

THEILERIOSIS IN NEWBORN CALVES IN MOSUL, IRAQ

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(Received 2 January 2013 ,Accepted 24 February 2013)

Keywords; newborn calves, *Theileria annulata*., temporal fossa

ABSTRACT

The objective of the present study were to evaluate hemogram, acute phase response, acid base balance and blood gas analysis in newborn calves infected with *Theileria annulata* . The study was conducted on 55 local newborn calves (male and female), 1-9 days old. The study was carried out in Mosul , Iraq. Forty five newborn calves were infected with *Theileria annulata*. and Ten clinically normal calves served as controls. Diseased calves showed sings of fever ,unilateral or bilateral visible swelling of superficial lymph nodes. pronounced upward bulging of temporal fossa, outward bulge of conjunctivae with exophthalmos, paleness and/or icteric mucus membranes with petechial hemorrhages on the conjunctiva, lacrimation, dyspnea and depression. There was a significant reduction in the mean values of erythrocytes count, Hb and PCV,in diseased newborn calves infected with *Theileria annulata* and anemia was of Macrocytic hypochromic type. The percentage of hemoparasitism (parasitemia) ranged between (3-18%) with a mean of (11.88%). Results also indicated significant increase in total leukocytes count as results of significant increase lymphocytes. Diagnosis of Theileriosis were confirmed by microscopical examination of Giemsa stained lymph node aspirate and peripheral blood smears. Results were also showed significant decrease in blood pH, P_{CO_2} ,Bicarbonate, Base access and Oxygen saturation percent(SO_2) ,However significant increase in Anion gab was found and Titritional metabolic acidosis were indicated. Moreover results show significant decrease in haptoglobin and fibrinogen in diseased newborns calves than in controls.It have been concluded that *Theileria annulata* infection in newborn calves resulting in serious effects and health deterioration, leading to substantial significant economic losses

INTRODUCTION

Theileriosis are those tick-borne protozoan diseases associated with *Theileria spp* in cattle, sheep, goats, buffaloes, camels as well as in some wild animals. The genus *Theileria* belongs to the sporozoa ,order : Piroplasmidia group which includes *Theileria* and *Cytauxzoon* (1). Tropical theileriosis is one of the commonest and economically important fatal diseases of cattle (2), and is highly prevalent in Africa, southern Europe, the Near and Far East and Central Asia, (3,4). The disease were also distributed in most regions of Iraq (5,6). However, the disease had considerable economic impact on livestock production due to the high morbidities and mortalities (7).

Infection with *Theileria* spp. can cause acute, subacute or chronic disease and characterized by emaciation, moderate to severe anemia, unilateral or bilateral exophthalmia, petechiae in conjunctiva, oral and nasal mucosa, and occasionally in the pinnae. Widespread subcutaneous nodules with a 0.5 to 3.0 cm diameter were also detected, as well as enlarged superficial lymph nodes (8, 9,10). The pathogenesis of anemia in Theileriosis is not clear but a hemolytic factor has been reported in the serum of acutely affected cattle. In addition, it has been shown that oxidative bursts of macrophages in experimentally infected cattle can damage red blood cells and this may contribute to the anemia (11,12). Furthermore, the pathogenesis of various forms of theileriosis is also dependent on the production of schizonts in lymphocytes and piroplasms in erythrocytes (13).

Intrauterine infection with *T. annulata* is strongly suggested and The infection appears to have taken place prenatal (14). *T. annulata* schizonts and/or piroplasms were detected in blood smears from 70 of 79 cows (on one farm) expected to calve within 30 days. Similarly, 33 of 59 calves born to the above cows also exhibited *T. annulata* schizonts on day 1 to day 19 post partum. The cows and calves were free of *Hyalomma anatolicum*, the vector of *T. annulata*, during the course of study in the winter months, when the activity of ticks is reduced to the minimum (15). Moreover, (16) added that As a result, we are of the opinion that congenital *Theileria annulata* may cause the congenital tropical theileriosis cases in newborn calves via intrauterine transmission and prognosis may be poor in this cases.

In the present study, infection of newborn calves with *Theileria annulata* were identified, 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 55 local newborn calves(male and female), 1-9 days old. The study was carried out in Mosul , Iraq. Forty five local newborn calves breed were infected with *Theileria annulata*. Animals are free from ticks infestation .Ten clinically normal calves served as controls. Careful clinical examination had been carried out in all animals.

Blood collection and analysis

Blood samples (10 mL) were obtained from each newborn calves by jugular veni-puncture. Blood mixed with EDTA (2.5 mL) used to determine erythrocyte count (ER), 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 (17). Another 2.5 mL of blood mixed with trisodium citrate (9:1 ratio) was used to determine fibrinogen using commercial kits (Biolabo, France).The remaining (5 mL)of blood were used for obtaining serum, Serum haptoglobin concentrations were assayed according to (18). Diagnosis of Theileriosis were confirmed by microscopical examination of Giemsa stained lymph node aspirate and peripheral blood smears.Lymph node aspiration was carried out by using a 16 gauge needle which was rapidly pushed into the substance of swollen lymph node (usually prescapular) the hub of the needle then covered with a finger and the needle withdrawn ,the needle contents were expelled onto a glass slide ,spread into smears and stained with Giemsa stain (19).

1 mL of blood mixed with heparin were drained separately from each newborn calves used to determine ,Blood pH, P_{CO_2} ,Bicarbonate , P_{O_2} ,Bass access, Anionic gab, Oxygen saturation percent(So_2),Sodium and potassium (Opti-critical care analyzer/ USA) according to (20).Serum chloride values were estimated according to (21).

Statistical analysis

The significance of variations between diseased and healthy newborn calves were statistically analyzed using T-test (SPSS), (22).

RESULTS

Diseased newborn calves show sings of fever ,unilateral or bilateral visible swelling of superficial lymph nodes. pronounced upward bulging of temporal fossa, outward bulge of conjunctivae with exophthalmos, paleness and/or icteric mucus membranes with petechial hemorrhages on the conjunctiva, lacrimation, dyspnea and depression. There was a significant reduction ($P<0.05$) in the mean values of ER, Hb and PCV, in diseased newborn calves infected with Theileriosis and anemia was of Macrocytic hypochromic type, Moreover percentage of hemoparasitism (parasitemia) ranged between (3-18%) with a mean of (11.88%). 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 newborn calves infected with Theileriosis and controls

Parameters	Control calves (n=10)	Infected calves(n=45)
ER ($\times 10^6$)	7.73 \pm 1.31	4.76 \pm 1.73 *
Hb (g/dl)	13.78 \pm 1.12	7.43 \pm 2.61 *
PCV (%)	32.55 \pm 2.23	22.45 \pm 5.76 *
MCV/fl	42.12 \pm 2.65	47.16 \pm 5.48*
MCHC /dl	42.33 \pm 4.64	33.12 \pm 6.74*
parasitemia	-----	11.88 \pm 2.34

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

Table(2) Total and absolute differential leukocyte count of newborn calves infected with Theileriosis and controls

Parameters	Control calves (n=10)	Infected calves(n=45)
TLC($\times 10^3$)	10.64 \pm 1.56	14.78 \pm 3.65 *
N/ absolute	4479 \pm 492.45	4420.33 \pm 645.11
L/ absolute	4589 \pm 551.41	8875 \pm 865.73*
M/ absolute	543 \pm 325	551 \pm 371
E/ absolute	386 \pm 44	380 \pm 66
B/ absolute	82 \pm 79	82 \pm 29

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

Theileriosis was diagnosed by observation of the parasites in the peripheral blood and the presence of schizonts in lymphocytes that were provided from swollen lymph nodes.Fig 1 and 2

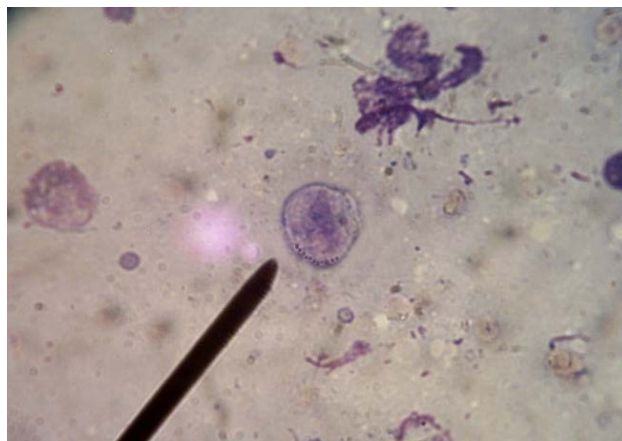


Fig 1: Macroshizonts of *Theileria annulata* in lymphocyte

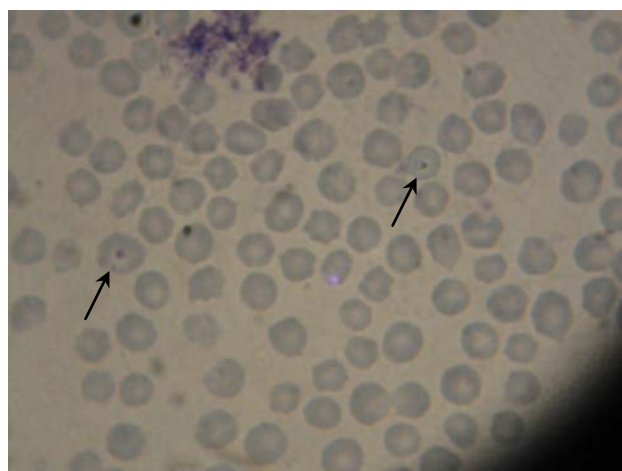


Fig 2: Different forms of *Theileria annulata* piroplasm in erythrocyte

Results were also showed significant decrease($p<0.05$)in blood pH, P_{CO_2} , Bicarbonate, Base excess and Oxygen saturation percent, however significant increase($p<0.05$) in Anion gap were indicated. Table 3.

Table 3: Acid-base balance and blood gas analysis of newborn calves infected with Theileriosis and controls

Parameters	Control calves (n=10)	Infected calves (n=45)
pH	7.44 ±0.15	6.94±0.37 *
P_{CO_2} /mm Hg	44.32±1.63	41.75±2.34 *
Bicarbonate mEq/L	22.12±2.87	18.33±1.53 *
P_{O_2} /mm Hg	149±2.72	149.54±6.23
Base excess /mEq/L	4.32 ± 1.11	(-5.87) ± 0.4*
So ₂ %	%94	%81*
Anion gap/ mEq/L	8.45±1.66	12.33±2.87*
Sodium/ mEq/L	137±3.38	136±4.45
Potassium /mEq/L	3.55±0.71	3.72±1.76
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 show significant decrease in haptoglobin and fibrinogen in diseased newborns calves than in controls, Table 4.

Table 4:Haptoglobin and fibrinogen values of newborn calves infected with Theileriosis and controls

Parameters	Control calves (n=10)	Infected calves (n=45)
Haptoglobin g/L	0.0212±0.16	0.0065±0.04 *
Fibrinogen g/L	3.07±0.03	2.13±0.05*

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

DISCUSSION

Transplacental transmission of *Theileria spp* has been reported with no known natural exposure to the tick vector in areas where transmitter tick vectors may be present (23,15,16). However, recent manifestations documented on transplacental *T. annulata* in a 1-3 days old cross breed calf were registered which supported that Infection of calves by *T.annulata in utero* can result in neonatal theileriosis (14). Moreover, Onoe *et al* (24)added that carrier dams can transmit *T.annulata* to their offspring and such calves born at term can have a massive parasitaemia.

Results of hemogram indicated a significant decrease in ER, HB, PCV, reflect Macrocytic hypochromic type of anemia, same results also recorded by (13, 25,26). The decline in RBCs, PCV and Hb may be attributed to the destruction of erythrocytes by macrophages in the lymph nodes, spleen and other organs of the monocyte macrophage system (27,11). A significant feature of Theileriosis is haemolytic anemia caused by an immune-mediated haemolysis which is indicated by the presence of a haemagglutinin (28, 29, 13,10). Although different evidence have been presented to explain the mechanism of the anaemia, the exact underlying mechanism is currently unknown (12). It is suggested that the activity of antioxidant enzymes such as superoxide dismutase is affected by the parasite and results in increased fragility of RBCs and thus, acceleration of erythrocytes clearance by phagocytic cells, since oxidating agents are responsible for the conversion of haemoglobin to methaemoglobin that results in decreased O₂ transmission, therefore the oxidative damage to the RBCs might play an important role in the pathogenesis of anaemia in bovine theileriosis (30,31). Moreover, Shizonts induced an indirect effect on RBCs resulting in production of haemagglutinin antibody, The RBCs exposed to antibodies are changed to different shape and size whose presence indicated marked immune-mediated hemolysis (32, 33, 34,35).

Leukogram showed significant increase in total leukocytic count and lymphocytosis were also indicated in comparison with healthy controls, Such changes in Leukogram might be attributed to harmful effects of toxic metabolites of *Theileria* on the haemopoietic organs especially bone marrow and their interference with the process of leukogenesis, Thereby indicated the stimulation of lymphoid tissues and stem cells in the bone marrow by the parasite and their toxins, However increase in numbers of lymphocytes reflects compensatory mechanism as target cells in response to their invasion with *Theileria* protozoan. Moreover, Omuse, (36) added that leukocytosis occur as a result to lymphoid depletion and disorganization with massive lymphocytes, Similar results were also observed by (11,37).

Acid-base disorders are those which are restricted to one primary alteration in CO₂ or HCO₃⁻ with or without a compensatory response (38). Newborn calves

infected with *Theileria annulata* in current study show decrease blood pH and bicarbonate which indicated Metabolic acidosis. Two types of metabolic acidosis, both are characterized by a decrease in the HCO_3^- but they differ in how that decrease occurs. Secretional metabolic acidosis is caused by loss of bicarbonate rich fluid such as diarrhea or saliva, whereas Titration metabolic acidosis is caused by the presence of non- CO_2 acids that titrate bicarbonate causing a decreased HCO_3^- . Titration type metabolic acidosis is a result of increased endogenous or exogenous acids in the plasma (39), Titration metabolic acidosis were indicated in the present study. Moreover, Ayers and Warrington (40) added that anemia will decreased blood perfusion therefore tissue hypoxia where follow, thereby anaerobic metabolism become a consequence of decreased perfusion, so lactic acid accumulates and Hyperlactemia will result. Furthermore (41) mentioned that the negative Base excess were also indicate metabolic acidosis as Base excess expresses the amount of strong acid that must be added to each liter of fully oxygenated blood to return the pH to 7.40. With titration metabolic acidosis, the anion gap is increased which were indicated in the present study (42). Blood gas analysis of newborn calves infected with Theileriosis 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 (43). In current study decrease P_{CO_2} have been encountered in diseased newborn calves. P_{CO_2} is a measurement of the respiratory component of acid-base balance and were decreased in hypoxia and metabolic acidosis (44).

Significant difference has been encountered in acute phase response and results show significant decrease in haptoglobin and fibrinogen in diseased newborns calves than in controls. The acute phase response is a non-specific reaction by an individual to different types of tissue damage (45). The acute phase proteins (APPs) are a group of blood proteins that change in concentration in animals subjected to external or internal challenges such as infection, inflammation, surgical trauma or stress (46). Furthermore, The APPs consist of negative and positive proteins that show a decrease and an increase in levels, respectively, in response to challenge, Moreover it is suggested that various infections and inflammatory processes may be associated with different APP pattern details, which may lead to APP parameters being used for diagnostic purposes (47). Glass *et al*(48,49) and Nazifi *et al* (2) reported that *T. annulata* causes severe pathology in susceptible cattle by inducing high levels of pro inflammatory cytokines such as IL-1, IL-6 and $\text{TNF}\alpha$, Therefore calves were respond differently to infection with *T. annulata* and produce markedly different levels of APPs.

Conclusions

Theileria annulata is an important protozoan affected newborn calves and exhibited different clinical signs, a significant changes were noticed between infected and control animals in hematological and some biochemical values, therefore all anemic / and or icteric calves born in endemic areas should be screened for neonatal Theileriosis .

Acknowledgement

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

داء الثايليريا في العجول حديثة الولادة في الموصل- العراق

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الخلاصة

تم في هذه الدراسة تقييم الصورة الدموية، استجابة الطور الحاد، التوازن الحمضي القاعدي وتحليل غازات الدم للعجول حديثة الولادة الخمجة بطفيلي *Theileria annulata* في الموصل - العراق. فحص 55 عجلا حديث الولادة بعمر (1-9) يوم ومن كلا الجنسين، شملت مجموعة حيوانات السيطرة عشرة عجول حديثة الولادة سوية سريريا، اما مجموعة العجول الخمجة فشملت 45 عجلا. اظهرت العجول الخمجة علامات سريرية تمثلت بارتفاع درجة حرارة الجسم، تضخم العقد اللمفية السطحية، انتفاخ الحفرة الصدغية وملتحمة العين وبروزهما، شحوب او اصفرار الاغشية المخاطية مع ملاحظة الانزفة الحبرية على ملتحمة العين، تدمع العينين، بهر التنفس والانخساف. اظهرت نتائج الدراسة انخفاض معنوي للعدد الكلي لكريات الدم الحمر وتركيز خضاب الدم وحجم خلايا الدم المرصوصة اذ كان فقر الدم من النوع ذي الكريات كبيرة الحجم قليلة الصباغ وتراوحت النسبة المئوية للتطفل الدموي بين (3-18%) وبمعدل (11.88%)، كما اتضح حدوث ارتفاع معنوي في معدلات العدد الكلي لخلايا الدم البيض بسبب الزيادة المعنوية للعد التفرقي للخلايا اللمفية. تم تأكيد اصابة العجول بالطفيلي بالفحص المجهرى للمسحات الدموية واللمفية المصبوغة بصبغة الكمزا. اظهرت نتائج الدراسة ايضا تناقص معنوي في معدلات باها الدم Blood ph الضغط الجزيئي لغاز ثاني اوكسيد الكربون Pco_2 ، تركيز ايون البيكاربونات، الزيادة القاعدية Base excess، نسبة تشبع الدم بالاوكسجين Oxygen saturation percent (So_2) في العجول الخمجة بالمقارنة مع مجموعة عجول السيطرة، في حين لوحظ تزايد معنوي في معدلات فجوة الصاعد Anionic gab اذ توضح ان نوع الحمض الايضي هو من النوع حماض ايضي معياري Titritonal metabolic acidosis فضلا عن ذلك فقد لوحظ تناقص معنوي في معدلات الهابتوكلوبين ومنشئي الليفين في العجول الخمجة بالمقارنة مع عجول مجموعة السيطرة. استنتج من هذه الدراسة ان الخمج بطفيلي *Theileria annulata* كان له اثر سلبي واضح وكبير في العجول حديثة الولادة مما قد يؤدي الى هلاكها .

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