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Clinical and Diagnostic Study of Equine Babesiosis in Drought Horses in Some Areas of Basrah Province

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Abstract: The objective of this study was to investigate the clinical, haematological, biochemical changes, percentage of hemoparasite and c-Elisa test in naturally infected drought horses with babesiosis. The study were conducted on (115), drought horses (male and female) (2-10) years old. About 90 drought horses were naturally infected with *Babesia* sp. and (25) clinically normal drought horses served as control group. Results indicated that diseased drought horses were affected with acute form of the disease and they exhibited, loss of appetite, sever sweating, congested mucous membranes with Petichial hemorrhages, disinclination to move and in coordination, Pale and/ricteric mucous membranes, edema of fetlock joint, panting, depression, colic with signs of diarrhea and constipation, hemoglobin urea, muscles tremor, coughing, dehydration with rough coat and ticks were detected on different body regions. Statistically significant increase ($p>0.01$) were encountered in body temperature, respiratory and heart rates, however capillary refilling time were also increased significantly in diseased horses. A statistically significant decrease ($p<0.01$) were encountered in the TRBCs, Hb and PCV values of diseased animals, anemia was normocytic normohromic type and a statistically significant increase ($p<0.01$) were also encountered in the sedimentation rate of RBCs in diseased animals. The percentage of hemoparasitism (parasitemia) ranged between (5-23%) with a mean of (18.72%). A statistically significant decrease ($p<0.01$) were encountered in platelets count and fibrinogen and a statistically significant increase ($p<0.01$) were encountered in clotting time, prothrombine time and activated partial thromboplastine time in diseased horses. The results also indicated a significant increase in WBCs as a result of significant increase in lymphocytes. Results of biochemichal tests revealed significant increase in AST, ALT, ALP total bilirubin, BUN, however significant decrease in total protein values, calcium and glucose were encountered in infected horses. Serum samples were tested spectrophotometrically using c-Elisa test to detected *Babesia equi* and *Babesia caballi*, as *Babesia equi* detected in 86.58% and *Babesia caballi* detected in 54.39%, moreover mixed infection were indicated in 16.1% for both species.

Key words: Babesiosis, drought horses, hematological finding, *biochemical changes*, c-Elisa, *B. equi*, *B. caballi*

INTRODUCTION

Equine babesiosis is a tick-borne protozoal disease caused by two intraerythrocytic parasites, *Babesia equi* and *Babesia caballi*, the disease affected horses, mules, donkeys and zebras and may occur in acute, sub acute or chronic form. It characterized by fever, anemia, icterus, hepato and splenomegaly, intravascular hemolysis and petechial hemorrhages of the mucous surfaces, hemoglobinemia and hemo-globinuria (Brooks *et al*., 1996; Radostitis *et al*., 2000). Equine babesiosis has a worldwide distribution, being endemic in most tropical and subtropical areas of the worlds as well as in some temperate climatic zones, once infected horses become carriers of *Babesia* sp. and potential disseminators of the parasite (Dewaal, 1992; Bruning, 1996).

The disease were endemic in Iraq, mostly in all parts and regions and caused economic losses specially in infected draught horses due to high morbidity and mortality rate (Al-Saad and Al-Mola, 2006). Studies of babesiosis in drought horses in Basrah-Iraq are very scarce and little information had been provided. Therefore, the objective of the present research was to study the clinical, hematological and some biochemical

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changes as well as the effect of babesiosis on blood clotting factors indices, c-Elisa were used to confirm the diagnosis in drought horses naturally infected with babesiosis in Basrah, Basrah-Iraq.

MATERIALS AND METHODS

Animals and study design: The study were conducted on (115) drought horses (male and female), 2-10 years old. The study was carried out in Basrah Province (Basrah-Iraq). About 90 animals were naturally infected with *Babesia* sp. (*B. equi* and/or *B. caballi*) and 25 clinically normal drought horses served as control group. Careful clinical examination had been carried out in all animals and fecal samples were screened for parasitic loud using standard technique.

Blood collection and hematological examination: About 10 mL 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), total and absolute differential leukocytes count, (Coles, 1986) and Erythro-cytes 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 AST, ALT, ALP, total bilirubin, Blood Urea Nitrogen (BUN) total protein, calcium, glucose and fibrinogen using available kids (Biomerex, France).

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

RESULTS AND DISCUSSION

Results of clinical sings showed by infected drought horses with babesiosis were loss of appetite 83%, sever sweating 68%, congested mucous membranes with petechial hemorr-hages which showed by 55% of infected horses were detected on conjunctivae and 3rd eyelid.

Table 1: Clinical sings of infected drought horses with babesiosis

Clinical singes	Percentage
Loss of appetite	83
Sever sweating	68
Congested mucous membranes	55
Petichial hemorrhages	55
Disinclination to move and in -coordination	48
Pale and/or Icteric mucous membranes	45
Edema of fetlock jont	37
Panting	35
Depression	33
Colicky sings	27
Hemoglobin urea	24
Presence of ticks on different body regions	23
Muscles tremor	17
Coughing	17
Diarrhea and /or constipation	24
Dehydration with rough coat	4

Table 2: Body temperature, respiratory rate, heart rate, capillary refilling time in infected drought horses with babesosis and control group

Parameters	Control	Infected
Body temperature C°	38.2±0.8760	39.5±1.223**
Respiratory rate min^{-1}	14.23±2.344	45.77±5.625**
Heart rate min^{-1}	29.82±3.224	77.23±6.622**
Capillary refilling time sec^{-1}	1.2±0.3200	4.7±1.324**

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

Diseased animals were also show disincli-nation to move 48% with rigidity of muscles and when do so it will be in coordinated and ataxic pale and or icteric mucus membranes were see in 45% of diseased horses, edema with pits on pressure of fetlock joint were detected in 37% of infected horses which also show sings of panting and depression in 35 and 33%, respectively.

Diseased horses were also suffering from sings of colic 27% in the form of restless, pawing with fore limb and watching its flanks. Hemoglobin-urea with passing of brown-coffee like urine were seen in 24% of infected horses. Ticks were noticed on different body region in 23%. Others non common clinical sings were also showed by diseased horses included, muscles tremor, coughing, diarrhea (with passing of watery fluids), constipation (with passing of dry feces covered some times with mucus), dehydration with rough coat were seen in 17, 17, 24 and 4% of diseased horses, respectively (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 shape inside the RBCs as maltase cross or double pears shape are prominent as well as oval, anaplasmoid, spherical, single pear and rod shape were also seen in stained blood films of infected drought horses (Fig. 1 and 2).

With respect to hemogram there was a significant decrease, ($p<0.01$) in TRBCs, HB and PCV reflecting normocytic normo-chromic type of anemia, significant increase in ESR values were also encountered in diseased

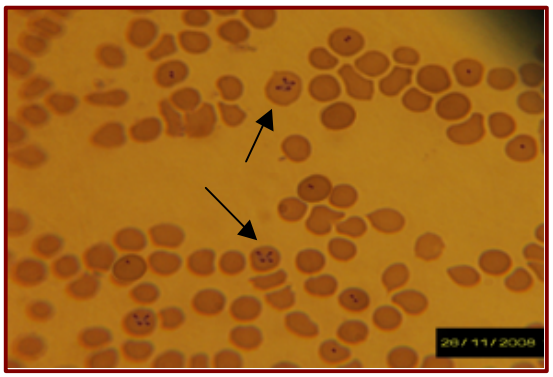

Fig. 1: *B. equi* in the blood smears, (Maltes cross), Giemsa stained 1000x

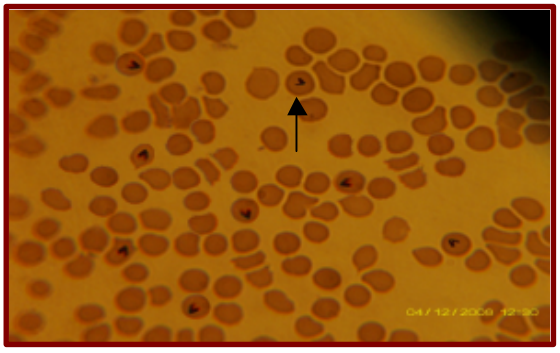

Fig. 2: *Babesia caballi* in the blood smears of horses (Double pear shape), giemsa stained 1000x

horses and parasitemia ranged between (5-23%) with mean values of (18.72%) (Table 3). Results also indicated significant increase ($p<0.01$) in total leukocytes count which were due to significant increase ($p>0.01$) lympho-sites (Table 4). Indices of clotting factors were also changed in infected horses 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 clotting time, prothrombin time and activated thromboplastine time (Table 5).

Results of biochemical changes indicated significant increase ($p<0.01$) in AST, ALT, ALP, total bilirubin and BUN, however significant decrease ($p>0.01$) were encountered in total protein values, calcium and glucose in infected drought horses compared with control animals (Table 6).

Serum samples were tested for c-Eliza for *B.equi* and *B. caballi* antibodies results showed that 78 (86.58%) were positive for *B. equi* whereas 49 (54.39%) were positive for *B. caballi* moreover there were 29 (16.1%) of

Table 3: Blood parameters in infected drought horses with babesiosis and control group

Parameters	Control group (Mean±SE)	Infected group (Mean±SE)
RBC×10^6	9.7±1.223	6.9±0.433**
HB gm dL^{-1}	12.98±2.543	8.88±1.44**
PCV (%)	35.6±2.554	25.62±3.721**
MCV fL^{-1}	36.38±3.224	37.86±5.664
MCHC gm dL^{-1}	36.46±4.221	34.66±2.553
ESR mm 20 min^{-1}	22.63±3.553	83.76±5.321
Parasitemia (%)	-	18.72±4.282**

($p<0.01$), Values are mean±standard error of mean

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

Parameters	Control (Mean±SE)	Infected (Mean±SE)
TLC×10^3	9.664±1.662	14.227±2.881**
Lymphocytes	4452±531.226	8812±768.224**
Nutrophiles	4432±432.5564	4525.563±455.831
Monocytes	534±255	566±277
Eosinophiles	392±22	398±22
Basophiles	82±72	83±69

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

Table 5: Clotting factors indices in drought horses infected with *Babesia* sp. and control group

Parameters	Control group	Infected group
Plt×10^3	566.233±80.764	311.273±63881**
Fibrinogen mg 100 mL^{-1}	374.546±54.336	254.543±74.883**
CT min^{-1}	2.986±1.653	5.473±1.824**
Prt time sec^{-1}	11.823±2.549	28.342±3.553**
Aptt time sec^{-1}	52.437±6.226	72.342±12.567**

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

Table 6: Biochemical changes in drought horses infected with *babesia* sp. and control group

Parameters	Control	Infected
AST IU L^{-1}	212.6±22.433	398.543±43.337**
ALT IU L^{-1}	22.34±5.66	47.36±10.782**
ALP IU L^{-1}	160.54±32.342	279.34±52.432**
BUN mg/100 mL	19.65±4.223	55.54±9.342**
Total bilirubin mg/100 mL	1.26±0.45	3.64±0.54**
Total protein gm/100 mL	6.88±0.874	3.54±0.77**
Calcium gm/100 mL	12.563±1.635	8.776±1.764**
Glucose gm/100 mL	118.3±8.3	85.4±8.6**

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

Table 7: c-Eliza serological optical density test of *B.equi, B.caballi* of infected drought horses with babesiosis

No.	c-Eliza	Seropositive (%)	Seronegative (%)	Total
A	*Babesia equi*	78 (86.58)	12 (13.42)	90
B	*Babesia caballi*	49 (54.39)	41 (45.61)	90
C	Mixed infection	29 (16.10)	151 (83.80)	180

samples give positive results for both *B.equi* and *B. caballi* (Table 7). Clinically infected horses show signs of fever, increase respiratory and heart rates accompanied with loss of appetite, sweating, congested mucous membranes with petechial hemorrhages.

The diseased horses were also disinclination to move and incoordinated if do so on the other hand pale and/or icteric mucus membranes with edema of fetlock joint were noticed when the disease progressed. Diseased horses also suffer from panting with labored respiration, depression, beside the passing of brownish-coffee like

urine, moreover colicky signs have been seen accompanied with diarrhea and/or constipation. Ticks were detected on different body regions less common signs such as muscles tremor, dehydration with rough hair coat were also seen. Those sings were in agreement with those described by Salem *et al.* (1986), Dewaal (1992), Hailat *et al.* (1997), Lewis *et al.* (1999), Radostitis *et al.* (2000) and Zobba *et al.* (2008).

An increase body temperature may indicate the liberation of endogenous pyrogens due to cellular lysis stimulating thermoregulatory centers of the hypo-thalamus (Svendson and Carter, 1984). However, Krause (2002) added that the severity of fever may depend on the severity of causative agent, the stage of the disease, type of lesion and generalized infection.

An increase heart and respiratory rate which have been detected in diseased horses were due to hypoxia (Anemic hypoxia) due to the fact that the decrease RBCs count and Hb concentration affected oxygen transmitted to body tissues, therefore a failure of tissues to receive an adequate supply of oxygen will occur and panting of affected animals were detected clinically (Radostitis *et al*., 2000).

Petichial hemorrhages detected on 3rd eye lid and conjunctivae refer to hemostasis disturbances which were reflected by increasing clotting time, Smith (1996). Moreover, Jain (1993) added that the thrombocytopenia and the disturbance of other clotting factors indices may enhance the distribution of theses hemorrhages. Paleness of mucous membranes indicated anemia, through the destruction and the removal of infected RBCs by reticulo-endothelial macrophages (Hailat *et al*., 1997; Zobba *et al*., 2008) whereas icteric mucus membranes reflected the progressive anemia and bilirubinemia developed in diseased horses (Dewaal, 1992; Al-Saad, 2009).

The accumulation of edematous fluids around fetlock joint may be attributed to the pressure differences of arterial hydrostatic pressure and venous osmotic pressure causing dialysis of fluids from blood vessels which was accumulated in distal parts of the body, moreover hypoprotenemia may also play a good role in the escaping and the accumulation of edematous fluids (Romerrio and Dyson, 1997) however, Smith (1996) refers to detected edematous fluids at abdomen (Ascitis), scrotum and lungs in horses infected with babesiosis.

Hemoglobin urea reflected the intravascular hemolysis of RBCs in infected animals which was also detected by Al-Saad and AL-Mola (2006); Zobba *et al.* (2008) who stated that the destruction of RBCs may occur due to the increase intracellular pressure of the infected cell during the multiplication of the protozoa, beside increase erythrocyte fragility which might occur due to the consumption of the phosphorus component of the cell wall by the protozoa. (Ambawat *et al*., 1999; Kawai *et al*., 1999) have observed important bio-chemical changes in the infected erythrocytes wall with *Babesia* sp. including the disturbance in the accumulation of proteins and fats and decrease sialic acid which may lead to increase RBCs fragility and more distraction will occur. Destruction of RBCs resulting in release of hemoglobin (Hemoglobinemia) which in turn passed through the kidney and discolored the urine to brownish or dark coffee like color (Radostitis *et al*., 2000). Colicky signs were detected in 27.75% of infected horses may occur due to the disturbances of intestinal movements either in the form of diarrhea or constipation, these signs were also mentioned by Dewaal (1992), Hailat *et al.* (1997) and Al-Saad and AL-Mola (2006). However, Radostitis *et al.* (2000) added that the lack of bile salts due to hepatic insufficiency may also increase digestive disturbances, moreover, frequent hemoglobin urea might result in glomerulo-nephrosis and renal damage reflecting colicky signs Zobba *et al.* (2008) on the other hand, Collatos (1997) refers to the microthrombosis which was detected in intestinal capillaries.

Detection of ticks on different body regions of diseased horses refers to the fact that ticks were an important transmitters of *Babesia* sp. this will agree with De Waal (1990). Dehydration appears on diseased horses may occur due to the lack of body fluids resulting in un urea or oligurea increase thirsty and rough hair coat, those signs were also mentioned by Lewis *et al.* (1999).

Examination of stained blood films in the current research revealed that *Babesia* sp. appears in different shape inside the RBCs as 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 horses and parasitemia ranged between (5-23%) with mean of (18.72±4.282), these results were in agreement with those described by Inci (1997).

The results showed decrease RBCs count, Hb and PCV which reflected normocytic normochromic anemia similer results were recorded by Selim *et al.* (1981) and Sellon (1997) whose stated that the cause of anemia during blood parasitic infection may be multifactorial, the direct effect of the parasite to the infected erythrocytes may be incrimi-nated or decrease life span of RBCs and also suppression of hemopoitic system.

Dewaal (1992) and Hailat *et al.* (1997) added that anemia in infected horses with babesiosis is due to extensive erythrocytic hemolysis, moreover Zobba *et al.* (2008) refer to the role of autoimmunity and the

anti-erythrocytic auto antibodies enhancing more erythrophago-cytosis and bone marrow depression. An increase in ESR values was in agreement with (Allen, 1988; Jain, 1993) who refers to the correlation between the sedimentation of RBCs and intensity of anemia and the increase settling of RBCs will take place when anemia are more intense.

The increase in WBC in the current study was also seen by (Al-Saad and AL-Mola, 2006; Zobba *et al*., 2008) which might occur due to the stimulation of lymphoid system and bone marrow as immune response against the parasite or their toxins, Jain (2000) added that leukocytosis occurs as a result to lymphoid depletion and the disorganization with massive lymphocytes. Lymphocytosis specially in equine babesiosis was also reported by (Salem *et al*., 1986) who stated that lympho-cytosis was marked during the formation of antibodies in response to antigen during babesial infection, on the other hand (Purnell, 1981) recorded monocytosis during acute babesiosis caused by *B. equi*, however Selim *et al*. (1981) recorded neutrophilia and shift to left in spleenactomesid animals affected with babesiosis. Changes in clotting factor indices which were indicated in current research were also recorded by (Al-Saad and Al-Mola, 2006; Zobba *et al*., 2008) as thrompocytopenia, hypofibrinogemia and increase clotting time of the blood reflected the petecheial hemorrhages seen on mucus membranes, however the depression of platelets number may occur due to the depression of bone marrow activity, spleenomegaly and platelets sequestration which may occur due to disorganization of hemostatic mechanism enhanced by disseminating intravascular coaggulopathy, causing microthrombosis and infarction of special organs such as brain, lungs and intestine.

The results of biochemical changes indicated significant increase in AST, ALT, ALP, total bilirubin, BUN and decrease level of total protein, those results were also recorded by Salem *et al*. (1986), Al-Saad and Al-Mola (2006) and Zobba *et al*. (2008) who stated that the damage to the skeletal or heart muscles, hepatic tissues and erythrocytes may result in considerable increase in the level of AST ALT and ALP due to the fact that bulk of those tissues throughout the body could be considered as an ample reservoir of enzymes liable to be released and detected during pathological situation (Hailat *et al*., 1997; Mohri and Sardari, 2000) added that hyperbilirubinemia which were seen in equine babesisosis resulting from the excessive destruction of RBCs and the indirect hepatocellular damage.

An increase level of BUN may indicate indirect damage of renal tissue and the presences of globins catabolites librated from hemoglobin lysis by reticulo-endothelial system through the process of erythrophagocytosis, Kohn and Chew (1987). There was significant reduction seen in the total protein values in this study which agreed with (Salem *et al*., 1986; Hailat *et al*., 1997; Al-Saad and Al-Mola, 2006) who stated that decrease protein levels during blood parasitic infection may occur due to the digestive disturbances, destruction of proteins due to fever as well as less production from liver.

The results also indicated slight hypocalcaemia which might be responsible for muscle tremors and some other signs shown by diseased drought horses this agreed by others (Nafie *et al*., 1981; Al-Saad, 2009), however Doxy (2006) mentions that calcium is responsible for the mineralization of bones, contractions of muscles and clotting processes, moreover hypoglycemia were also observed by Nel *et al*. (2004) in sever complicated babesioses which might be attributed to starvation, malabsorbtion and hepatic depletion.

In the current study serum samples were tested spectrophotometrically using c-Elisa test to detect *B. equi* and *B. caballi* as *B. equi* detected in 86.58% and *B. caballi* detected in 54.39%, moreover mixed infection was found in 16.1%, these results were higher than those indicated by Sevinc *et al*. (2008) in Turkey whose recorded the percent rate of infection of 16.21 and 0.43% for *B. equi* and *B. caballi*, respectively however mixed infection were seen in 1.46%. This might be explained that control programs were less applied to ticks in Basrah which plays a good role in the increase of ticks populations and higher infection rate. Results also showed that the infection rate with *B. equi* was more than that of *B. caballi*, these results were also recorded by Katz *et al*. (2000) which may reflect the more prominent acute style of babesiosis affected horses in Basrah, Moreover, Knowles (1996) refers that *B. equi* were more pathogenic than *B. caballi* and more common in endemic countries.

CONCLUSION

Babesiosis were adversely affected drought horses in Basrah province (Basrah-Iraq) and exhibited different clinical signs, a significant changes were noticed between the infected and control animals in hematological and bio-chemical values with differences indicated in indices of clotting factors. It has been shown that c-Eilsa was an important, accurate and easily diagnostic tool for detection of both *Babesia equi* and *Babesia caballi* which could be used as screening test.

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