CONCOMITANT OF ANAPLASMOSIS WITH ACID-BASE BALANCE ,BLOOD GAS ANALYSIS , ACUTE PHASE RESPONSE AND HEMOGRAM IN CATTLE

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Keywords; Anaplasmosis ,Cattle , Blood.
(Received 26May 2013,Accepted 5 June 2013)

ABSTRACT

The objective of the present work was to evaluate Hemogram, acid-base balance, blood gas analysis and acute phase response in cattle infected with anaplasmosis in Mosul,Iraq.The study were conducted on (167), local cattle breed 2-5 years old of both sexes. Eighty local cattle breed out of (167) were clinically infected with *Aanaplasam marginale* since the causative Rickettsia diagnosed by Giemsa stained blood smears and confirmed by indirect Elisa test. (20) clinically normal cattle were served as controls. Results indicated statistically significant decrease ($P<0.05$) in TRBCs, Hb, and PCV values of diseased animals, Macrocytic hypochromic type of anemia was indicated and the percentage of Rickettsemia (Hemoparasitism) ranged between (12-21%) with a mean of (15.57%). Results also indicated a significant increase in TLC as a result of significant increase lymphocytes. *A marginale* were detected in 96.25% of diseased cattle .There were no significant difference encountered in clotting factor indices. Statistically significant decrease were encountered in Pco_2, Oxygen saturation percent (SO_2), bicarbonate ions, Base excess and blood pH in diseased cattle than in controls, However statistically increase in Anionic gap have been detected, Moreover Titritional metabolic acidosis were registered . Statistically significant difference have been encountered in acute phase response as haptoglobin and fibrinogen values were increased in clinically infected cattle than in controls.

Key words: Anaplasmosis , Cattle, hemogram, Acute phase response, Acid base balance, Blood gas analysis.

INTRODUCTION

Anaplasmosis is an infectious, non contagious, tick born disease of domesticated and wild ruminants. Progressive anemia, emaciation , digestive disturbances, and increase body temperature are the main characteristics of this disease (1,2).

The disease were globally distribution, especially over the tropical and sub tropical regions, Nevertheless it was also recorded in some temperate areas(3). The disease either seen sporadically or as outbreaks leading to a deleterious significant economic losses (4). In Iraq, The disease has wide distribution especially at the north parts (5,6,7,8,9,).

Studies of anaplasmosis in local cattle breed at Mosul, Iraq concerning evaluation of acute phase response, acid –base balance and blood gas analysis are very limited and little information had been provided. Therefore the main objects of this study was to investigate, hematological observation, and some biochemical

changes as well as the effect of anaplasmosis on clotting factors indices in cattle naturally infected with *Anaplasma marginale*.

MATERIALS AND METHODS

The study were conducted on (167), local cattle breed 2-5 years old of both sexes in Mosul , Iraq . Eighty local cattle breed out of (167) were clinically infected with *Aanaplasma marginale* since the causative Rickettsia diagnosed by Giemsa stained blood smears and confirmed by indirect Elisa test ,(Svanova- Sweden). (20) clinically normal cattle served as controls. Clinical examination of all animals had been carried out, and fecal samples were screened for parasitic loud using standard technique.

Blood samples (11 ml) were obtained from each animal via jugular vein-puncture. 2.5 ml of Blood mixed with EDTA used to determine erythrocyte count (TRBs), haemoglobin (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 leukocyte counts (TLC) on an automatic full digital cell counter (Beckman,USA). Clotting time (CT) was also estimated according to (10).

Giemsa-stained blood smears were used to evaluated differential leukocyte counts (11), Moreover another 2.5 ml of blood mixed with trisodium citrate was used to determine fibrinogen, prothrobin time (Prt) and activated partial thrompoplastine time (Appt) using commercial kits (Biolabo, France).The remaining (5mL) of blood were used for obtaining serum, Serum haptoglobin concentrations were assayed according to (12). 1 mL of blood mixed with heparin were drained separately from each cattle used to determine Pco_2 , Po_2 ,Oxygen saturation percent(So_2), Bicarbonate, Bass access , Anionic gap and Blood pH, Sodium and potassium (Opti-critical care analyzer/ USA) according to (13).Serum chloride values were estimated according to (14).

Statistical analysis The significance of variations between diseased and healthy cattle were statistically analyzed using T-test (SPSS), (15).

RESULTS

Clinically infected cattle show sings of paleness of mucus membranes, fever, loss of appetite, emaciation, rough hair coat, lacrimation with discharging serious ocular discharge, furthermore ticks were detected on different regions of the body.

Anaplasma marginale appears as spherical granules near periphery of infected red blood cells and Rickettsemia ranged between (12-21%) with a mean of (15.57) , *Fig.1*.

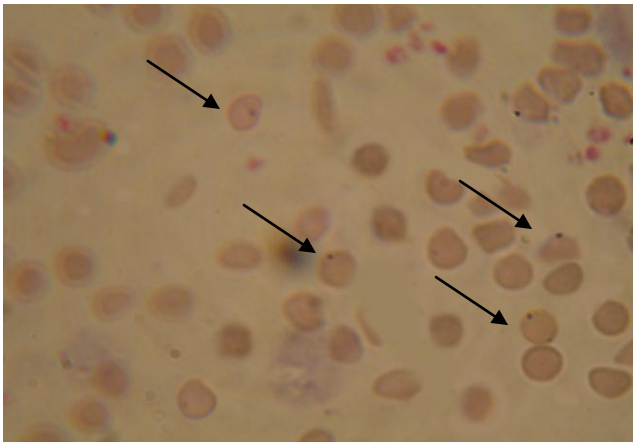

***Fig.1: A. marginale* in blood smear of cow erythrocytes**

There was a significant decrease (P<0.05) in the mean values of TRBs, Hb and PCV, in diseased cattle infected with Anaplasmosis and anemia was of Macrocytic hypochromic type. Results also indicated significant increase (p<0.05) in total leukocytes count as result of significant increase (p<0.05) lymphocytes. Table 1.and 2.

Table (1): Blood parameters of cattle infected cattle with Anaplasmosis and controls

Parameters	Control cattle	Infected Cattle
TRBs ($\times 10^6$)	8.34 ± 0.67	4.83 ± 0.89 *
Hb (g/dl)	10.87 ± 0.61	6.23 ± 0.39 *
PCV (%)	34.36 ±1.16	22.75 ±1.43 *
MCV/fl	41.19 ±1.57	47.12 ±1.46*
MCHC /dl	31.63± 0.45	27.38 ±5.57*
Parasitemia	----------	15.57 ± 6.81*

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

Table(2): Total and absolute differential leukocyte count of cattle infected with anaplasmosis and controls

Parameters	Control cattle	Infected cattle
TLC($\times 10^3$)	10.48 ± 0.35	13.66 ± 1.75*
N/ *absolute*	4673.7±144.16	3982.2±50.67 *
L/ *absolute*	4481.7 ±143.1	7055 ±726.36*
M/ *absolute*	422.76±113.66	566 ±108.78
E/ *absolute*	514.66 ± 204.65	565.21± 53.76
B/ *absolute*	73.43 ± 77.33	75±77.3

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

Moreover there was no significant difference were encountered in clotting factors indices among infected and control groups of cattle,Table 3.

Table(3):Clotting factors indices of infected cattle with anaplasmosis and controls

Parameters	Control cattle	Infected cattle
Plt $\times 10^3$	408.23 ± 50.62	392.21± 70.65
MPV /fl	9.32 ± 1.26	9.63 ± 3.22
PDW %	17.52 ± 2.51	18.76 ± 4.24
CT / mint	3.22 ± 0.78	3.87 ± 2.49
Prt / sec	11.52 ± 4.51	12.43 ± 5.57
Aptt /sec	60.82 ± 3.359	63.51 ± 7.463

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

A marginale was diagnosed on the basis of Giemsa stained blood smears and was confirmed by Indirect ELISA test and results showed that out of 80 samples tested (96.25%) of cattle were positive, whereas (3.75%) were detected as negative .

Results were also showed significant decrease(p<0.05)in Pco_2, Oxygen saturation percent, Bicarbonate, Base access and blood pH, however significant increase(p<0.05) in Anion gap were indicated, Moreover Titritional metabolic acidosis were registered. Table 4.

Table(4): Acid-base balance and blood gas analysis of cattle infected with anaplasmosis and controls

Parameters	Control cattle	Infected cattle
Pco_2/mm Hg	46.91± 1.65	39.61 ± 3.76 *
Po_2/mm Hg	152.61 ± 5.61	152.87 ± 6.81
So_2 %	94%	83% *
Bicarbonate mEq/L	26.65 ± 3.76	20.84 ± 2.57 *
Base access /mEq/L	4.72±1.32	-5.94±0.43 *
Blood pH	7.53 ± 0.11	6.93 ± 0.58 *
Anion gap/ mEq/L	7.22 ± 2.64	13.48 ± 2.76 *
Sodium/ mEq/L	137 ± 4.55	136 ± 6.28
Potassium /mEq/L	4.77 ± 0.65	4.64 ± 0.68
Chloride /mEq/L	94.75 ± 2.43	95.77 ± 5.38

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

Furthermore significant increase (p<0.05) in haptoglobin and fibrinogen have been encountered in diseased cattle than in controls, Table 5.

Table(5):Haptoglobin and fibrinogen values of cattle infected with Anaplasmosis and controls

Parameters	Control cattle	Infected cattle
Haptoglobin mg/100mL	0.24±0.6	0.83±.27*
Fibrinogen mg/100mL	242.28±36.32	301.11±43.66*

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

DISCUSSION

Results of hemogram indicated a significant decrease in TRBC, HB, PCV, reflecting Macrocytic hypochromic type of anemia, similar results were recorded by (5, 16,17). The cause of anemia during anaplasmosis may be multifactorial, the direct effect of the Ricketsia to the infected erythrocytes may be incriminated or decrease life span of RBCs and also suppression of hemopoitic system (18,19), Moreover extensive erythrophagocytosis initiated by *A.marginale* to erythrocytes and the anti-erythrocytic auto antibodies changes in bone marrow are an indication to bone marrow depression followed by anemia ,Therefore Hemoglobinuria was an unusual

clinical sign of anaplasmosis, because anemia results from extravascular opsonization and phagocytosis of parasitized erythrocytes by reticuloendothelial cells (20).
Examination of stained blood smears in the current work revealed that *Anaplasma marginale* appears as spherical bodies,dark red in color , near periphery of infected erythrocytes, these results were similar to those seen by (21,7.22). Leukocytosis which accompanied by increase in the lymphocytes were in agreement with that reported by (23,24). The increase in WBC is due to stimulation of lymphoid tissues and stem cells in the bone marrow by the causative agent and their reactant factors , Moreover Allison and Meınkoth (25) added that leukocytosis occur as a result to lymphoid depletion and disorganization with massive lymphocytes. lymphocytosis especially in Anaplasma infected cattle agree with that recorded by Aubry and Geale (2) whose stated that lymphocytosis was marked during the formation of antibodies in response to antigen and during anaplasma infection.

There were no significant difference encountered in clotting factors indices in current study, similar results were detected by (26, 7,27).

Results of Indirect Elisa test revealed that (96.25%) of tested cattle were seropositive to *A.marginale* , similar results were also recorded by Coetzee (28) whose stated that Indirect Elisa test may be an alternative for increased and sensitive detection of acute and latent anaplasmosis , Moreover Molloy(29) and Hornok(30) added that ELISA using recombinant antigens which were developed as a more specific method for the serodiagnosis of Anaplasmosis .

For maintaining the pH range differences , Acid-base balance is a critical and required for various enzyme systems to function ideally in the body, However disorders are restricted to an alteration in CO_2 or HCO_3^- with or without a compensatory response (31). In current study results showed decrease blood pH and bicarbonate in infected cattle which indicated Metabolic acidosis, similarly results also mentioned by(32,33). Two types of metabolic acidosis have been mention previously , Secretional metabolic acidosis which caused by loss of bicarbonate rich fluid such as diarrhea or saliva and Titrational metabolic acidosis which caused by the presence of non-CO_2 acids that titrate bicarbonate causing a decreased HCO_3^- (34) .Titration type metabolic acidosis were indicated when endogenous or exogenous acids in the plasma will increased (35,36), Titrational metabolic acidosis were detected in the current work.

It have been mentioned before that hypovolemia result in decreased blood perfusion and hypoxia were follow , therefore anaerobic metabolism become a consequence, thereby lactic acid accumulates and Hyperlactemia will result (37,38) . Furthermore the negative Base excess were also indicate metabolic acidosis (39) .
With Titritional metabolic acidosis, the anion gap is increased which were indicated in the present study, since anion gap is a reflected value based on the principle of electroneutrality which states that the total anions in the body must be equal to the total cations (40,41).

Blood gas analysis of infected cattle 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(42).Moreover Pco_2 reflects the amount of carbon dioxide gas dissolved in the blood, Therefore is an independent measure of the respiratory component of acid-base balance and were decreased in hypoxia and metabolic acidosis, (4,1).

The reactive Inflammatory response to any tissue injury is a mechanism through which the host sets up defense against further injury and starts the healing process (43) ,The early and immediate set of inflammatory reactions is known as acute phase

response (APR)(44). One of the predominant features of APR is changes in the concentrations of a number of plasma proteins associated with the host response(45). These changes are mainly the result of alterations in acute phase proteins synthesis in the liver (46). Haptoglobin has been one of the acute phase proteins most commonly monitored as a marker of inflammation in cattle (47,48).The function of the APR is to prevent tissue damage, and initiation of APR most commonly starts by the release of inflammatory mediators from tissue macrophages or blood monocyte cells that gather at the site of damage, these inflammatory mediators set off both the local and systemic inflammatory processes(49). The main function of haptoglobin is binding free hemoglobin and the hemoglobin binding property has a bacteriostatic effect, as it limits free iron available for bacteria (44). In current study results showed increase values of haptoglobin in cattle affected with Anaplasmosis ,same results were also mentioned by (50).

In current work increase fibrinogen level were also indicated .Fibrinogen is a plasma protein that considers as an acute phase protein in most species, including cattle, Therefore evaluation of this protein was found to be particularly useful in detecting inflammatory diseases (51).

CONCLUSIONS

Anaplasmosis were affected cattle and exhibited different clinical signs, a significant changes were noticed between diseased and control animals in hemogram ,acid base balance and blood gas analysis. Furthermore acute phase response were also detected in diseased cattle .Therefore The disease is responsible for substantial significant economic losses in endemic areas.

Acknowledegments

This article was supported by the College of Veterinary Medicine, University of Mosul, Iraq

ترابط حدوثية داء الانابلازموسز مع التوازن الحمضي–القاعدي،تحليل غازات الدم،استجابة الطور الحاد والصورة الدموية للأبقار

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الخلاصة

كان الهدف من هذه الدراسة تقييم الصورة الدموية، التوازن الحمضي القاعدي، تحليل غـــازات الدم وقياس استجابة الطور الحاد للأبقار المحلية المصابة سريرياً بداء الانابلازموسز في الموصل–العـــراق.إذ فحص (167) حيواناً من الأبقار المحلية بأعمار مختلفة تراوحت بين 2–5 سنوات ومن كلا الجنسين، شملت مجموعة الأبقار المصابة (80) حيواناً أظهرت علامات سريرية لداء الانابلازموسز الحاد وتم تأكيد اصـــابتها باستخدام مسحات دموية مصبوغة بصيغة الكمزا فضلا عن استخدام اختبار الاليـــزا غيـــر المباشـــر . أمـــا مجموعة حيوانات السيطرة فشملت (20) حيواناً سليماً سريرياً. أظهرت نتائج الدراسة انخفاض معنوي للعـــدد الكلي لكريات الدم الحمر وتركيز خضاب الدم وحجم خلايا الدم المرصوصة بالمقارنة مع مجموعة السيطرة، كما لوحظ ارتفاع معنوي لمعدلات الحجم الكروي وانخفاض معدلات تركيز خضاب الدم الكروي في الأبقـــار المصابة بداء الانابلازموسز بالمقارنة مع مجموعة السيطرة إذ كان فقر الدم من النوع ذي الكريـــات كبيـــرة الحجم قليلة الصباغ، وتراوحت النسبة المئوية للتطفل الدموي بين (12–21%) وبمعـــدل (15.57%). كمـــا تبين حدوث زيادة ملحوظة في معدلات العدد الكلي لخلايا الدم البيض في الأبقار المصابة بداء الانابلازموسز

وكانت هذه الزيادة ناتجة من زيادة العدد التفريقي للخلايا اللمفيـــة. كمـــا تأكـــد إن 96.25**%** مـــن الأبقـــار المفحوصة بتقنية الاليزا كانت مصابة بالنوع *A.marginale* .لم تظهر نتائج الدراسة أي اختلافات معنويـــة في معدلات معايير قيم عوامل تخثر الدم في حيوانات الدراسة ، كما تناقصت معنوياً معدلات الضغط الجزيئي لغاز ثاني اوكسيد الكاربون Pco_2، نسبة تشبع الـــدم بالاوكســـجين Oxygen saturation percent(SO_2)، تركيز ايون البيكاربونات، الزيادة القاعدية Base excess، باها الدم Blood pH في الابقار المصـــابة بـــداء الكمثريات بالمقارنة مع مجموعة السيطرة، في حين لوحظ تزايد معنوي في معدلات فجوة الصاعد Anionic gap. إذ توضح إن نوع الحماض الايضي هو من النوع حماض ايضي معيـــاري Titrational metabolic acidosis. وقد سجل الاختلاف في استجابة بروتينات الطور الحاد حيث لوحظ ارتفاع معنوي فـــي معـــدلات الهابتوكلوبين Haptoglobin ومنشيء الليفين Fibrinogen فـــي الأبقـــار المصـــابة بـــداء الانابلازموســـز بالمقارنة مع مجموعة السيطرة.

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