999), by using 1 mL of heparinized blood was aspirated separately from each diseased horse to determine the potential of hydrogen (blood pH), PCO_2, bicarbonate, PO_2, base excess, anionic gap, oxygen saturation percent (SO_2), sodium, and potassium using the Opti-critical care analyzer (USA) Serum chloride levels were also assessed according to Katsuhiko (2002). Samples were immediately sent for laboratory analysis to exclude any harmful changes.

Molecular diagnosis: Quantitative real time PCR (qPCR)

Using a Blood Genomic Extraction Kit (Geneaid, Taiwan), DNA was extracted from 200 µL of blood according to the manufacturer's instructions. We used the NanoDrop device (Thermo Fisher, USA) to determine the amount of DNA in the sample and its purity. Finally, the eluted DNA was stored at −20 ˚C until it was used for quantitative real time PCR.

Specific primers and SYBR Green were used for the detection of the *16S RNA* gene of *M. haemofelis* based on the forward primer: CGGCCAAGGTTAGTGGCAAACGG, and the reverse primer: TCCCTCAGCGCCCGAAGGCT (Amplicon size 170 base pairs).

Quantitative real time PCR was performed using Qiagen HotStarTaq Master Mix (UK). The reaction mixture (total volume 25 µL) contained 200 nM primers, 100 nM SYBR Green (diluted 1:100,000; Sigma-Aldrich), 4.5 mM $MgCl_2$, and 5 µL of DNA. All reactants were mixed into a master mix, then aliquoted into 96-well PCR plates (Thermofast, Abgene). The 5 µL DNA sample was added last. A negative control (water) was included in each run. The PCR was performed on an iCycler IQ (QuantStudio 5, Applied Biosystems) with an initial denaturation at 95 ˚C for 10 minutes, followed by 55 cycles of 95 ˚C for 10 seconds and 60 ˚C for 60 seconds, with fluorescence data collection.

For SYBR Green analysis, the protocol was extended with stepwise heating from 75 ˚C to 95 ˚C in 0.5 ˚C increments, holding for 15 seconds at each step to record a melting curve.

Statistical analysis

Statistical analysis was performed using the SPSS software, version 16. A student's t-test (unpaired) was used to calculate the statistical differences between diseased and healthy control horses. Values are expressed as Mean±SE. A significant value was set at $P<0.05$ (Leech et al., 2015).

Results

Out of 100 suspected horses infected with *M. haemofelis*-like organisms, 88 (88%) were identified as positive through microscopic examination. Clinically infected horses displayed various clinical manifestations, including partial or complete loss of appetite (91%); anemia characterized by paleness and/or icteration of mucous membranes observed on the conjunctiva, nictitating membrane, and vaginal mucous membranes (with icterus being more prominent on the scleral tissue) (88.6%); labored and rapid breathing (86.3%); a rough coat (79.5%); lethargy (69.3%); weight loss (67%); and poor performance. Edema of the lower hind limbs (fetlock area) was also noted in some infected animals (62.5%). Ticks were seen parasitizing various parts of the horse's body (61.3%), and hemoglobinurea was detected in 22.7% of diseased horses (Table 1). Moreover, a significant rise ($P<0.05$) in body temperature, respiratory rate, heart rate, and capillary refilling time was recorded in diseased horses compared with the controls (Table 2).

M. haemofelis is a parasite of the erythrocyte cell membrane, seen as spherical or rod-like. However, it is found alone or in a chain on the RBC's membrane. Infected RBCs are anisocytic and/ or poikilocytic in shape with high parasitemia (Figure 1). On the other hand, cytologi-

Table 1. Clinical manifestations of diseased horses with equine hemomycoplasmosis

Clinical Manifestation	No. (%)
	Diseased Horses (n=88)
Partial or complete lack of appetite	80 (91)
Anaemia with pale and/or icteric mucous membranes	78(88.6)
Labored and rapid breathing	76(86.3)
Rough coat	70(79.5)
Weight loss	61(69.3)
Poor performance	59(67)
Edema of the lower hind limbs (fetlock area)	55(62.5)
Ticks parasitizing various parts of the horse's body	54(61.3)
Hemoglobinurea	20(22.7)

cal diagnosis of the causative agent using fluorescent microscopy was also confirmed using acridine dye, as the bacterium appears fluorescent to invade the cell wall of erythrocytes (Figure 2).

Furthermore, molecular diagnosis using quantitative real-time PCR was performed for the causative agent. The results indicated that out of 88 diseased horses, 76(86.23%) tested positive for a *M. haemofelis*-like organism (Figures 3 and 4).

Concerning the hematological analysis of diseased horses infected with *M. haemofelis*-like organisms compared to the controls, the results indicated a type of anemia known as macrocytic hypochromic. The results showed a significant decrease ($P \leq 0.05$) in complete erythrocyte count, hemoglobin (Hb) concentration, and packed cell volume (PCV) values compared to the healthy control horses. Consequently, the values of mean corpuscular volume (MCV) and mean corpuscular Hb concentration (MCHC) were also different. On the other hand, the total leukocyte count of diseased horses was found to increase as a result of a significant increase ($P \leq 0.05$) in lymphocyte count of infected horses with *M. haemofelis*-like organism compared with healthy control animals (Table 3).

The present work also indicated a significant difference ($P \leq 0.05$) in the values of clotting factor indices between horses infected with *M. haemofelis*-like organism and controls (Table 4). Furthermore, the values of blood pH, PCO_2, bicarbonate, base excess, and oxygen saturation percentage were lowered, while a significant increase ($P \leq 0.05$) was observed in the anion gap of infected horses with *M. haemofelis* compared to controls (Table 5).

Biochemical analysis of horses infected with *M. haemofelis*-like organism (Table 6) indicated a significant increase ($P \leq 0.05$) in AST, GGT, ALP, total bilirubin, and troponin I levels. Conversely, significantly lower

Table 2. Vital clinical signs of diseased horses with equine hemomycoplasmosis

Parameters	Mean±SE	
	Controls (n=25)	Diseased Horses (n=88)
Body temperature (°C)	37.36±0.29	39.4±3.7*
Respiratory rate (brpm)	21.12±4.31	61.3±6.4*
Heart rate/min (bpm)	39.3±4.8	97.2±11.3*
Capillary refilling time (sec)	1.31±0.54	5.43±0.71*

*$P<0.05$.

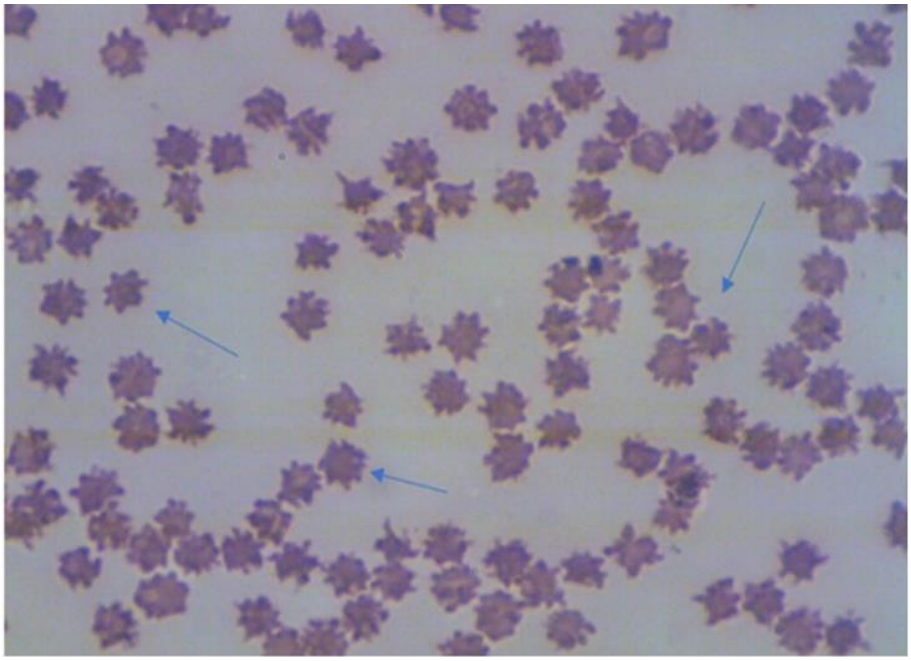

Figure 1. Erythrocyte cell membrane infected with *M. haemofelis*-like organisms, showing anisocytic and/or poikilocytic shapes and high parasitemia. Giemsa stain blood smear (×1000)

($P \leq 0.05$) values were observed for total protein, ferritin, and glucose in diseased horses compared to controls.

Discussion

Equine hemomycoplasmosis is a common blood infection in horses because of its pathological significance, which may sometimes be severe for the infected animal and may lead to death (Dieckmann et al., 2012). *Hemomycoplasma* spp. can infect most domestic farm animals (Constable et al., 2017; Al-Shamo & Esmaeel, 2025). Nonetheless, it is sometimes characterized by a silent infection that mostly occurs in stressed individuals (Esmaeel & Albadrani, 2019; Messick, 2024).

Infected horses exhibited different clinical manifestations, with a significant difference in vital signs of diseased animals. Anemia, lack of performance, and difficulty in respiration were the most obvious signs indicated, which is consistent with other studies (Kahn et al., 2010; Dieckmann et al., 2012; Hasan, 2012; Maggi, 2013; Ballados-González et al., 2025).

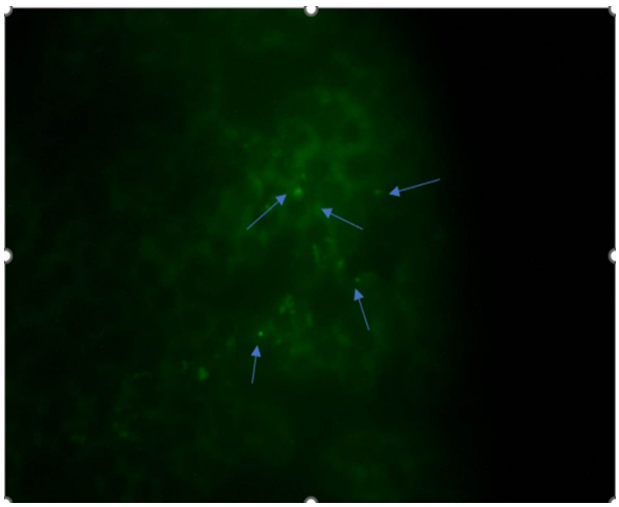

Figure 2. Blood smear showing *M. haemofelis*-like organisms invading the cell membrane of erythrocytes (Fluorescent microscopy; 0.01% acridine orange staining; ×1000)

Table 3. Hematological analysis for horses infected with *M. haemofelis*-like organism and controls

Hematological Parameters	Mean±SE	
	Controls (n=25)	Diseased Horses (n=88)
RBC (×10^6/µL)	9.32±0.4	6.81±1.2*
PCV (%)	33.56±3.64	27.32±6.47*
Hb (g/dL)	13.31±1.76	9.84±1.22*
MCV (fL)	36.±2.51	40.11±4.56*
MCHC (g/dL)	39.66±6.62	36.01±5.82*
TLC (×10^3/µL)	10.11±7.34	14.36±8.11*
Absolute neutrophil count (×10^9/L)	4481±54.81	4427.11±23.41
Absolute lymphocyte count (×10^9/L)	4554±421.2 2	8818±822.31*
Absolute monocyte count (×10^9/L)	563±215	581±361
Absolute eosinophil count (×10^9/L)	381±47	392±51
Absolute basophil count (×10^9/L)	82±77	81±27

*P<0.05.

The first cytologic diagnosis of the causative agent was made via blood smear. The Giemsa stain technique revealed that *M. haemofelis*-like organisms appeared as single small coccoid or rod-shaped organisms, present in short or long chains on the RBC wall. Similar results were also indicated by other researchers (Urquhart et al., 2003; Hampel et al., 2014; Al Matwari et al., 2022; Ballados- González, 2025). Moreover, the confirmatory diagnosis of the causative Hemomycoplasma was done using fluorescent microscopy to detect the organism stained with acridine orange. This technique, used for detecting microorganisms in clinical specimens, analyzes the cell cycle, whereby the stain interacts with nucleic acids, causing them to fluoresce (Ciancaglini et al., 2004).

On the other hand, quantitative real-time technique confirmed the diagnosis of infected organisms, which has also been used in other research (Dieckmann et al., 2012; Al Matwari et al., 2022; Ballados-González et al., 2025). The PCR results exhibit higher sensitivity and specificity than serological and cytological investigation for the detection of *H. mycoplasma* infections. Moreover, it amplifies specific DNA sequences of the mycoplasma, offering high sensitivity and specificity, and can also identify the specific species of *H. mycoplasma* (Kahn et al., 2010).

Table 4. Clotting factor indices in horses infected with *M. haemofelis*-like organism and controls

Parameter	Mean±SE	
	Controls (n=25)	Diseased Horses (n=88)
Total thrombocyte counts (×10^3)	546.8±17.3	259.6±34.2*
Mean thrombocyte volume (fL)	9.22±0.8	13.6±3.8*
Thrombocyte distribution with (%)	15.2±0.71	20.3±1.8*
Prothrombin time (sec)	2.8±0.6	6.81±2.4*
Activated partial thromboplastin time (sec)	11.2±0.31	24.8±3.4*
Fibrinogen time (sec)	51.2±0.6	39.4±5.2*

*P<0.05.

Table 5. Blood gas analysis and the acid-base balance of the horses infected with *M. haemofelis*-like organism and controls

Parameter	Mean±SE	
	Controls (n=25)	Diseased Horses (n=88)
pH	7.22±0.12	7.12±3.33*
PCO_2 (mm/Hg)	41.72±1.23	38.45±3.51*
Bicarbonate (mEq/L)	25.13±1.45	21.23±3.23*
PO_2 (mm/Hg)	149.43±2.55	149.61±7.72
Base excess (mEq/L)	4.31±1.11	-5.43±0.4*
Blood oxygen saturation (SO_2) (%)	91	82*
Anion gap (mEq/L)	8.16±1.72	13.78±3.54*
Sodium (mEq/L)	135±2.18	136±9.23
Potassium (mEq/L)	4.34±0.42	4.51±0.61
Chloride (mEq/L)	103.33±1.12	102.21±5.33

*P<0.05.

Horses infected with *M. haemofelis*-like organisms exhibited a macrocytic hypochromic anemia, characterized by significantly lower values of total RBCs (TRBs), Hb, and PCV. These findings are consistent with those of other researchers (Dieckmann et al., 2012; Jarad & Alsaad, 2016; Abed & Alsaad, 2017), who reported that the hemolytic type of anemia resulting from such infections could induce extravascular hemolysis, erythrocyte clumping, and ultimately lead to regenerative anemia, as indicated by the presence of immature RBCs. However, other scientific opinions suggest that additional causes of anemia in animals infected with blood parasites may include a shortened lifespan of erythrocytes and decreased activity of the hemopoietic system. Furthermore, other researchers (Alsaad et al., 2010; Sudan et al., 2012; Jamwal et al., 2020; Ballados-González et al., 2025) indicated that anemia in diseased horses with blood parasite infections was also worsened by phagocytosis resulting from the development of anti-erythrocyte autoantibodies. Regarding the leukocyte profile of diseased horses compared to healthy controls, the results show significant leukocytosis with lymphocytosis. This

Table 6. Biochemical analysis of horses infected with *M. haemofelis*-like organism and controls

Biochemical Elements	Mean±SE	
	Controls (n=25)	Diseased Horses (n=88)
AST (U/L)	226.4±6.17	423.2±23.44*
GGT (U/L)	23.41±2.45	76.34±8.67*
ALP (IU/L)	523.33±11.54	724±22.56
Total protein (g/100 mL)	6.51±0.4	5.4±1.87*
Total bilirubin (mg/ dL)	0.63±0.27	0.87±0.18*
Ferritin (ng/mL)	62.13±2.87	39.45±6.93*
Troponin I (ng/mL)	0.21±0.07	18.33±4.8*
Glucose (mg/dL)	92.33±3.22	72.56±10.45*

*P<0.05.

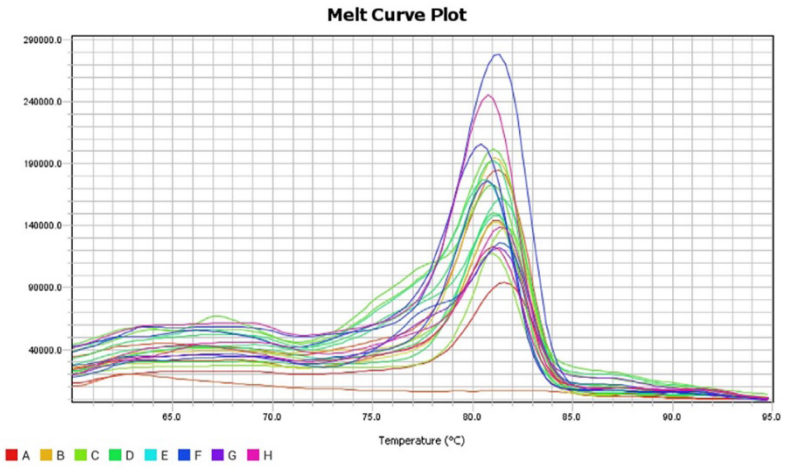

Figure 3. Melting curve for the *16S rRNA* gene of *M. haemofelis*-like organism-positive samples

could be attributed to the stimulation of stem cells and lymphoid tissues in the bone marrow, as well as the ability of *M. haemofelis* to establish persistent infections, which might produce superantigens that stimulate large numbers of lymphocytes (Tagawa et al., 2010; Suzanne et al., 2011; Dieckmann et al., 2012). Conversely Zobba et al. (2008) suggest that lymphocytosis could also result from lymphoid depletion and disorganization, leading to an abnormal lymphocyte response.

Hemomycoplasma spp. have been proven to alter the clotting factor indices of diseased animals (Dieckmann et al., 2012; Jarad & Alsaad, 2016; Al Matwari et al., 2022). The coagulation system mechanism will be changed, leading to the development of disseminated intravascular coagulation syndrome (DIC), which can be supported by differences in blood hemoconcentration and the activation of coagulation activators, accompanied by a reduction in the activities of coagulation inhibitors. Furthermore, hypo- and hyper-coagulation statuses are the prevalent conditions always associated with DIC, which mostly depend on the platelet aggregation state and the stage of anemia (Bick, 2003; Zobba et al., 2008; Hou et al., 2015; Jarad & Alsaad, 2016). Accordingly, petechial hemorrhages indicated on the mucous membranes of diseased horses and the internal organs of the carcass could reflect thrombocytopenia and hypofibrinogenemia resulting from the release of endogenous mediators, like platelet-activating factor in inflammatory disorders. Moreover, it is important to note that any bleeding process that occurs in the body is followed by a clotting mechanism due to the stimulation and activation of the existing clotting factors, which are activated by the influence of special chemical factors (Pantanowitz, 2003; Dieckmann et al., 2012; Constabile et al., 2017).

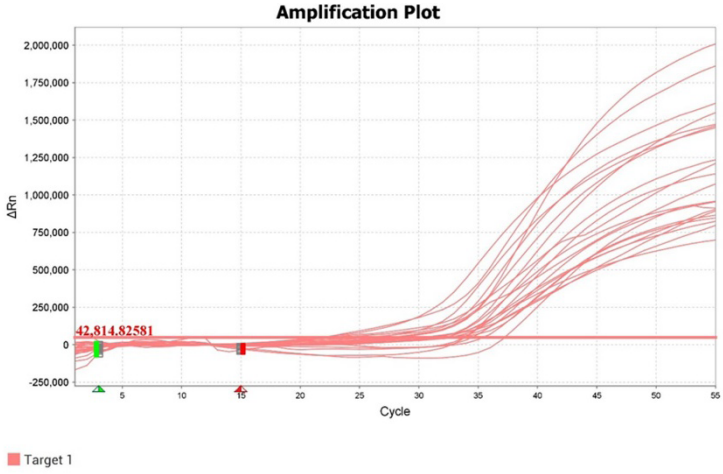

Figure 4. Amplification plot for the *16S rRNA* gene of *M. haemofelis*-like organism-positive samples

It has been shown that blood parasitic infection might affect the acid-base and blood gas balance in diseased animals. Consequently, it has been proven that acid-base balance is crucial for maintaining the optimal potential of hydrogen (pH) range, which is necessary for different enzyme systems to function ideally in the body. Therefore, the results of the current study indicate that diseased horses infected with *M. haemofelis*-like organisms suffered from acidemia (a decrease in pH values) due to an increase in hydrogen ion concentration (Ayers & Warrington, 2008).

When systemic acidosis occurs (as indicated in the present study), it represents an abnormal state characterized by an increased concentration of hydrogen ions, which decreases the pH. This condition can be of respiratory or metabolic origin, reflecting the acid-base level disturbance that always needs compensatory responses. The increased CO_2 level is expressed as PCO_2, which reflects the occurrence of respiratory acidosis. On the contrary, In contrast, a decrease in HCO_3 is referred to as metabolic acidosis, as both conditions may alter the buffer equation and shift it to the right, resulting in increased hydrogen ions and decreased pH (Johnson, 1995). The same results were also mentioned in another research (Leisewitz et al., 2001).

There are two types of metabolic acidosis: the sectional and the titrational types. Both are induced by the loss of bicarbonate fluid, which might be enhanced due to excessive salivation and diarrhea, reflecting the presence of non-CO_2 acids that titrate bicarbonate and cause decreased HCO_3 (Ayers & Warrington, 2008). Moreover, it was shown that hemolytic anemia, as indicated in the present study, leads to decreased blood perfusion, causing severe hypoxia and producing an anaerobic metabolic state, which increases the accumulation of lactic acid and results in harmful hyperlactatemia that may be fatal, whereby hydrogen ions increase as lactate production accumulates (Leisewitz et al., 2001). On the other hand, a depressed (negative) base excess may also be observed in metabolic acidosis, since base excess reflects the amount of acid that must be added to balance the acidity (pH) and return it to its normal levels (Johnson, 1995).

It was documented that the anion gap increases in all cases of titrational metabolic acidosis (as indicated in this study). This type of metabolic acidosis is associated with high levels of endogenous and/or exogenous acids; when bicarbonate is consumed to buffer these organic acids, the chloride level is not affected. Furthermore, an increased anion gap confirms the occurrence of metabolic acidosis (Lewis, 2025).

The partial pressure of carbon dioxide refers to the amount of the gas that is dissolved in the blood. However, it reflects the independent quantities of the acid-base balance that are lowered in metabolic acidosis and hypoxia (Johnson, 1995).

The results of the present study indicated significant differences in certain biochemical changes in diseased horses compared to healthy controls. The levels of AST, GGT, ALP, total bilirubin, and troponin I were increased, while total protein, ferritin, and glucose levels were significantly decreased in infected horses. Similar findings have been observed and documented in other research (Dieckmann et al., 2012; Al Matwari et al., 2022; Ballados-González et al., 2025).

When those researchers demonstrated that damage to skeletal or heart muscle fibers, along with harmful effects on the liver, such as cholestatic liver disease and hepatocyte damage, and RBCs, is followed by a noticeable rise in AST, GGT, and ALP levels, this was attributed to the abundance of these enzymes in such tissues, where they also serve as indicators of pathological conditions (Kaneko, 2014). Furthermore indirect hyperbilirubinemia detected in blood parasitic infections reflects excessive destruction of erythrocytes by the causative organisms (Barrelet & Ricketts, 2002; Constable et al., 2017; Ballados-González et al., 2025). Moreover, it has been proven that troponins I and T are considered the gold standard for diagnosing myocardial injury in both animals and humans due to their high specificity and sensitivity to cardiac tissues. As these proteins are responsible for maintaining calcium-mediated interactions between myosin and actin, a significant increase in troponin I in the current study might indicate myocardial damage, serving as one of the earliest biochemical markers of such injury since it is tissue-specific to the heart.

Serum ferritin in horses is thought to be the best indicator of body iron stores (Abeni et al., 2013), with a specific correlation between ferritin, anemia, and hypoglycemia. The lower values of ferritin detected in the present work might indicate depressed iron levels, iron deficiency anemia, inadequate dietary iron or absorption, excessive blood loss as in hemolytic anemia, and the hypochromic type of anemia (Barrelet & Ricketts, 2002; Asgari & Amniattalab, 2023).

Decreased protein levels (hypoproteinemia), which are detected in horses infected with *M. haemofelis*-like organisms, could reflect digestive disturbances and starvation affecting diseased horses, as evidenced by partial or complete loss of appetite. Additionally, malabsorption

and high fever could also play a significant role. Also, abundant protein may be destroyed when macrophages are activated in the liver and spleen, enhancing the secretion of tumor necrosis factor (TNF-alpha) (Alsaad et al., 2010; Abeni et al., 2013; Burlikowska et al., 2015).

Hypoglycemia with low glucose levels observed in diseased horses in the current study could be caused by decreased food intake, hepatic and gastrointestinal disorders impairing glucose production, glycogenolysis, or gluconeogenesis, as well as increased glucose utilization (glucose depletion) (Ollis et al., 2007; Aleman et al., 2018; Nouri et al., 2021). Consequently, these animals may exhibit poor performance, weakness, lethargy, and even collapse in severe cases.

Conclusion

Equine *Hemomycoplasma* spp. infection is present in the Iraqi environment in all domestic animals. Therefore, early diagnosis and implementation of control measures are strongly recommended.

Ethical Considerations

Compliance with ethical guidelines

The Animal Ethics Committee granted final approval and permission to conduct this scientific research, as stated in the official document issued by the College of Veterinary Medicine, University of Basrah, Iraq (Code: No. VET 93/37/2025).

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Authors' contributions

Data collection, experiments, and investigation: Ali Jarad; Statistical analysis and writing: Kamal Alsaad; Final approval: All authors.

Conflict of interest

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References

Abed, F. A., & Alsaad, K. M. (2017). Clinical, hematological and diagnostic studies of hemomycoplasma infection (Mycoplasma Ovis) in sheep of Basrah governorate. *Basrah Journal of Veterinary Science, 16*(2), 284-301. [DOI:10.33762/bvetr.2017.143551]

Abeni, F., Prà, A. D., Bertin, G., & Calamari, L. (2013). Serum protein fraction in mature horses and relationship with metabolic and hematological parameters. *Journal of Equine Veterinary Science, 33*(11), 905-911. [DOI:10.1016/j.jevs.2013.01.006]

Akbari, H., Basaki, M., Imani Baran, A., & Akbarzadeh, Z. (2024). Molecular study of Anaplasma spp. in horses, sheep, and goats with phylogenetic analysis in northwest Iran. *Archives of Razi Institute, 79*(2), 327–334. [DOI:10.32592/ari.2024.79.2.327] [PMID]

Al Matwari, E. H., Ahmed, J. A., & Al Saad, K. M. (2022). Hemomycoplasmosis (Eperythrozoonosis) in domestic animals (a review). *International Organization of Scientific Research 15*(7), 14-19. [DOI:10.9790/2380-1507011419]

Aleman, M., Costa, L. R. R., Crowe, C., & Kass, P. H. (2018). Presumed Neuroglycopenia Caused by Severe Hypoglycemia in Horses. *Journal of Veterinary Internal Medicine, 32*(5), 1731–1739. [DOI:10.1111/jvim.15245] [PMID]

Alsaad, K. M., Alsaad, E. A., & Al-Derawie, H. A. (2010). Clinical and diagnostic study of equine babesiosis in drought horses in some areas of Basrah Province. *Research Journal of Animal Science, 4*(1), 16-22. [DOI:10.3923/rjnasci.2010.16.22]

Al-Shamo, S., & Esmaeel, S. A. (2025). Prevalence of hemoplasmosis in sheep in Mosul city, Iraqi. *Iraqi Journal of Veterinary Science, 39* (3), 475-481. [DOI:10.33899/ijvs.2025.157058.4106]

Asgari, P., & Amniattalab, A. (2023). Immunohistochemical assessment of GDNF and chromogranin a expression in erosive and granulomatous lesions in glandular region of equine stomach. *Archives of Razi Institute, 78*(4), 1365–1377. [DOI:10.32592/ari.2023.78.4.1365] [PMID]

Ayers, P., & Warrington, L. (2008). Diagnosis and treatment of simple acid-base disorders. *Nutrition in Clinical Practice, 23*(2), 122-127.[DOI:10.1177/0884533608314534]

Ballados-González, G. G., Cruz-Romero, A., Martínez-Hernández, J. M., Aguilar-Domínguez, M., Vieira, R. F. C., & Grostieta, E., et al. (2025). Confirmation of the presence of Hemotropic Mycoplasma species in working equids from Veracruz, Mexico. *Tropical Animal Health and Production, 57*(5), 225. [DOI:10.1007/s11250-025-04465-w] [PMID]

Barker, E. N., Tasker, S., Day, M. J., Warman, S. M., Woolley, K., & Birtles, R., et al. (2010). Development and use of real-time PCR to detect and quantify Mycoplasma haemocanis and "Candidatus Mycoplasma haematoparvum" in dogs. *Veterinary Microbiology, 140*(1-2), 167–170. [DOI:10.1016/j.vetmic.2009.07.006] [PMID]

Barrelet, A., & Ricketts, S. (2002). Haematology and blood biochemistry in the horse: A guide to interpretation. *In Practice, 24*(6), 318-327. [DOI:10.1136/inpract.24.6.318]

Bick, R. L. (2003). Disseminated intravascular coagulation current concepts of etiology, pathophysiology, diagnosis, and treatment. *Hematology/Oncology Clinics of North America, 17*(1), 149–176. [DOI:10.1016/s0889-8588(02)00102-8] [PMID]

Burlikowska, K., Bogusławska-Tryk, M., Szymeczko, R., & Piotrowska, A. (2015). Haematological and biochemical blood parameters in horses used for sport and recreation. *Journal of Central European Agriculture, 16*(4), 370-382. [DOI:10.5513/JCEA01/16.4.1634]

Ciancaglini, E., Fazii, P., & Sforza, G. R. (2004). The use of a differential fluorescent staining method to detect bacteriuria. *Clinical Laboratory, 50*(11-12), 685–688. [PMID]

Constable, P. D., Hinchcliff, K. W., Done, S. H., & Grünberg, W. (2017). *Veterinary medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats*. Amsterdam: Elsevier. [Link]

Dieckmann, S. M., Hoelzle, K., Dieckmann, M. P., Straube, I., Hofmann-Lehmann, R., & Hoelzle, L. E. (2012). Occurrence of hemotrophic mycoplasmas in horses with correlation to hematological findings. *Veterinary Microbiology, 160*(1-2), 43–52. [DOI:10.1016/j.vetmic.2012.05.016] [PMID]

Esmaeel, S. A., & Albadrani, B. (2019). Prevalence and some risk factors of bovine heamotropic mycoplasma in Nineveh province-Iraq. *Iraqi Journal of Veterinary Science, 33*(2), 427-431. [DOI:10.33899/ijvs.2019.163170]

Hampel, J. A., Spath, S. N., Bergin, I. L., Lim, A., Bolin, S. R., & Dyson, M. C. (2014). Prevalence and diagnosis of hemotrophic mycoplasma infection in research sheep and its effects on hematology variables and erythrocyte membrane fragility. *Comparative Medicine, 64*(6), 478–485. [PMID]

Hasan, M. H. (2012). Diagnosis of some blood parasites in cattle and sheep in Mosul, Iraq. Iraqi Journal of Veterinary Science. 26. 57-61.

Hornok, S., Földvári, G., Elek, V., Naranjo, V., Farkas, R., & de la Fuente, J. (2008). Molecular identification of Anaplasma marginale and rickettsial endosymbionts in blood-sucking flies (Diptera: Tabanidae, Muscidae) and hard ticks (Acari: Ixodidae). *Veterinary Parasitology, 154*(3-4), 354-359. [DOI:10.1016/j.vetpar.2008.03.019]

Hou, Y., Carrim, N., Wang, Y., Gallant, R. C., Marshall, A., & Ni, H. (2015). Platelets in hemostasis and thrombosis: Novel mechanisms of fibrinogen-independent platelet aggregation and fibronectin-mediated protein wave of hemostasis. *Journal of Biomedical Research, 29*(6), 437–444. [DOI:10.7555/jbr.29.20150121] [PMID]

Jamwal, M., Sharma, P., & Das, R. (2020). Laboratory approach to hemolytic anemia. *Indian Journal of Pediatrics, 87*(1), 66–74. [DOI:10.1007/s12098-019-03119-8] [PMID]

Jarad, A., & Alsaad, K. M. (2016). Clinical, hematological, and diagnostic studies of Mycoplasma Wenyonii infection in cattle of Basrah governorate, Basrah, Iraq. *Basrah Journal ofVeterinary. Research,15*(4), 37-53. [Link]

Johnson P. J. (1995). Electrolyte and acid-base disturbances in the horse. The veterinary clinics of North America. *Equine Practice, 11*(3), 491–514. [DOI:10.1016/s0749-0739(17)30312-7] [PMID]

Kahn, C. M., Allen, D. G., Constable, P. D., Quesenberry, K. E, Reeves, P. T., & Sharma, J. M., et al. (2010). *The Merck Veterinary Manual*. New Jersey: Merck & Co., Inc; 2010. [Link]

Kalantari, M., Sharifiyazdi, H., Ghane, M., & Nazifi, S. (2020). The occurrence of hemotropic Mycoplasma ovis-like species in horses. *Preventive Veterinary Medicine, 175,* 104877. [DOI:10.1016/j.prevetmed.2019.104877]

Kaneko, J. J. (2014). *Clinical Biochemistry of Domestic Animals*. Massachusett: Academic Press. [Link]

Yokoi, K. (2002). Colorimetric determination of chloride in biological samples by using mercuric nitrate and diphenylcarbazone. *Biological Trace Element Research, 85*(1), 87–9. [DOI:10.1385/bter:85:1:87] [PMID]

Leech, N., Barrett, K., & Morgan, G. A. (2015). *SPSS for intermediate statistics: Use and interpretation*. New York: Routledge. [DOI:10.4324/9781410616739]

Leisewitz, A. L., Jacobson, L. S., de Morais, H. S., & Reyers, F. (2001). The mixed acid-base disturbances of severe canine babesiosis. *Journal of Veterinary Internal Medicine, 15*(5), 445–452. [DOI:10.1892/0891-6640(2001)015%3C0445:tmados%3E2.3.co;2] [PMID]

Lewis, J. L. (2025). *Overview of Acid-Base Balance*. Rahway: MSD. [Link]

Maggi, R. G., Compton, S. M., Trull, C. L., Mascarelli, P. E., Mozayeni, B. R., & Breitschwerdt, E. B. (2013). Infection with hemotropic Mycoplasma species in patients with or without extensive arthropod or animal contact. *Journal of Clinical Microbiology, 51*(10), 3237–3241. [DOI:10.1128/jcm.01125-13] [PMID]

Mandal, S. C. (2025). *Textbook of Veterinary Parasitology*. Berlin: Springer. [Link]

Messick, J. B. (2004). Hemotrophic mycoplasmas (hemoplasmas): A review and new insights into pathogenic potential. *Veterinary Clinical Pathology, 33*(1), 2–13. [DOI:10.1111/j.1939-165x.2004.tb00342.x] [PMID]

Neimark, H., Johansson, K. E., Rikihisa, Y., & Tully, J. G. (2001). Proposal to transfer some members of the genera Haemobartonella and Eperythrozoon to the genus Mycoplasma with descriptions of 'Candidatus Mycoplasma haemofelis', 'Candidatus Mycoplasma haemomuris', 'Candidatus Mycoplasma haemosuis' and 'Candidatus Mycoplasma wenyonii'. *International Journal of Systematic and Evolutionary Microbiology, 51*(Pt 3), 891–899. [DOI:10.1099/00207713-51-3-891] [PMID]

Nouri, A., Bashashati, M., Mirzaie, S. G., Shoshtari, A., & Banani, M. (2021). Isolation, identification and antimicrobial susceptibility of Avibacterium Paragallinarum from backyard chicken in retail markets of Karaj and Tehran cities, Iran. *Archives of Razi Institute, 76*(4), 1047–1053. [DOI:10.22092/ari.2020.343173.1502] [PMID]

Hollis, A. R., Boston, R. C., & Corley, K. T. (2007). Blood glucose in horses with acute abdominal disease. *Journal of Veterinary Internal Medicine, 21*(5), 1099–1103. [DOI:10.1892/0891-6640(2007)21[1099:bgihwa]2.0.co;2] [PMID]

Pantanowitz, L. (2003). Mechanism of thrombocytopenia in tick born diseases. *International Journal of Infectious Diseases, 2,* 1-7. [Link]

Peters, I. R., Helps, C. R., McAuliffe, L., Neimark, H., Lappin, M. R., & Gruffydd-Jones, T. J., et al. (2008). RNase P RNA gene (rnpB) phylogeny of Hemoplasmas and other Mycoplasma species. *Journal of Clinical Microbiology, 46*(5), 1873–1877. [DOI:10.1128/jcm.01859-07] [PMID]

Saki, C. E. (2009). Clinical Eperythrozoon wenyoni (Adler and Ellenbogan,1934) and Heamobartonella bovis (Donatin) and Lestoquard,1934) infection in a cattle. *Fırat Üniversitesi Sağlık Bilimleri Veteriner Dergisi, 23*(2), 117-118. [Link]

Shiroshita, Y., Tanaka, R., Shibazaki, A., & Yamane, Y. (1999). Accuracy of a portable blood gas analyzer incorporating optodes for canine blood. *Journal of Veterinary Internal Medicine, 13*(6), 597–600. [DOI:10.1892/0891-6640(1999)013%3C0597:aoapbg%3E2.3.co;2] [PMID]

Stockham, S. L., & Scott, M. A. (2025). *Fundamentals of Veterinary Clinical Pathology*. New Jersey: John Wiley & Sons. [Link]

Vikrant Sudan, Sharma, R. L., Gupta, S. R., Borah, M. K., & Mishra, R. (2012). An occurrence of clinical eperythrozoonosis in a German Shepherd dog and its therapeutic management. *Journal of Parasitic Diseases: Official Organ of the Indian Society for Parasitology, 36*(2), 181–183. [DOI:10.1007/s12639-012-0100-9] [PMID]

Genova, S. G., Streeter, R. N., Velguth, K. E., Snider, T. A., Kocan, K. M., & Simpson, K. M. (2011). Severe anemia associated with Mycoplasma wenyonii infection in a mature cow. The *Canadian Veterinary Journal, 52*(9), 1018–1021. [PMID]

Tagawa, M., Matsumoto, K., Yokoyama, N., & Inokuma, H. (2010). Comparison of the effect of two hemoplasma species on hematological parameters in cattle. *The Journal of Veterinary Medical Science, 72*(1), 113–115. [DOI:10.1292/jvms.09-0304] [PMID]

Urquhart, G. M., Armour, J., Duncan, J. L., Dunn, A. M., & Jennings, F. W. (2003). *Veterinary parasitology*. Massachusetts: Blackwell Science Ltd. [Link]

Willi, B., Novacco, M., Meli, M., Wolf-Jäckel, G., Boretti, F., & Wengi, N., et al. (2010). Haemotropic mycoplasmas of cats and dogs: Transmission, diagnosis, prevalence and importance in Europe. *Schweizer Archiv fur Tierheilkunde, 152*(5), 237–244. [DOI:10.1024/0036-7281/a000055] [PMID]

Zobba, R., Ardu, M., Niccolini, S., Chessa, B., Manna, L., & Cocco, R., et al. (2008). Clinical and laboratory findings in equine piroplasmosis. *Journal of Equine Veterinary Science, 28*(5), 301-308. [DOI:10.1016/j.jevs.2008.03.005]