J Parasit Dis (Apr-June 2017) 41(2):538–542
DOI 10.1007/s12639-016-0846-6

ORIGINAL ARTICLE

Haematological and biochemical indicators of tropical theileriosis diseased cattle in wilaya of Sétif (North East Algeria)

Ouarda Ayadi[1] · Mohamed Gharbi[2] · Mohammed Cherif Benchikh-Elfegoun[1]

Received: 6 December 2015 / Accepted: 21 September 2016 / Published online: 28 September 2016

Abstract The authors investigated biochemical and haematological parameters in 40 healthy and 40 tropical theileriosis (*Theileria annulata*) clinically infected cattle in El Eulma region (North East Algeria). The mean haematological and biochemical parameters including the number of erythrocytes, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin concentration and mean corpuscular haemoglobin were estimated. The mean hematological and biochemical parameters including the number of leukocytes, erythrocytes, hemoglobin, hematocrit, glucose, albumin, total proteins decreased significantly ($p = 0.005$; 0.008; 0.03 0.048; 0.002; 0.027 and 0.018; respectively) in Montbéliard breed clinically infected with *T. annulata*. In all diseased animals, the concentration of total and direct bilirubin increased significantly ($p < 0.001$). In cross breed cattle, the erythrocytes, hemoglobin, hematocrit means decreased significantly ($p = 0.027$; 0.003 and <0.001, respectively). Two types of anemia were detected: a microcytic hypochromic anemia in Montbéliard breed clinically infected cattle and normocytic normochromic anemia in local and cross breed cattle. Tropical theileriosis induces severe biochemical and haematological alterations that should be taken into consideration by field veterinarians for both the diagnosis of the disease and its treatment.

✉ Ouarda Ayadi
ayadioird@yahoo.com

[1] Laboratoire de Parasitologie, Institut des Sciences Vétérinaire El Khroub, Université des Frères Mentouri, Constantine, Algeria

[2] Laboratoire de Parasitologie, Ecole Nationale de Médecine Vétérinaire de Sidi Thabet, Univ. Manouba, 2020 Sidi Thabet, Tunisia

Keywords *Theileria annulata* · Cattle · Haematology · Biochemistry · Algeria

Introduction

Bovine tropical theileriosis (*Theileria annulata* infection) is a tick-borne disease transmitted by *Hyalomma* genus ticks. The infection is due to the presence and multiplication in leukocytes then erythrocytes of the parasite resulting in progressive and severe anemia (Gharbi et al. 2012). In the Maghreb region, this parasite is the most important summer cattle disease (Ziam and Benaouf, 2004). Tropical theileriosis induces hematologic and biochemical changes associated with anemia, these changes are related to the virulence of the strain, the infectious dose, the animal (breed, immune status), but also to climatic local factors (Mahmmod et al. 2011; Singh et al. 2001).

In Algeria, to our best of knowledge, there are no studies concerning the biochemical and hematological changes induced by *T. annulata* infection. Based on the presence of tropical theileriosis clinical signs, 80 cattle consisting of 40 *T. annulata* naturally infected and 40 clinically healthy cattle have been hematologically and biochemically studied.

Materials and methods

Study area

The present study was realized in El Eulma region, wilaya of Sétif (North East Algeria). The altitude of the region

varies between 800 and 1300 m. It is a semi-arid continental region with high summer temperature with a mean temperature of 22.8 °C. Winter is rainy and cold with a mean temperature of 8.5 °C. The mean annual rainfall is 482 mm (Sétif meteorological center, 2014). The total cattle population in El Eulma region is estimated to 30,000 composed of exotic (Montbéliard, Holstein Frisian, Pie Rouge and Fleckvieh) and local breeds conducted under a semi-extensive and intensive mode for milking and beef cattle respectively (Fig. 1).

Animals and sampling

The present study was carried out during the tropical theileriosis transmission season (from May to September 2013). A total number of 40 healthy and 40 naturally infected cattle presenting typical clinical tropical theileriosis symptoms (fever between 40 and 42 °C, lymph node enlargement, anemia and anorexia) were included in the present survey. All the clinical cases were confirmed by positive Giemsa stained blood smears. Animals were either local, exotic (Montbéliard) or crossbred cattle, aged between 10 months and 5 years were included in the survey.

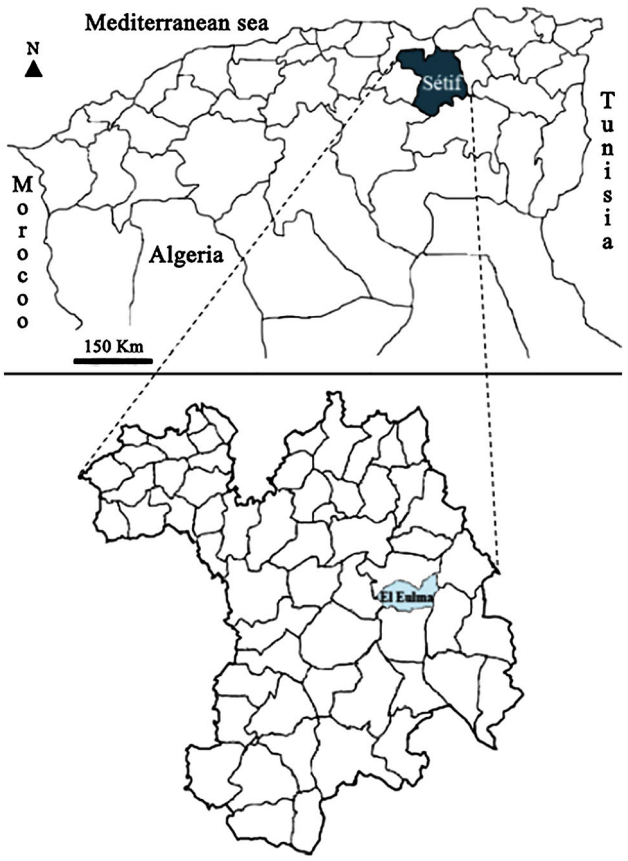

Fig. 1 Geographical location of El Eulma region, Algeria

The animals were blood sampled during the morning from the jugular vein before being treated. The samples were analyzed within one hour of field collection for leukocytes, erythrocytes, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean concentration of hemoglobin (MCH), heparin, glycaemia, albumin, total proteins and total and direct bilirubin.

Laboratory assays

Collected heparinized blood samples were centrifuged and the sera were collected. Glucose, total and direct bilirubin, total protein and albumin concentrations were estimated by GLUCOSE Spinreact (BSIS17-I, Girona, Spain), BILIRUBIN Spinreact (BSDTT36, Girona, Spain), TOTAL PROTEIN Spinreact (BSIS30-E, Girona, Spain) and ALBUMIN Spinreact (BSIS02-F, Girona, Spain) kits, respectively. The ISO (International Organization for Standardization, Ed. 2004, Spain) references were used as standards. The results were read with a BA-88A Semi-Auto Chemistry Analyzer (Shenzhen, China) spectrophotometer. Hematological parameters were estimated from EDTA collected blood samples with a hematology analyzer (Pentra ES 60, Kyoto, Japan).

In order to confirm the presence of *T. annulata* piroplasms, Giemsa stained blood smears were prepared then examined with an optical microscope at $\times 1000$ magnification with immersion oil.

Statistical analysis

The means were compared using Excel 11 software with the Student *t* test at 5 % threshold.

Results

The mean hematological and biochemical parameters including the number of leukocytes, erythrocytes, hemoglobin, hematocrit, glucose, albumin, total proteins decreased significantly ($p = 0.005$; 0.008; 0.03; 0.048; 0.002; 0.027 and 0.018, respectively) in Montbéliard breed clinically infected with *T. annulata* (Tables 1, 2) The concentration of total and direct bilirubin increased significantly in diseased animals ($p < 0.001$) in Montbéliard, local and crossbred infected cattle. The erythrocytes, hemoglobin, hematocrit means decreased significantly ($p = 0.027$; 0.003 and <0.001, respectively) in crossbred cattle.

Whilst, the levels of leucocytes, glycaemia, total proteins and albumin did not change significantly ($p = 0.441$; 0.420; 0.242 and 0.156, respectively) in local

Table 1 Biochemical parameters in cattle with tropical theileriosis and non-diseased cattle

Breed (number)	Glucose (g/L)	Albumin (g/L)	Total proteins (g/L)	Total bilirubin (mg/dL)	Direct bilirubin (mg/dL)
Montbéliard cattle (27)					
Non diseased cattle (13)	0.79 ± 0.11	33.46 ± 5.56	79.69 ± 5.99	0.66 ± 0.22	0.04 ± 0.07
Diseased cattle (14)	0.68 ± 0.06	29.06 ± 8.78	69.28 ± 10.06	5.52 ± 1.24	1.18 ± 0.44
p value	**0.005***	**0.008***	**0.03***	**<0.001**	**<0.001**
Crossbreed cattle (21)					
Non diseased cattle (11)	0.75 ± 0.11	32.63 ± 3.44	78.36 ± 8.36	0.75 ± 0.20	0.05 ± 0.09
Diseased cattle (10)	0.67 ± 0.07	30.30 ± 5.39	77.31 ± 12.38	3.89 ± 1.34	1.24 ± 0.72
p value	0.081	0.140	0.340	**<0.001**	**<0.001**
Local cattle (32)					
Non diseased cattle (16)	0.74 ± 0.08	30.56 ± 3.30	79.81 ± 5.43	0.72 ± 0.14	0.045 ± 0.09
Diseased cattle (16)	0.73 ± 0.18	28.92 ± 5.03	75.96 ± 21.19	2.38 ± 1.19	0.08 ± 0.42
p value	0.420	0.156	0.242	**<0.001**	**<0.001**

* In bolded characters: statistically significant values

Table 2 Hematological parameters in cattle with tropical theileriosis and non-diseased cattle

Breed (number)	Leukocytes (10^3/mm^3)	Erythrocytes (10^6/mm^3)	Hemoglobin (g/dL)	Hematocrit (%)	MCV (μm^3)	CCMH (%)	MCH (pg)
Monbéliard cattle (27)							
Non diseased cattle (13)	16.98 ± 23.2	7.05 ± 1.4	10.51 ± 1.8	31.07 ± 5.5	45.61 ± 5.9	34.37 ± 1.2	15.53 ± 2
Diseased cattle (14)	5.42 ± 2.5	5.10 ± 1.1	7.16 ± 1.3	21 ± 4.1	39.53 ± 5.3	33.48 ± 1	14.02 ± 1.6
p value	**0.048***	**0.002***	**<0.001**	**<0.001**	**0.002***	**0.027***	**0.018***
Crossbreed cattle (21)							
Non diseased cattle (11)	8.23 ± 1.96	6.97 ± 1.09	10.20 ± 0.72	30.00 ± 2.09	43,9 ± 8.52	34.23 ± 1.34	15.54 ± 2.29
Diseased cattle (10)	10.35 ± 5.69	5.69 ± 1.04	8.8 ± 1.61	24. 89 ± 4.64	41.02 ± 3.55	35.09 ± 1.24	14.69 ± 0.99
p value	0.172	**0.027***	**0.003***	**<0.001**	0.09	0.149	0.051
Local cattle (32)							
Non diseased cattle (16)	14.46 ± 21.07	6.87 ± 1.15	9.82 ± 1.06	28.81 ± 2.66	38.93 ± 15.47	34.05 ± 0.97	15.44 ± 3.13
Diseased cattle (16)	15.53 ± 16.25	6.53 ± 1.28	9.62 ± 1.65	27.75 ± 4.64	37.33 ± 6.26	34.48 ± 1.20	14.37 ± 1.10
p value	0.441	0.263	0.378	0.264	0.349	0.151	0.085

* In bolded characters: statistically significant values

and crossbred animals ($p = 0.172$; 0.081; 0.340 and 0.140, respectively).

Based on the classification criteria's of Weiss et al. (2010), the significantly decrease in MCV, MCHC, MCH levels ($p = 0.002$; $p < 0.001$; $p < 0.001$, respectively) showed a microcytic hypochromic anemia in Montbéliard clinically infected cattle and the non significantly changes in MCV, MCHC, MCH levels showed a normocytic normochromic anemia ($p = 0.349$; 0.151 and 0.085, respectively) in local and crossbred clinically infected cattle ($p = 0.09$; 0.149 and 0.051, respectively) (Table 2).

Discussion

According to Singh et al. (2001) and Omer et al. (2003), the decrease in the total proteins concentration was due to hypoalbuminemia and hypoglobulinaemia. These authors found a significant decrease of mean concentration of total proteins, which was similar with those estimated in Montbéliard breed cattle in present survey.

The significant decrease in the concentration of hemoglobin, hematocrit and red blood cells may be due to erythrocytes destruction by macrophages in the spleen and other mononuclear phagocyte system organs (Singh et al.

2001). In experimentally infected crossbred calves, a significant progressive decrease in the concentration of hemoglobin, hematocrit and red blood cells, leukopenia followed by leukocytosis were reported (Sandhu et al. 1998).

Several authors reported a progressive decrease in hemoglobin, hematocrit and red blood cells in acute *T. annulata* infections (Dhar and Gautam 1979; Sharma 1979; Mehta et al. 1988; Rayula and Hafeez 1995). A progressive decrease in hemoglobin and hematocrit associated to marked reticulocytosis were also reported by Singh et al. (2001).

In the present study, there was no significant decrease in glycaemia in infected Montbéliard breed, this result is consistent with the observations of Sandhu et al. (1998) in calves expressing tropical theileriosis. Collar and Uslu (2006) indicated that the decrease of glycaemia could be due to utilization of glucose by *Theileria* in the blood and hepatic dysfunction consecutive to *T. annulata* infection. Similar findings were reported in *T. annulata*-infected cattle and water buffalos (El-Deeb and Younis 2009).

In this study, the results showed a significant decrease in Montbéliard cattle leukocytes concentration compared to control animals. A leukopenia was reported by Osman and Al-Gaabary (2007) in tropical theileriosis of buffalo, similar results have been found in infected cattle (Omer et al. 2002; Bell-Sakyi et al. 2004; Aulakh and Singla 2006; Modi et al. 2015).

However, Sandhu et al. (1998) observed an immediate increase in white blood cell counts, followed by a significant decrease, several days after the beginning of the infection. Ugalmugle et al. (2010) reported 40 % cases with leucocytosis and 20 % cases with leukopenia in crossbred cattle. The insignificant leukocytosis was observed in crossbred cattle in this survey.

Leukocytosis result from lymphocytes proliferation in lymphoid organs as a defensive reaction to the invasion and multiplication of *T. annulata* (Modi et al. 2015) while, leukopenia may be the consequence of the destruction of white blood cells by the parasites.

In the present study, there was no difference in leucocytes, glycaemia, total proteins and albumin was found between diseased and non-infected local and crossbred cattle, this discrepancy could be explained by the higher immunization of the latters for this disease.

Indigenous breeds are more resistant against *T. annulata* infection than exotic breeds such as Frisian and Holstein cattle (Gharbi et al. 2014; Saleem et al. 2014). This difference in sensitivity is attributed to the difference in the immune response to produce pro-inflammatory cytokine which is higher in exotic breeds (Glass et al. 2005; Jensen et al. 2008).

The significant increase in the concentration of total and direct bilirubin could be due to two mechanisms: (1) hepatic dysfunction where a significant increase in the concentration of direct bilirubin is observed and (2) hemolytic anemia (Hooshmand-Rad 1976; Yadav and Sharma 1986; Sandhu et al. 1998; Singh et al. 2001; Omer et al. 2003).

This study revealed the presence of two types of anemia, a microcytic hypochromic observed in infected Montbéliard breeds and normocytic normochromic anemia in local and crossbred cattle. The latter results were similar to those of Sandhu et al. (1998) who reported a normocytic normochromic anemia in crossbred calves. A macrocytic hypochromic anemia was reported in Holstein (Omer et al. 2002; Durrani Kamal 2008) and crossbred cattle (Modi et al. 2015). Ganguly et al. (2015) reported a normocytic hypochromic anemia in *T. annulata* infected crossbred cattle. This anemia could be due to both an autoimmune reaction (Hooshmand-Rad 1976) and the effect of intra-erythrocytic piroplasms (Preston et al. 1992).

Tropical theileriosis induced important changes in hematological and biochemical parameters, which varies from one region to another and were influenced by the characteristics, the dose and virulence of the strain of *T. annulata* in each country. These biochemical and hematological changes should be taken into consideration by the field veterinarians in the interpretation of results and the therapeutic decision.

Acknowledgments We thank the staff of the Laboratory of Parasitology, Ecole Nationale de M"decine Vétérinaire de Sidi Thabet, Tunisia for their help in analyzing the blood samples. The authors also thank Pr. Brarhi El Hassan, director of Institut Supérieur Vétérinaire de Constantine, University Constantine and Dr. Kars Abd El Ali for their support, and all cattle farmers who agreed to let us handle their animals.

References

Aulakh GS, Singla LD (2006) Clinico-haematobiochemical observations on bovines naturally infected with *Theileria annulata*. J Vet Parasitol 20:49–52

Bell-Sakyi L (2004) *Ehrlichia ruminantium* grows in cell lines from four ixodid tick genera. J Comp Pathol 130:285–293. doi: 10.1016/j.jcpa.2003.12.002

Col R, Uslu U (2006) Haematological and coagulation profiles during severe tropical theileriosis in cattle. Turk J Vet Anim Sci 30:577–582

Dhar S, Gautam OP (1979) Observations on anemia in experimentally induced *Theileria annulata* infection of calves. Indian J Anim Sci 49:511–516

Durrani AZ, Kamal N (2008) Identification of ticks and detection of blood protozoa in Friesian cattle by polymerase chain reaction test and estimation of blood parameters in district Kasur, Pakistan. Trop Anim Health Prod 40:441–447

El-Deeb W, Younis E (2009) Clinical and biochemical studies on *Theileria annulata* in Egyptian buffaloes (*Bubalus bubalis*) with

particular orientation to oxidative stress and ketosis relationship. Vet Parasitol 164:301–305

Ganguly A, Bhanot V, Bisla RS, Ganguly I, Singh H, Chaudhri SS (2015) Hemato-biochemical alterations and direct blood polymerase chain reaction detection of *Theileria annulata* in naturally infected crossbred cows. Vet World 8:24–28. doi: 10.14202/vetworld.2015.24-28

Gharbi M, Mhadhbi M, Darghouth MA (2012) Diagnostic de la theilériose tropicale du boeuf (infection par *Theileria annulata*) en Afrique du Nord. Rev Méd Vét 163:563–571

Gharbi M, Rjeibi MR, Darghouth MA (2014) Epidémiologie de la theilériose tropicale bovine (infection par *Theileria annulata*) en Tunisie: une synthèse. Rev D'Elev Méd Vét Pays Trop 67:241–247

Glass EJ, Preston PM, Springbett A, Craigmile S, Kirvar E, Wilkie G, Brown CGD (2005) *Bos taurus* and *Bos indicus* (Sahiwal) calves respond differently to infection with *Theileria annulata* and produce markedly different levels of acute phase proteins. Int J Parasitol 35:337–347. doi:10.1016/j.ijpara.2004.12.006

Hooshmand-Rad P (1976) The pathogenesis of anaemia in *Theileria annulata* infection. Res Vet Sci 20:324–329

Jensen K, Paxton E, Waddington D, Talbot R, Darghouth MA, Glass EJ (2008) Differences in the transcriptional responses induced by *Theileria annulata* infection in bovine monocytes derived from resistant and susceptible cattle breeds. Int J Parasitol 38:313–325. doi:10.1016/j.ijpara.2007.08.007

Mahmmod YS, Elbalkemy FA, Klaas IC, Elmekkawy MF, Monazie AM (2011) Clinical and haematological study on water buffaloes (Bubalus bubalis) and crossbred cattle naturally infected with *Theileria annulata* in Sharkia province, Egypt. Ticks Tick-Borne Dis 2:168–171. doi:10.1016/j.ttbdis.2011.05.001

Mehta HK, Sisodia RS, Misraula RS (1988) Clinical and haematological observations in experimentally induced cases of bovine theileriosis. Indian J Anim Sci 58:584–587

Modi DV, Bhadesiya CM, Mandali GC (2015) Hematobiochemical changes in crossbred cattle infected with *Theileria annulata* in Banaskantha district of Gujarat. Int J Sci Res 5(1):1–4

Omer OH, El-Malik KH, Mahmoud OM, Haroun EM, Hawas A, Sweeney D, Magzoub M (2002) Haematological profiles in purebred cattle naturally infected with *Theileria annulata* in Saudi Arabia. Vet Parasitol 107:161–168

Omer OH, El-Malik KH, Magzoub M, Mahmoud OM, Haroun EM, Hawas A, Omar HM (2003) Biochemical profiles in Friesian cattle naturally infected with *Theileria annulata* in Saudi Arabia. Vet Res Commun 27:15–25

Osman SA, Al-Gaabary MH (2007) Clinical, haematological and therapeutic studies on tropical theileriosis in water buffaloes (*Bubalus bubalis*) in Egypt. Vet Parasitol 146:337–340. doi: 10.1016/j.vetpar.2007.03.012

Preston PM, Brown CGD, Bell-Sakyi L, Richardson W, Sanderson A (1992) Tropical theileriosis in *Bos taurus* and *Bos taurus* cross *Bos indicus* calves: response to infection with graded doses of sporozoites of *Theileria annulata*. Res Vet Sci 53:230–243

Rayula V, Hafeez MD (1995) Haematological values in cattle infected with *Theileria annulata*. Indian J Anim Sci 65:1202–1203

Saleem MI, Tariq A, Shazad A, Mahfooz SA (2014) Clinical, epidemiological and therapeutic studies on bovine tropical theileriosis in Faisalabad, Pakistan. Iraqi J Vet Sci 28:87–93

Sandhu GS, Grewal AS, Singh A, Kondal JK, Singh J, Brar RS (1998) Haematological and biochemical studies on experimental *Theileria annulata* infection in crossbred calves. Vet Res Commun 22:347–354

Sharma NN (1979) Haematological observations in bovine theileriosis, anaplasmosis and in mixed infections. Indian J Parasitol 3:153–156

Singh A, Singh J, Grewal AS, Brar RS (2001) Studies on some blood parameters of crossbred calves with experimental *Theileria annulata* infection. Vet Res Commun 25:289–300

Ugalmugle SS, Jayraw AK, Gatne ML (2010) Prevalence and clinical pathology of bovine tropical theileriosis in cross-bred population of Ahmednagar district of Maharashtra. J Vet Parasitol 24:141–145

Weiss DJ, Wardrop KJ, Schalm OW (2010) Schalm's veterinary hematology, 6th edn. Wiley, Ames, IA, pp 155–833

Yadav CK, Sharma NN (1986) Changes in blood chemical components during experimentally induced *Theileria annulata* infections in cattle. Vet Parasitol 21:91–98

Ziam H, Benaouf H (2004) Prevalence of blood parasites in cattle from wilayates of Annaba and El Tarf, East Algeria. Arch Inst Pasteur Tunis 81:27–30