

Hemato-Biochemical Alterations in Water Buffaloes Clinically Infected with Bovine Theileriosis Before and After Treatment by Buparvaquone

Ahmed N F NEAMAT-ALLAH

Department of Clinical Pathology, Faculty of Veterinary Medicine, Zagazig University, 1 Alzeraa Street
Postal Code 44511, Zagazig City, Sharkia Province, Egypt

* Corresponding author: drnemovete@yahoo.com, a_neamatallah@yahoo.com

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Abstract

Most of researches about theileriosis have been done about its effect in cows and derelict it in water buffaloes. So this investigation is aimed to study the hematological and serum constituents alterations in buffaloes affected with bovine theileriosis in Egypt. A total of thirty six buffaloes about 3-5 years old were examined in this study. Twenty six were naturally infected by theileria. Another ten buffaloes of the same age and apparent healthy were kept as control. The infected buffaloes were treated by buparvaquone (50 mg/20 kg B.W). Blood were taken from the healthy, infected non treated buffaloes and then post one and two weeks from buparvaquone administration. The affected buffaloes revealed high fever, corneal opacity, lacrimation, enlargement of the prescapular lymph node. Blood smears confirm presence of piroplasms of *T. annulata* in red blood corpuscles. Hematological analysis showed a significant reduction in erythrocytes count (RBCs), packed cell volume (PCV) and hemoglobin concentration (Hb) in non treated buffaloes infected by theileria. Normocytic normochromic anemia was reported rapidly with the first appearance of signs. Latterly, macrocytic hypochromic anemia. Total leucocyte count significantly increased soon after infection and persists until the first week post buparvaquone treatment. Theileria infected non treated buffaloes revealed an increase in the serum activities of aminotransferase (AST and ALT). Also serum levels of total bilirubin, unconjugated bilirubin (UB), conjugated bilirubin (CB), creatinine and blood urea nitrogen level were increased. In the opposite, side, the total serum proteins and albumin levels significantly decreased. Significant increase in malonyldialdehyde (MDA) activity was recorded with a significant decrease in the catalase activity (CAT) in *T. annulata* clinically infected buffaloes. These alterations were improved by treatment of the diseased buffaloes by buparvaquone two weeks post administration.

Keywords: *biochemistry, buffaloes, Buparvaquone, hematology, theileriosis*

INTRODUCTION

Blood parasites represent a major health problem in cattle breeding due to severe economic losses. That, lead to a decrease of animal production and an increase both of susceptibility to other infections and mortalities (Miodrag *et al.*, 2012). Theileriosis is transmitted by vector ticks of the genus *Hyalomma*, so it is one of a tick borne disease of cattle (Glass *et al.*, 2003). It is called tropical theileriosis or Mediterranean or even Egyptian fever. It is caused by *Theileria annulata* (*T. annulata*). It spread from the Mediterranean

and Middle East area, from Morocco to Western parts of India and China (Razmi *et al.*, 2003). *T. annulata* is one of the most destructive blood parasite affecting cattle, buffaloes, and even sheep.

Early diagnosis of *T. annulata* infection in bovines is mostly based on clinical signs as well as the detection of piroplasms in red blood corpuscles in Giemsa stained blood smears (Kaufmann, 1996). Early therapeutic approach can prevent the high mortality rates (Modi and Bhadesiya, 2014).

Basic control of tropical theileriosis depends on acaricides and chemotherapy (Tait and Hall,

1990). Buparvaquone is currently the most specific used chemotherapeutic treatment against theileriosis from 1980 till now (Müller *et al.*, 2015).

Many studies have been done about theileriosis in cows and derelict its effect in water buffaloes, so the present study is to estimate the effects of bovine theileriosis in buffaloes and the efficacy of buparvaquone as anti theilerial drug. The evaluation will be done by hematological, biochemical and antioxidant examination.

MATERIALS AND METHODS

Animal

The present study was carried out on twenty six clinically diseased buffaloes showed clinical symptoms of theileriosis and confirmed by detection of intraerythrocytic stages of the hemoparasite and another ten clinically have been used as apparent healthy buffaloes.

Treatment of infected animals:

The diseased buffaloes were treated with BUTAJECT, (Adwia Pharmaceuticals, Egypt 50 mg buparvaquone /ml) by recommended dose 50 mg/20 kg body weight by intramuscular in the neck region.

Blood film:

Thin blood smears from the ear vein of diseased buffaloes. The smears were dried off then fixed by methyl alcohol and stained with commercial Giemsa stain (Coles, 1986). They have been examined under oil immersion lens of microscope at (100X) according to (Kaufmann, 1996). Examination of blood smear for theileria was done by cross-section method to give representative examination according to (Ali and Radwan, 2011).

Samples:

Blood sample was collected from jugular vein of each buffalo and divided into two portions. The first portion of the blood was collected in EDTA tubes for hematological parameters. The second was collected without anticoagulant for serum separation for biochemical parameters analysis. Samples were taken from the healthy, infected non treated buffaloes and then post one and two weeks from buparvaquone administration.

Hematological studies:

Blood samples for hematological examination were sent to laboratory within two hours for determination of (RBCs, Hb, PCV, MCV, MCHC, MCH

and WBCs) using full automatic digital cell counter (Hospitex Hemascreeen 18, Italy).

Serum biochemical studies:

The serum was separated by centrifugation at 3000 rpm for 25 min and stored at -20 °C until used, then have been tested spectrophotometrically for the biochemical parameters. Total proteins ,albumin and globulins were measured (Doumas *et al.*, 1981; Drupt, 1974; Coles, 1986) respectively. Activities of serum aminotransferases (ALT and AST) were determined (Reitman and Frankel, 1957), while serum creatinine and blood urea nitrogen levels were estimated ; {Putton, 1977, Determination of serum blood urea nitrogen} Putton and Crouch, 1977). Serum reduced GSH was determined according to the method of Beutler *et al.* (1963). While, malonyldialdehyde (MAD) was determined according to the method of Ohkawa *et al.* (1979). Catalase (CAT) was determined by the method of Aebi (1984).

Statistical analysis:

By using SPSS version 8 for windows, the obtained data were statistically analyzed. One way analysis of variance (ANOVA) have been applied (Tamhane and Dunlop, 2000). Means at the same rows followed by distinct letters were significantly distinct. The letter (a) was representing the highest value.

RESULTS AND DISCUSSION

Mediterranean coast fever or tropical theileriosis caused by *T. annulata*. It is one of the most important diseases of cattle in Egypt, which lead to severe economic losses among them (Abou-El-Naga *et al.*, 2005). The most obvious clinical symptoms on theileria infected buffaloes were fever (>40°C), anorexia, enlargement of lymph nodes, corneal opacity and lacrimation (Fig. 1 and 2). These clinical signs are similar to those reported in theileria infected cows (AL-Hosary *et al.*, 2015). Blood smears revealed presence of theileria trophozoite (Fig. 3) in side erythrocytes in the present study.

Results of erythrogram (Tab. 1) revealed that theileria infected non treated buffaloes showed a significant decrease in erythrocytes count, packed cell volume and hemoglobin when compared with the healthy control. This reduction may probably because of severe damage caused by the organisms inside the erythrocyte during their multiplication (Ganguly *et al.*, 2015). The

obtained data of the mean corpuscular volume (MCV) and the mean corpuscular hemoglobin concentration (MCHC) were classified the produced anemia as a normocytic normochromic in infected non treated buffaloes. Macrocytic hypochromic anemia was pronounced in the buparvaquone treated buffaloes one and two weeks post buparvaquone administration. This picture may be a signs of regenerative anemia (Coles, 1986). Mean corpuscular hemoglobin (MCH) revealed a significant increase in the theileria infected non treated buffaloes compared with the healthy control that confirm hemolytic condition (Feldman *et al.*, 2000). The erythrogram returned toward the normal values two weeks post buparvaquone administration. This indicated

that the treated buffaloes start the resumption to their normal status. The recuperation could be due to the helps of buparvaquone in elimination of both piroplasmic and lymphocytic stages of the protozoan parasites from both blood and lymph nodes (AL-Hosary *et al.*, 2015).

Leucogram (Tab. 2) revealed leukocytosis in the theileria infected non treated buffaloes which is entirely due to lymphocytes proliferation in the lymphoid organs and granulocyte as a defensive response to invading protozoans (Modi *et al.*, 2015). Also the lymphocytosis may be due to that the intra-lymphocytic theilerial parasites stimulate the host cells, leading to growth of lymphocytes (Yamaguchi *et al.*, 2010). The same results were obtained by (Stockham *et al.*, 2000).

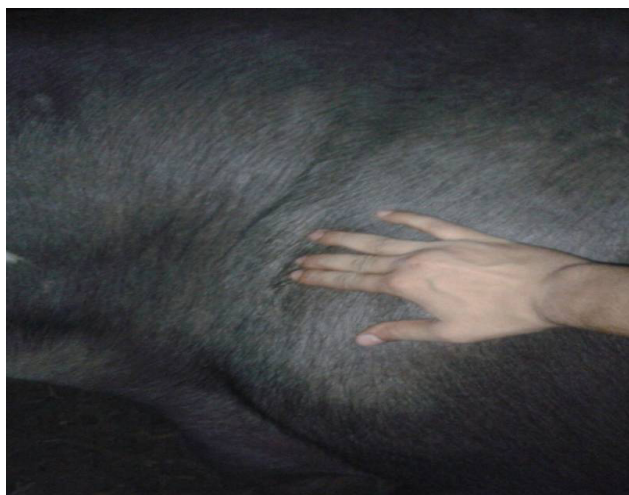


Fig. 1. Water buffalo showing enlargement of prescapular lymph node



Fig. 2. Water buffalo showing corneal opacity and watery lacrimation

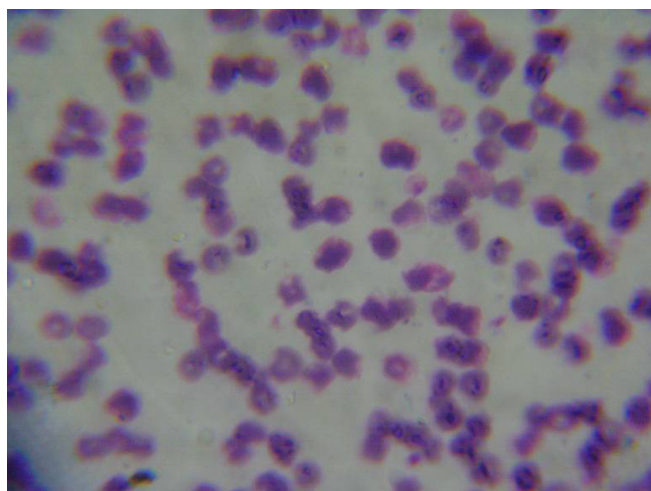


Fig. 3. Showing intra erythrocytic trophozoite of *Theileria annulata*.

On the contrary, (Omer *et al.*, 2002) who reported a pan leucopenia in pure bred cattle infected with theileriosis in Saudi Arabia. Similar findings were obtained by (Ghanem *et al.*, 2013) who reported that Egyptian water buffaloes clinically diagnosed as theileriosis showed a reduction in the total leucocyte counts. This variance in the leucogram may be due to the different infection stages in

the different investigations, since within an acute phase of infection; there is a wave of leucocytic response, which gradually drop as the infection becomes chronic.

Disturbances in the liver and kidneys functions (Tab. 3 and 4) demonstrated a significant increase in the serum activities of aminotransferases (AST and ALT) in the theileria infected non treated buffaloes.

Tab. 1. Erythrogram in the normal buffaloes and clinically infected with *T. annulata* (mean values \pm SE).

Parameters	Groups				F test
	Healthy buf- faloes	Theileria infected buffaloes			
		Non treated	1 week post bu- parvaquone administration	2 week post bu- parvaquone Administration	
RBCs (x 10 ⁶ /μl)	8.15 ^a ±0.11	6.44 ^c ±0.05	7.71 ^b ±0.26	8.23 ^a ±0.05	**
PCV %	40.04 ^a ±0.49	32.04 ^c ±0.30	38.86 ^b ±0.41	42.3 ^a ±0.48	**
Hb gm/dl	13.56 ^a ±0.02	11.76 ^c ±0.10	13.20 ^b ±0.40	13.54 ^a ±0.03	**
MCV fl	49.12 ^a ±0.19	49.75 ^a ±0.16	50.40 ^b ±10.40	51.39 ^b ±0.13	**
MCHC %	33.92 ^a ±0.45	33.87 ^a ±0.34	33.68 ^a ±0.44	31.90 ^b ±0.70	*
MCH ng	16.66 ^b ±0.24	18.24 ^a ±0.24	17.12 ^{ab} ±0.08	16.46 ^b ±0.19	**

Means at the same row followed by distinct letters were significantly distinct and the letter *a.* was representing to the highest value.

**Highly significant at $P \leq 0.01$

* significant at $P \leq 0.05$

RBC = red blood corpuscles

Hb = hemoglobin

PCV = packed cell volume

MCH = mean corpuscular hemoglobin

MCV = mean corpuscular volume

MCHC = mean corpuscular hemoglobin concentration

Tab. 2. Leucogram in the normal buffaloes and clinically infected with *T. annulata* (mean values \pm SE).

Parameters	Groups				F test
	Healthy buffaloes	Theileria infected buffaloes			
		Non treated	1 week post buparvaquone administration	2 week post buparvaquone administration	
WBC (×10 ³ /μl)	8.44 ^c ±0.05	10.02 ^a ±0.18	9.00 ^b ±0.24	8.45 ^c ±0.06	**
LYM (×10 ³ /μl)	5.00 ^b ±0.16	5.66 ^a ±0.20	4.91 ^c ±0.01	5.21 ^b ±0.17	**
MID (×10 ³ /μl)	1.94 ^a ±0.02	1.04 ^b ±0.03	1.66 ^a ±0.21	1.92 ^a ±0.03	*
GRA (×10 ³ /u)	1.50 ^c ±0.13	3.30 ^a ±0.11	2.43 ^b ±0.45	1.31 ^c ±0.12	**

Means at the same row followed by distinct letters were significantly distinct and the letter *a.* was representing to the highest value.

**Highly significant at $P \leq 0.01$

* Significant at $P \leq 0.05$

WBC= White blood cells

LYM = lymphocytes

GRA= neutrophils, eosinophil's and basophils

MID = monocytes and some eosinophil's

Also, serum levels of total bilirubin, unconjugated bilirubin (UB), conjugated bilirubin (CB), creatinine and blood urea nitrogen level showed a significant increase. In contrary, total serum proteins and albumin levels showed significant decrease. These changes probably indicated an inflammatory changes in hepatic and glomerular cells that in turn affected their functions (Abou-El-Naga *et al.*, 2005). These results are in agreement with Sandhu *et al.* (1998) who reported a hepatic and renal damages in liver and kidneys of calves infected *T.annulata* that appeared in macroscopic and microscopic evaluation. These results were returned to the normal level two weeks post buparvaquone

administration compared to the apparent healthy values.

Antioxidants play complementary roles in many aspects of pathogenesis of *T. annulata* infection. The results presented in Tab. 5 revealed a significant increase in malonyldialdehyde (MDA) activity and a significant decrease in catalase activity (CAT) in theileria infected non treated buffaloes compared with healthy one. Reduction of CAT activity may be attributed to the reduction of antioxidant enzymes as they are used by excessive free radicals in the infected buffaloes (Hassanpour *et al.*, 2013). The data obtained in the present study, regarding MDA levels were correlated with

Tab. 3. Total proteins, albumin and globulin levels in the normal buffaloes and clinically infected with *T. annulata* (mean values \pm SE).

Parameters	Groups				F test
	Healthy buffaloes	Theileria infected buffaloes			
		Non treated	1 week post buparvaquone administration	2 week post buparvaquone administration	
Total proteins (g/dl)	7.02 ^a ±0.05	6.07 ^c ±0.13	6.39 ^c ±0.11	7.25 ^a ±0.09	**
Albumin (g/dl)	3.80 ^a ±0.10	2.97 ^c ±0.03	3.18 ^b ±0.10	3.83 ^a ±0.03	**
Globulin (g/dl)	3.22 ^a ±0.08	3.10 ^a ±0.09	3.21 ^a ±0.05	3.42 ^a ±0.05	N.S

Means at the same row followed by distinct letters were significantly distinct and the letter *a.* was representing to the highest value.

**Highly significant at $P \leq 0.01$

N.S = non significant

Tab. 4. Some biochemical parameters in the normal buffaloes and clinically infected with *T.annulata* (mean values \pm SE).

Parameters	Groups				F test
	Healthy buf- faloes	Theileria infected buffaloes			
		Non treated	1 week post bu- parvaquone administration	2 week post bu- parvaquone administration	
AST (U/L)	41.81 ^c ±0.63	85.79 ^a ±1.23	74.33 ^b ±1.59	44.27 ^c ±1.40	**
ALT (U/L)	17.25 ^c ±0.80	57.24 ^a ±1.04	47.14 ^b ±1.96	18.77 ^c ±1.66	**
Total bilirubin (mg/dl)	0.37 ^d ±0.05	1.06 ^a ±0.06	0.87 ^b ±0.08	0.46 ^{cd} ±0.04	**
Direct bilirubin (mg/ dl)	0.15 ^c ±0.02	0.36 ^a ±0.01	0.30 ^b ±0.03	0.15 ^c ±0.02	**
Indirect bilirubin (mg/dl)	0.22 ^d ±0.07	0.70 ^a ±0.06	0.57 ^b ±0.03	0.31 ^{cd} ±0.04	**
Creatinine (mg/dl)	0.53 ^b ±0.02	1.49 ^a ±0.09	1.46 ^a ±0.06	0.55 ^b ±0.07	**
Urea (mg/dl)	16.21 ^b ±1.04	20.23 ^a ±0.63	20.11 ^a ±0.68	15.94 ^b ±1.12	**

Means at the same row followed by distinct letters were significantly distinct and the letter *a.* was representing to the highest value.

**Highly significant at 0.01 probabilities

ALT = alanine aminotransferase

AST = aspartate aminotransferase

Tab. 5. Catalase and malonyldialdehyde levels in the normal buffaloes and clinically infected with *T. annulata* (mean values \pm SE).

Parameters	Groups				F test
	Healthy buffaloes	Theileria infected buffaloes			
		Non treated	1 week post buparvaquone administration	2 week post buparvaquone administration	
Catalase (U/L)	2.61 ^a ±0.07	1.49 ^c ±0.11	1.79 ^b ±0.07	2.56 ^a ±0.05	**
Malonyldialdehyde (nmol/ml)	0.87 ^c ±0.06	1.46 ^a ±0.17	1.29 ^b ±0.12	0.90 ^c ±0.03	**

Means at the same row followed by distinct letters were significantly distinct and the letter *a.* was representing to the highest value.

** : Highly significant at 0.01 probabilities

those presented by Grewal *et al.* (2005). MDA level indicated that lipid peroxidation in erythrocytes of affected buffaloes was significantly higher than those of apparent healthy. After treatment by buparvaquone, these results were completely varied, the level of MDA has been significantly decreased and a significant increase in the activity of CAT has been recorded.

CONCLUSION

It could be concluded that *T. annulata* infected non treated buffaloes showed adverse alterations on the hematological profile, biochemical parameters of liver, kidneys and oxidative stress. These alterations were significantly improved two weeks after buparvaquone administration.

CONFLICT OF INTEREST: not exist.

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