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EVALUATION OF HEMOGRAM, ACUTE PHASE RESPONSE, ACID BASE BALANCE AND BLOOD GAS ANALYSIS IN NEWBORN FOALS INFECTED WITH BABESIOSIS

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ABSTRACT

The aims of the present work were to evaluate hemogram, acute phase response acid base balance and blood gas analysis in newborn foals infected with Babesiosis. Thirty three newborn foals were infected with Babesia spp. and ten clinically normal foals served as controls. There was a significant reduction in the mean values, TRBc, Hb, PCV in diseased newborn foals infected with Babesiosis and anemia was of Macrocytic hypochromic type. Results also indicated significant increase in total leukocytes count as significant increase in lymphocytes. Infection with Babesia spp. was diagnosed on the basis of Giemsa stained blood smears and was confirmed by competitive c-ELISA test and results showed that out of 33 samples tested (100%) of foals were seropositive for *B. equi*, whereas no antibodies were detected for *B. caballi*. Results were also showed significant decrease in blood pH, Pco_2, Bicarbonate, Base access and Oxygen saturation percent (So_2), However significant increase in Anion gab was found and Titritional metabolic acidosis was indicated. Furthermore significant decrease in haptoglobin and fibrinogen have been encountered in diseased newborns foals. It have been concluded that Babesiosis causes economic loss, therefore all anemic foals born in endemic areas should be screened for Babesia spp.

Key words: Babesiosis, newborn foals, hemogram, Acute phase response, Acid base balance, Blood gas analysis.

INTRODUCTION

Equine babesiosis is an acute, subacute, or chronic infectious disease, globally distributed and responsible for heavy economic losses, caused by the tick-borne protozoan (Radostitis *et al.*, 2007). In foals, as in horses, it caused by *Babesia equi* and *Babesia caballi*, (Solusby, 1986), since susceptibility does not appear to vary with age, (Acici *et al*. 2008). The disease is also known as equine piroplasmosis and endemic in most tropical and subtropical regions of the world, this infection has been documented in horses, mules, donkeys, and zebras, (Bruning, 1996; Smith, 2004). Occurrence of equine pyroplasmosis were always related to distribution and seasonal activity of the biological vectors, Ticks in the genus *Rhipicephalus*, *Dermacentor*, and *Hyalomma* are responsible for transmission , (Battur *et al* . 2001).

The disease characterized by fever, inappetence, anemia, jaundice and some time sudden death, (Seifi *et al.* 2000; Uilenberg, 2006) and are distributed in most regions of Iraq, (Alsaad *et al*. 2010; Alsaad *et al*. 2012).

Intra-uterine transmission of Babesia parasites seems to be rare, and might be accidental, however it was reported in horses infected by *B. equi* and the fetus may be infected with Babesia parasites at any stage furthermore in enzootic areas it may cause abortions whereas evidence of *B. caballi* infection in utero is lack (Allsopp *et al*., 2007; USDA.–APHIS, 2009; Georges *et al*., 2011).

In the present study, infection of newborn foals with babesia species were detected for the first time in Mosul ,Iraq with evaluation of hemogram, acute phase response, acid base balance and blood gas analysis.

MATERIALS AND METHODS

Animals and study design: The study was conducted on 62 local newborn foals (male and female), 1-5 days old. in Mosul , Iraq. Thirty three out of sixty two local newborn foals were infected with Babesia spp. Infection with Babesia spp. was diagnosed on the basis of Giemsa stained blood smears and was confirmed by competitive ELISA test (c-ELISA test) (VMRD, Inc, Pullman, WA 99163 USA). Animals are free from ticks infestation .Ten clinically normal foals served as controls. Routine clinical examination had been carried out in all animals. complete history was obtained upon presentation in the clinic and emphasis was placed on clinical signs observed moreover body temperature, respiratory and heart rate were calculated.

Blood collection and analysis: Blood were drained from each newborn foals by jugular puncture. Blood mixed with EDTA (2.5 mL) were used to determine total erythrocyte count (TRBc), haemoglobin (HB), packed cell volume (PCV), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin concentration (MCHC), total leukocyte counts (TLC) on an automatic full digital cell counter (Beckman, USA). Giemsa-stained blood smears were used for differential leukocyte counts (Weiss

and Wardrop, 2010). Another 2.5 mL of blood mixed with trisodium citrate were used to determine fibrinogen using commercial kits (Biolabo, France). Serum haptoglobin concentrations were analyzed according to (Hiss *et al*. 2004). One mL of blood mixed with heparin were obtained separately from each newborn foal, used to determine Blood pH, Pco_2, Bicarbonate , Po_2, Bass access, Anionic gab, Oxygen saturation percent(So_2), Sodium and potassium (Opti-critical care analyzer /USA) according to (Shiroshita *et al*. 1999). Serum chloride values were estimated according to (Katsuhiko, 2002).

Statistical analysis: The significance of variations between diseased and healthy newborn foals were statistically analyzed using (SPSS) T-test, (Leech *et al.* 2007).

RESULTS

Diseased foals showed sings of paleness and/or icteric mucus membranes with petechial hemorrhages on the conjunctiva, increase body temperature, labored or rapid respiration, colicky sings, unable to suck, rough hair coat, and lacrimation. Moreover, some diseased foals were suffering from muscles tremors, in coordination and hind limbs paralysis.

There were a significant reduction ($P<0.05$) in the mean values of TRBc, Hb and PCV, in diseased newborn foals infected with Babesiosis and anemia was of Macrocytic hypochromic type. Results also indicated significant increase ($p<0.05$) in total leukocytes count and lymphocytes. Table 1.

Table 1. Blood parameters of new born foals infected with Babesiosis and controls.

Parameters	Control foals (n=10)	Infected foals (n=33)
TRBc ($\times 10^6$)	7.66 ± 1.42	4.21 ± 1.35*
Hb (g/dl)	13.63 ±1.53	7.56 ±2.73*
PCV %	32.61 ±3.43	23.17 ±4.68*
MCV/fl	42.55±3.58	54.29±4.22*
MCHC /dl	41.61±6.62	31.87±5.82*
TLC($\times 10^3$)	10.53 ±1.63	14.43 ±2.23*
Lymphocytes /absolute	4554 ± 421.2 2	8892 ± 788.8 2*

* ($P<0.05$), Values are mean ± standard error of mean

Results showed that out of 33 samples tested (100%) of newborn foals were positive for B. equi, whereas (0%) no antibodies were detected for B. caballi.

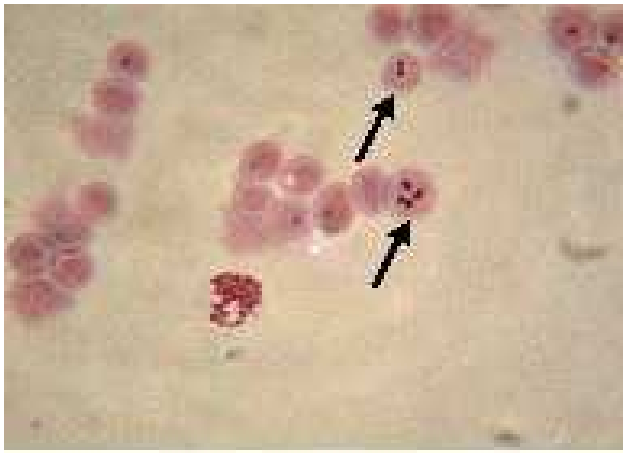

Fig. 1. Babesia equi in newborn foal blood smear

Significant decrease ($p<0.05$) in blood pH, Pco_2, Bicarbonate, Base access and Oxygen saturation percent were observed, However significant increase ($p<0.05$) in Anion gab were indicated Table 2.

Table 2. Acid-base balance and blood gas analysis of newborn foals infected with Babesiosis and controls.

Parameters	Control foals (n=10)	Infected foals (n=33)
pH	7.32 ±0.11	6.91±0.33 *
Pco_2/mm Hg	43.85±1.75	40.88±2.19 *
Bicarbonate mEq/L	23.17±2.77	18.46±1.23 *
Po_2/mm Hg	151±3.82	151.67±5.72
Base access /mEq/L	4.11 ± 1.33	(-5.73) ± 0.3*
So_2 %	%96	%83*
Anion gab/ mEq/L	8.24±1.84	12.28±2.72*
Sodium/ mEq/L	137±3.38	136±4.45
Potassium /mEq/L	3.55±0.71	3.72±0.76
Chloride /mEq/L	97.53±1.14	98.44±4.13

* ($P<0.05$), Values are mean ± standard error of mean

Moreover significant difference have been encountered in acute phase response and results showed significant decrease in haptoglobin and fibrinogen in diseased newborns foals than in controls, Table 3.

Table 3. Haptoglobin and fibrinogen values of new born foals infected with Babesiosis and controls.

Parameters	Control foals (n=10)	Infected foals (n=33)
Haptoglobin g/L	0.0213±0.15	0.0063±0.05*
Fibrinogen g/L	3.08±0.04	2.33±0.04*

* ($P<0.05$),Values are mean ± standard error of mean

DISCUSSION

Reports of transplacental transmission of *B. equi* has been diagnosed in asymptomatic horses with no known natural exposure to the ticks, which indicate that carrier animals may act as reservoirs to prove equine piroplasmosis in some areas where tick vectors may be present, Moreover intrauterine Infection of foals with *B. equi* can result in still births, abortions, or the birth of live foals with neonatal piroplasmosis, Phipps and Otter (2004), In addition evidence of intrauterine Infection with *B. caballi* were not reported, (Allsopp *et al*. 2007). Furthermore Babesiosis were detected in clinically normal horses in Mosul, Iraq, (Allsaad *et al*. 2012) However results revealed that all diseased foals were infected with *B.equi* these results were in support of data reported also by (Allsopp *et al.* 2007) who suggested that neonatal equine Babesiosis may be acquired during days 40 – 150 of gestation which congruent to the histotrophic nutrition when the embryo is exposed to a mixture of uterine gland secretions, maternal erythrocytes and desquamated epithelial cells. Moreover (Georges *et al*. 2011) added that carrier mares can transmit *B. equi* to their foals and such foals born at term can have a massive parasitaemia, On the other hand, (De Waal, 1992; De Waal and Van Heerden , 2004) mention that although anti piroplasims drugs suppress the parasite but do not eliminate completely therefore carrier mares may transmit the organism to their breed and it is assumed that once the parasite crosses the placenta and infects the fetus, the result is either an abortion or a foal born with neonatal Babesiosis. Nevertheless, It has been suggested before that transplacental transmission results from abnormal placentation as placental damage may permitting the maternal and fetal blood to mix, or that reverse erythroblastosis fetalis may occur, allowing parasites to cross the placenta, (Phipps and Otter , 2004). In contrast (Allsopp *et al*. 2007) suggest that parasite transmission can also occurs during pregnancies where placentation is normal.

Significant decrease in TRBc, HB, PCV, reflect Macrocytic hypochromic type of anemia, same results also recorded by (Zobba *et al*. 2008; Allsaad, 2009). However the degree of true anemia may not be reflected on foal's hemograme because of the sharp increase in erythrocyte parameters at birth During the first 12 hrs, transfusion of placental blood to the foal from the mare increases the foals RBCs which then decreased sharply over 12-24 hrs, (Weiss and Wardrop, 2010). The cause of anemia in blood pyroplasmal infection may be multi factorial, the direct effect of the parasite to the infected erythrocytes may be incriminated or decrease life span of RBCs beside suppression of hemopoitic system (Dewaal , 1992; Halat *et al*. 1997), Moreover (Oladosa and Oll Feml , 1992), refers to the role of auto immunity and the anti erythrocytic auto antibodies enhancing more erythrophagocytosis and bone marrow depression. Same type of anemia were also stated by (Nafie *et al*. 1981; Alsaad and A-Mola , 2006) whose stated that Macrocytic hypochromic type of anemia indicated regenerative type of anemia and reticulocytosis were detected in blood stream.

Leukocytosis in current study were also seen by (Alsaad and Al-Mola, 2006; Zobba *et al*. 2008) which may occur due to stimulation of bone marrow response and lymphoid system against the parasite, (Mohri and Sardari , 2000) added that leukocytosis occur as a result to lymphoid depletion and disorganization with massive lymphocytes. Lymphocytosis in equine piroplasmosis were also noted by (Salem *et al*. 1986) whose reported that increase lymphocyte numbers were detected during the formation of antibodies in response to babesia antigen.

Results of c-ELISA revealed that all infected foals were seropositive to *B. equi* and no *B. cabalii* antibodies were detected, similar results were also recorded by (Shkap *et al.* 1998; Sevinc *et al.* 2008) they observed that that competitive ELISA test might be an alternative for increased and sensitive detection of acute and also latent babesial infections, Moreover (Salim *et al*. 2008; Sar *et al*. 2010) added that ELISA using recombinant antigens which were developed as a more specific method for the serodiagnosis of piroplasmosis, Furthermore , (Knowles *et al*. 1996; Katz *et al*. 2000) refers that *B. equi* were more pathogenic than *B. caballi* and more common and distributed in endemic countries.

In the current study results showed decrease blood pH and bicarbonate in newborn foals infected with Babesiosis which indicated Metabolic acidosis, similarly results also mentioned by (Button, 1979; Johnson, 1995; Leisewitz *et al.* 2001; Sherlock *et al.* 2003). Titrational metabolic acidosis were indicated in the present study. Moreover (McMichael *et al*. 2005; Ayers and Warrington, 2008) added that in cases of anemia, and hypovolemic shock, blood perfusion is decreased and tissue hypoxia where follow therefore anaerobic metabolism become a consequence of decreased perfusion, so lactic acid accumulates and Hyperlactemia will result, Furthermore (Radostitis *et al.* 2007) refers that the negative Base excess were also indicate metabolic acidosis since Base excess were reflected the amount of strong acid which must be added to each liter of full oxygenated blood to return the pH to normal .

The anion gap is a calculated value based on the principle of electroneutrality which refer to that the total anions in the body must be equal to the total cations , (Dibartolam, 2006; LeRoy 2005).With titrational metabolic acidosis, the anion gap is increased which were indicated in the present study .

Blood gas analysis of newborn foals infected with Babesiosis were also indicated the tissue hypoxia via decrease level of Percent of Oxygen Saturation which

consider as an indicator of the percentage of hemoglobin saturated with oxygen at the time of the measurement , (Beall *et al*. 1999).

In current study decrease Pco_2 were indicated in diseased newborn foals, (Radostitis *et al*. 2007) mention that Pco_2 reflects the amount of carbon dioxide gas dissolved in the blood, Moreover Pco_2 is an independent measure of the respiratory component of acid-base balance and were decreased in hypoxia and metabolic acidosis, (Smith , 2004).

Haptoglobin is a plasma protein that binds free hemoglobin released from red blood cells with affinity, thereby inhibits its oxidative activity, (Jain *et al*. 2011). Decrease haptoglobin values were indicated in newborn foals infected with Babesiosis in this study as haptoglobin levels will be decreased in hemolytic anemia in the process of binding hemoglobin, because haptoglobin sequesters the iron within hemoglobin, preventing iron-utilizing organism from benefiting from hemolysis , (Tecles *et al*. 2005; Cary *et al*. 2009). The present study was clarify the importance of intrauterine infection of Babesiosis in newborn foals therefore all anemic / and or icteric foals born in endemic areas should be screened for neonatal piroplasmosis.

Conclusions: Babesiosis is an important disease affected newborn foals and exhibited different clinical signs, a significant changes were noticed between infected and control animals in hematological and some biochemical values.

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