

Acute Babesiosis in Foals

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Abstract: The objective of this study was to investigate the clinical, haematological and some biochemical changes in natural infected foals with babesiosis. A total of 105 local foals breed 1-8 months old from both sexes were investigated, 90 local foals breed were naturally infected with *Babesia equi* and *Babesia caballi*, 15 clinically normal local foals breed served as control. The results indicated that the clinically infected foals showed signs of congested mucus membranes with petechial hemorrhages on the conjunctivae, labored or rapid respiration, unable to suck, loss of appetite, colicky sings, diarrhea and/or constipation, rough hair coat, paleness and/or icteric mucus membranes and lacrimation. However, some diseased foals were suffering from unable to rise, inco-ordination muscles tremors and hind limbs paralysis. Ticks were detected on different regions of the body. High body temperature, respiratory and heart rates were also recorded in addition increase capillary refilling time were also noticed. The statistical analysis appeared significant decrease in the TRBCs, HB and PCV, while a significant increase in MCV and ESR were encountered in infected foals. Macrocytic hypochromic type of anemia was registered and the percentage of parasitemia ranged between (8-33%). Statistically significant decrease were encountered in platelets count and fibrinogen and a statistically significant increase were encountered in clotting time, prothrombine time and activated partial thromboplastine time in diseased foals. The results also, indicated a significant increase in WBC as a result of significant increase in lymphocytes. The biochemical changes revealed significant decrease in total protein and calcium. Results of c-ELISA showed that (81.11%) and (18.88%) of diseased foals were positive for *B. equi* and *B. caballi*, respectively.

Key words: Babesiosis, foals, hematological finding, c-ELISA, *B. equi*, *B. caballi*

INTRODUCTION

Equine babesiosis is an acute, subacute, or chronic infectious hemolytic disease caused by the intraerythrocytic protozoa (Radostitis *et al.*, 2000). In foals, like in horses, it caused by *Babesia equi* and *Babesia caballi* (Solusby, 1986). The disease is also known as equine piroplasmosis and biliary fever. Endemic in most tropical and subtropical regions of the world, this infection has been documented in horses, mules, donkeys and zebras (Bruning, 1996; Smith, 1996). The occurrence of equine babesiosis has been tied closely with the geographic distribution and seasonal activity of its biological vectors, species of ticks in the genus *dermacentor*, *rhhipicephalus* and *hyalomma* (Dewaai, 1992).

The disease characterized by fever, inappetence, malaise, anemia, jaundice and sudden death (Seifi *et al.*, 2000; Uilenberg, 2006) and are mostly distributed in all parts of Iraq (Al-Saad and Al-Mola, 2006).

Studies of babesiosis in foals are very scarce and little information had been provided in Iraq. Therefore, the objective of the present research was done to study the clinical, hematological and some biochemical changes as

well as the effect of babesiosis on blood clotting indices, c-ELISA were used to confirm the diagnosis, in foals naturally infected with babesiosis in Mosul, Mosul-Iraq.

MATERIALS AND METHODS

Animals and study design: The study were conducted on 105 local foals breed (male and female), 1-8 months old. The study was carried out in Mosul (Mosul-Iraq). Ninety local foals breed were naturally infected with *Babesia* sp. (*B. equi* and/or *B. caballi*) and 15 clinically normal foals served as control group. Careful clinical examination had been carried out in all animals and fecal samples were screened for parasitic load using standard technique.

Blood collection and hematology: Ten milliliter of blood were drained from each animal by jugular vein-puncture, from these 2.5 mL of blood mixed with EDTA used to determine Total erythrocyte count (TRBCs), Hemoglobin concentration (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC), Platelets count (Plt), Mean Platelets Volume (MPV), Platelets Distribution

Width (PDW), total and differential leukocytes count (Automatic full digital cell counter, Beckman, USA) and Erythrocytes Sedimentation Rate (ESR) by westergren method (Meyer and Harvey, 1998), another 2.5 mL of blood mixed with Trisodium citrate (used plasma) were used to determine Prothrombine time (Prt) and Activated partial thromboplastine time (Aptt) (Coles, 1986). Clotting Time (CT) was also estimated according to Bush (1975).

Infection with *Babesia* sp. was diagnosed on the basis of Giemsa staining blood smears and was confirmed by competitive ELISA test (c-ELISA test) VMRD, Inc, Pullman, WA 99163 USA.

Blood serum samples were tested spectrophotometrically for total protein, calcium and fibrinogen, using available kits (Biomere, France).

Statistical analysis: Statistical analysis were done using two way analysis of variance and t-test (Stell and Torrie, 1985).

RESULTS

Clinically infected foals showed signs of congested mucus membranes with petechial hemorrhages on the conjunctivae, labored or rapid respiration, unable to suck, loss of appetite, colicky signs, diarrhea with passing of watery fecal materials and/or constipation with dry feces covered some times with mucus, rough hair coat, paleness and/or icteric mucus membranes and lacrimation. However, some diseased foals were suffering from nervous signs manifested by unable to rise, incoordination muscles tremors and hind limbs paralysis. Ticks were detected on different regions of the body (Table 1).

Statistically significant increase ($p < 0.01$) were encountered in body temperature, respiratory and heart rates. However, capillary refilling time was also increased significantly (Table 2).

Babesia sp. appears in different shapes inside the RBCs, whereas maltase cross and double pears shape are prominent as well as, oval, anaplasmoised, spherical, single pear and rod shape were also seen in stained blood films of infected foals (Fig. 1 and 2).

With respect to hemogram there was a significant decrease ($p < 0.01$) in TRBCs, Hb and PCV, reflecting macrocytic hypochromic type of anemia, significant increase in ESR values were also encountered in diseased foals. Parasitemia ranged between (8-33%) with mean values of (23.82%). Results also indicated significant increase ($p < 0.01$) in total leukocytes count, which were due to significant increase ($p < 0.01$) lymphocytes (Table 3 and 4).

Table 1: Clinical signs of infected foals with babesiosis

Parameters	Frequency	%
Congested mucus membranes with petechial hemorrhages	61	67.00
Labored or rapid respiration	77	85.50
Unable to suck with loss of appetite	56	62.20
Colicky signs	32	35.50
Diarrhea and/or constipation	17	18.80
Rough hair coat	22	24.40
Paleness and/or icteric mucus membranes	31	34.40
Lacrimation	55	61.11
Nervous signs	19	21.11
Presence of ticks on different body regions	36	40.00

Table 2: Body temperature, respiratory and heart rate and capillary refilling time of infected foals and control group

Parameters	Control $\pm$ SE	Infected $\pm$ SE
Body temperature ($^{\circ}$ C)	38.68 $\pm$ 0.64	40.8 $\pm$ 1.4 ***
Respiratory (rate min^{-1})	23.4 $\pm$ 2.11	64.8 $\pm$ 8.39 ***
Heart (rate min^{-1})	56.2 $\pm$ 3.6	89.7 $\pm$ 10.8 ***
Capillary refilling (time min^{-1})	1.31 $\pm$ 0.64	5.73 $\pm$ 1.88 ***

*** $p < 0.01$, Values are mean $\pm$ standard error of mean

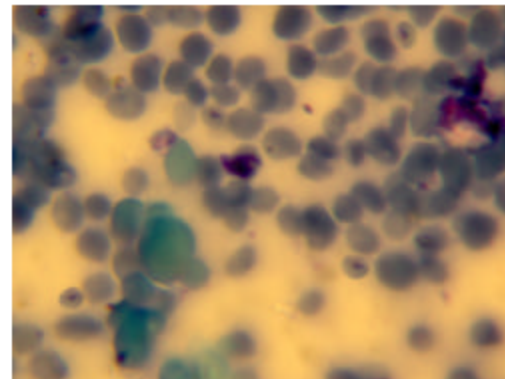


Fig. 1: *B. caballi*, double pear shape

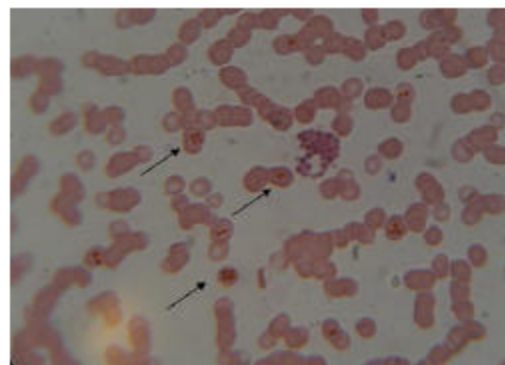


Fig. 2: *B. equi*, maltase cross

Changes of blood clotting indices were also noticed in infected foals compared with control animals and the results showed significant decrease ($p < 0.01$) in the mean values of total platelets count and fibrinogen, whereas significant increase ($p < 0.01$) were encountered in platelets

Table 3: Blood parameters of infected foals with babesiosis and control group

Parameters	Control±SE	Infected±SE
RBC $\times 10^6$	7.78±1.36	4.82±1.82**
HB (g dL ⁻¹)	12.7±2.43	7.41±1.56**
PCV (%)	31.4±4.58	25.3±3.76**
MCV (fl)	40.3±4.44	52.48±3.32**
MCHC (dL)	40.4±7.81	29.28±6.11**
ESR (mm/8h)	25.23±2.54	61.65±5.77**

Table 4: Total and absolute differential leukocytes count in foals infected with babesiosis and control group

Parameters	Control	Infected
TLC $\times 10^3$	9.765±2.662	14.628±3.881**
Lymphocytes	4362±431.326	8922±868.724**
Neutrophils	4322±532.322	4515.563±675.531
Monocytes	555±325	567±257
Eosinophiles	396±22	399±32
Basophiles	83±72	84±79

Table 5: Indices of clotting factors in foals infected with babesiosis and control group

Parameters	Control group	Infected group
Plt $\times 10^3$	589.253±80.764	332.273±63.881**
MPV (fl)	9.337±3.263	14.357±3.887**
PDW (%)	16.7544±2.825	22.882±3.792**
Fibrinogen (mg/100 mL)	346.546±54.346	233.593±74.883**
Ct (min)	2.624±1.653	5.213±1.824**
Prt (sec)	12.325±2.549	29.345±3.553**
Aptt (sec)	54.837±6.226	71.944±12.567**

Table 6: Biochemical changes in foals infected with Babesiosis and control group

Parameters	Control	Infected
Total protein (g/100 mL)	6.32±0.549	3.832±0.732**
Calcium (g/100 mL)	12.442±1.663	8.234±1.63**

**p<0.01, Values are mean±standard error of mean

Table 7: Results of c-ELISA serological test of *B. equi* and *B. caballi* of infected foals with babesiosis

No.	c-ELISA	Sero positive (%)	Sero negative (%)	Total
A	<i>Babesia equi</i>	73 (81.11)	17 (18.88)	90
B	<i>Babesia caballi</i>	17 (18.88)	73 (81.11)	90

volume, platelets distribution width, clotting time, pro-thrombin time and activated partial thromboplastin time (Table 5).

Results of biochemical changes indicated significant decrease ($p>0.01$) in total protein values and calcium in infected foals compared with control animals (Table 6).

Ninety serum samples were tested with c-ELISA, for detection of *B. equi* and *B. caballi* antibodies, results showed that 81.11% of foals were positive for *B. equi*, whereas 18.88% were positive for *B. caballi* (Table 7).

DISCUSSION

Naturally infected foals showed different clinical signs of the disease, which were in agreement with others (Erbsloh, 1975; Dewaal, 1992; Abubakr and Fadlalla, 2003). As Petichial hemorrhages detected on conjunctivae refers to hemostasis disturbances, which reflected by increasing clotting time (Smith, 1996). Moreover, Allan *et al.* (1975),

Jain (1993) and Al-Saad and Al-Mola (2006) added that thrombocytopenia and disturbance of other clotting factors indices may enhance distribution of these hemorrhages.

Laboured or rapid respiration, which have been detected in diseased foals, were due to hypoxia (Anemic hypoxia), due to the fact that decrease RBCs count and Hb concentration were affected the oxygen transmitted to body tissues, their for failure of tissues to receive an adequate supply of oxygen will occur and panting of affected animals were detected clinically (Radositis *et al.*, 2000; Kahn, 2005).

Colicky sings were detected in 35.5% of infected foals may occur due to disturbances of intestinal movements either in the form of diarrhea with passing of watery fluids or constipation with dry, mucus covered feces, these sings were also mentioned by Hailat *et al.* (1997).

Paleness of mucus membranes were exhibited the development of anemia and reduction of hemo-globin concentration and total erythrocytes count, was due to destruction and removal of infected erythrocytes by the reticulo-endothelial system (Sellon, 1997). Whereas icteric mucus membranes reflected the progressive anemia and bilirubinemia, developed in diseased foals (Nafie *et al.*, 1981).

Increase body temperature may indicated libration of endogenous pyrogens due to cellular lysis stimulating thermoregulatory centers of the hypo-thalamus (Svendson and Carter, 1984).

Results of hemogram revealed a significant decrease in TRBCs, HB, PCV, reflecting macrocytic hypochromic type of anemia, similar results were recorded by Sellon (1997), Sanjay *et al.* (2008) and Zobba *et al.* (2008). The cause of anemia during blood parasitic infection may be multi-factorial, the direct effect of the parasite to the infected erythrocytes may be incriminated or decrease life span of RBCs and also suppression of hemopoietic system (Dewaal, 1992; Hailat *et al.*, 1997), also (Oladosa and Oll-Feml, 1992), refers to the role of auto-immunity and the anti-erythrocytic auto antibodies enhancing more erythrophago-cytosis and bone marrow depression.

The type of anemia in current study were in agreement with (Nafie *et al.*, 1981; Al-Saad and Al-Mola, 2006) whose stated that macrocytic hypo-chromic type of anemia indicated regenerative form of anemia and the number of reticulocytes will increase in blood stream.

Increase in ESR values were in agreement with (Allen, 1988; Jain, 1993) whose refer to the correlation between the sedimentation of RBCs and intensity of anemia and increase settling of RBCs will tack place when, anemia are more intense.

The increase in WBC in current study were also seen by Al-Saad and Al-Mola (2006) and Zobba *et al.* (2008), which might occur due to stimulation of lymphoid system and bone marrow as immune response against the parasite or their toxins (Omuse, 1987) added that leukocytosis occur as a result to lymphoid depletion and disorganization with massive lymphocytes. Lymphocytosis specially, in equine babesiosis were also reported by Salem *et al.* (1986) whose stated that lympho-cytosis was marked during the formation of antibodies in response to antigen during babesia infection.

Examination of stained blood films in the current research revealed that *Babesia* sp. appears in different shapes inside the RBCs, whereas maltase cross and double pear shape are prominent as well as, oval, anaplasmoisd, spherical, single pear and rod shape were also seen in stained blood films of infected foals and parasitemia ranged between (8-33%), this results were in agreement with those described by Inci (1997) and Butler *et al.* (2005).

Changes in clotting factor indices, which were indicated in current work was also recorded by Al-Saad and AL-Mola (2006) and Zobba *et al.* (2008), as thrompocytopenia, hypofibrinogemia and decrease clotting time of the blood were reflected the petechial hemorrhages seen on mucus membranes. However, the depression of platelets number may occur due to depression of bone marrow activity, spleenomegaly and platelets sequestration, which may occur due to disorganization of hemostatic mechanism enhanced by Disseminating intra-vascular coagulopathy, causing micro thrombosis and infarction of special organs such as brain, lungs and intestine (Allen *et al.*, 1975).

There were significant reduction seen in total protein values in this study, which were agree with (Salem *et al.*, 1986; Hailat *et al.*, 1997; Al-Saad and Al-Mola, 2006), whose stated that decrease protein levels during blood parasitic infection may occur due to digestive disturbances, distruction of proteins due to fever as well as less production from liver. Results were also indicated slight hypo-calcaemia, which might be responsible for muscle tremors and some other sings showed by diseased foals, this were agreed by others (Nafie *et al.*, 1981; Nel *et al.*, 2004). However, Doxey (2006) mention that calcium are responsible for mineralization of bones, contractions of muscles and clotting processes.

Results of c-ELISA revealed (81.11%) of infected foals were serologically positive to *B. equi* and (18.88%) were serologically positive to *B. cabalii*, similar results were also recorded by Shkap *et al.* (1998) and Sevinc *et al.*

(2008) whose stated that c-ELISA may be an alternative for increased and sensitive detection of acute and latent babesial infections. Furthermore, Rhalem *et al.* (2001) added that ELISA using recombinant antigens, which were developed as a more specific method than CFT or IFAT for the serodiagnosis of piroplasmosis, where as (Knowles, 1996; Katz *et al.*, 2000) refers that *B. equi* were more pathogenic than *B. caballi* and more common in endemic countries.

CONCLUSION

Babesiosis were affected foals and exhibited different clinical signs, a significant changes were noticed between the infected and control foals in hematological and biochemical values with differences indicated in blood clotting indices.

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