

## HEMATOLOGIC CHANGES IN HORSES INFECTED WITH THEILERIOSIS IN TIARET, WEST OF ALGERIA

FADHÉLA<sup>1,2</sup> Smail, Mira CHIKHAOUI<sup>1,2</sup>, Fouzia ADDA<sup>1</sup>, Mokhtaria KOUIDRI<sup>1,2</sup> \*, Mustapha BELMEDJAHED<sup>3</sup>

<sup>1</sup>Institute of Veterinary Sciences, University of Tiaret, Algeria

<sup>2</sup>Laboratory of Research on Local Animal Products, University of Tiaret, Algeria

<sup>3</sup>Chaou Chaoua National Stud, Tiaret, Algeria

\*Corresponding Author: mokhtariakouidri@yahoo.fr

**Abstract.** The objective of this study was to determine the alterations in hematological parameters in horses naturally infested with *Theileria equi*, in the Tiaret area (Algeria) and the sensitive parameters such as age group, sex and breed influencing these changes in the group of horses most susceptible to this disease. Forty-two horses naturally infested with *T. equi* and ten clinically healthy (controls) were examined in this study. Blood samples were collected from November 2016 to April 2017 directly from the jugular vein in tubes containing an EDTA anticoagulant. Blood smears were examined for *T. equi* by microscopical examination of Giemsa-stained slides. A hematology analyzer for veterinary application (Mythic 18 Vet - Orphee) was used to study several hematological parameters. 33.07% of samples were positive for *Theileria equi*, females are more affected than males and horses less than 4 years old have a higher prevalence (52.38%). Significant reductions ( $p < 0.001$ ) in RBC, Hb and PCV and PLT were noted in the 42 horses in the patient sample compared to the control group showing the presence of anemia. A significant increase ( $p < 0.001$ ) was mentioned in the MCV, MCHC and MCH demonstrating the hypochromic macrocytic type of anemia. Highly significant thrombocytopenia was also observed. The present study is the first to report hematological changes in Barb horses and Arab Thoroughbreds infected with *T. equi* in Tiaret. The results of this research highlight the need to control horses for clinical piroplasmiasis caused by *T. equi*.

**Keywords:** Blood samples, Barb horse, Arab thoroughbred, Piroplasmiasis, *Theileria equi*, Anemia.

### INTRODUCTION

Equine piroplasmiasis is one of the most common tick-borne hemoprotozoan diseases that pose serious threats to the equids including horses, ponies, mules, donkeys and zebras (Aziz and Al-Barwary, 2019). It is found in most tropical and subtropical areas, and some temperate zones of the world (Mahmoud et al., 2016). This disease has high economic repercussions in the equine industry worldwide (Benfenatki et al., 2016) include the cost of treatment decreased equids production, still birth, lack of performance or death (Aziz and Al-Barwary, 2019) and has important implications for the international movement of horses (Bahrami et al., 2014). Two agents are responsible for causing Equine piroplasmiasis belonging in apicomplexa protozoan parasites *Babesia caballi* and *Theileria* (previously designated as *Babesia*) *equi* (Kamyngkird et al., 2014). Nevertheless *Theileria equi* is considered a more virulent species than *Babesia caballi* (Bahrami et al., 2014; Mahmoud et al., 2016). Additionally, Equine theileriosis caused by tick-borne apicomplexan protozoan *Theileria equi* is included in a single list of notifiable terrestrial and aquatic animal diseases, infections and infestations of the Office International des Epizooties/The World Organization for Animal Health (Ali et al., 2019).

These piroplasms are usually transmitted by tick vectors (Oliveira et al., 2019); altogether, 21 species of ixodid ticks of the genera *Dermacentor*, *Hyalomma* and *Rhipicephalus* are listed as vectors for equine piroplasms (Bahrami et al., 2014). Nevertheless, recent evidence shows that other genera such as *Ixodes*, *Haemaphysalis*, and *Ambloyomma* are capable of transmitting the equine piroplamosis parasites (Scoles and Ueti, 2015; Onyiche et al., 2019). Moreover, mechanical or iatrogenic transmission has been reported (Wise et al., 2013; Onyiche et al., 2019), via contaminated needles and syringes, blood transfusion, and surgical instruments (Onyiche et al., 2019). Likewise, several studies have noted the transplacental transmission of *T. equi* or *B. caballi* from pregnant mares to fetus mares in most cases leading to abortion (Sudan et al., 2015; Sousa et al., 2017; Onyiche et al., 2019), neonatal death in mare infected by *T. equi* (Rüegg et al., 2007), or foals birthed with neonatal theileriosis (Vianna et al., 2018). Clinical disease can take four forms, which could either be per-acute, acute, sub-acute or chronic (Ali et al., 2019; Onyiche et al., 2019). This disease is characterized by a variety of symptoms such as inappetence (Vianna et al., 2018; Ali et al., 2019; Onyiche et al., 2019), fever, anemia, icterus, hematuria (Bahrami et al., 2014; Mahmoud et al., 2016; Benfenatki et al., 2016; Montes Cortés et al., 2017; Vianna et al., 2018; Onyiche et al., 2019), edema (Onyiche et al., 2019), Lymphadenopathy (Montes Cortés et al., 2017), hepatomegaly, splenomegaly (Vianna et al., 2018; Onyiche et al., 2019), loss of weight (Ali et al., 2019; Mahmoud et al., 2016) and death in some cases (Benfenatki et al., 2016; Vianna et al., 2018; Onyiche et al., 2019).

In addition, Onyiche et al. (2019) reported that most equines, regardless of the clinical form of the infection, have some degree of anemia resulting from the hemolysis of infected erythrocytes. These same authors noted hematological changes during equine piroplasmosis. This pathology is classified as a probable cause of hematological anomalies in horses in Egypt (Zobba et al., 2008; Javed et al., 2014; Razi Jalali et al., 2015; Al-Obaidi et al., 2015; Mahmoud et al., 2016).

The objective of this study was to determine the alterations in hematological parameters in horses naturally infested with *Theileria equi*, in the Tiaret area (Algeria) and the sensitive parameters such as age group, sex and breed influencing these changes in the group of horses most susceptible to this disease.

## MATERIALS AND METHODS

**Study Area.** The study was conducted from November 2016 to April 2017 in Tiaret area. The latter is located 340 km from the capital Algiers in the north-west of Algeria at latitude of 35°15' N and longitude of 1°26' E. Climatologically, this region is a semi-arid area characterized by cold and humid winter and hot and dry summer.

**Horses and samples.** A total of 127 horses of the two breeds (Arab thoroughbred and Barb), both sexes and aged between 2-25 years were tested for detection of *T. equi*. The horses belong to the stud farm Chaouchaoua- Tiaret, Algeria. At the time of sampling, animals were examined by practicing veterinarians for clinical disease (pulse rate, respiratory rate, superficial lymph nodes, visible mucous membrane status, chest auscultation and fecal sample examination; the rectal temperature and clinical presentation were recorded) and presence of ticks.

**Blood samples.** One hundred twenty seven blood samples were collected from horses' jugular veins into tube contained ethylene diamine tetra acetic acid (EDTA) for preparation of thin blood smears and hematological analysis. After collection, the blood samples were transferred in iceboxes to the biochemistry laboratory, Veterinary Institute- University of Tiaret-Algeria. The blood samples were collected to check the disease status of the animal. Every individual sample was also accompanied by a questionnaire to collect information related to risk factors for theileriosis in horses consisting of month, season, sex, age, breed and tick infestation.

**Collection and identification of Ixodidae ticks.** The ticks were located by visual appraising. All visible ticks were manually removed from the body of the infected horses, taking care to avoid damage to the mouthparts. The ticks collected from each animal were stored into tubes contained alcohol and transferred to the parasitology laboratory of the veterinary institute (University of Tiaret), and identified under a stereomicroscope according to general identification keys as given by Meddour-Bouderda and Meddour (2006).

**Microscopic examination.** Giemsa staining technique was used for microscopical detection of *T. equi* in blood smears. Thin blood smears of 127 horses were fixed in ethanol, stained with Giemsa, and examined under microscopy in the biochemistry Laboratory of veterinary institute, University of Tiaret. At least 100 microscopical fields were carefully examined for *T. equi* using an oil-immersion  $\times 100$  objective. The presence of a single *T. equi* was considered positive.

**Hematological analysis.** Hematological analyses were performed by an automate hematology analyzer for veterinary application (Mythic 18 Vet - Orphee). Sixteen hematological parameters were studied, namely: red blood count (RBC), hemoglobin level (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), white blood count (WBC), neutrophils (Neutro), eosinophils (Eosino), basophils (Baso), lymphocyt (Lympho), monocyt (Mono), platelets (PLT), mean platelet volume (MPV), thrombocrit (THT) and platelet distribution width (PDW).

**Statistical analysis.** In the present study statistical analysis was performed by R software (version 3.4.4). Differences between Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post hoc test. In addition,  $P < 0.05$  was considered statistically significant.

## RESULTS AND DISCUSSIONS

Theileriosis can be diagnosed by clinical signs associated with blood smears stained with Giemsa for parasite visualization in red blood cells. However, this method is flawed because of non-detection of the parasite in the blood of clinically normal and negligibly parasitized animals (Vianna et al., 2019). These reasons prompted us to investigate the most affected hematological parameters in animals with equine theileriosis.

**Microscopic examination.** Microscopic observation of stained blood smears showed that 33.07% (42/127) of samples were positive for *Theileria equi*. Lower result was obtained by Benfenatki et al. (2016) in the same country and by the same technique with a rate of 15.9%. Likewise, Mahmoud et al. (2016) reported a rate of

11.4% among horses in Egypt by direct microscopic examination. While Salib et al. (2013) and Farah et al. (2003) in Egypt noted similar results of 34% and 38.8% respectively for *T. equi* in horses using a similar microscopic examination method.

However, Malekifard et al. (2014) in Iran and Guven et al. (2017) in Turkey also obtained a low prevalence rate by microscopic examination of stained blood smears with rates of 6.25% and 4.8%, respectively.

Prevalence of *T. equi* shows differences between males and females. Indeed, the rate in females (61.9%) is higher than that of males (39.1%) of subjects suffering from this pathology. Benfenatki et al. (2016) found that females (39.3%) are more affected than males (24.6%), which is in agreement with our study (females 61.9%; males 39.1%). These authors showed statistically that the female sex was a risk factor that could be attributed to the pregnant state of female, which could cause immunosuppression. However, Salem and El-Sherif, (2015) recorded that the stallions appear to be as infected as the mares. Also, other conflicting reports have shown a higher infection rate in males than in females (Salib et al., 2013). Sex does not seem to play a role in the infection rate (Kouam et al. 2010).

The horses aged less than 4 years also show a higher prevalence (52.38%) regarding the others age groups. Similar to our study, Dahiya et al. (2018) reported that 60% of equines aged 0 to 1 year presented with equine piroplasmosis. This rate can be explained by the fact that foals are born in good health and their passive immunity is transient and decreases after 63 to 77 days after parturition. As a result, foals become susceptible to natural *T. equi* infection (Kumar et al., 2008). Moreover, females are more affected than males, so it is natural that foals can be infected by ticks infected with *T. equi* deposited from their infected mothers (Dahiya et al., 2018). Additionally, Piroplasms of *T. equi* can be transmitted across the equine placenta and once a horse is infected, it appears to remain a lifelong carrier, since anti-theilerial drugs suppress but do not eliminate the parasite. Carrier mares may transmit the organism to their offspring and this may result in abortion or neonatal piroplasmosis, but observations by some researchers suggest that foals may be born as carriers yet remain apparently healthy (Allsopp et al., 2007).

Contrary to our result, Benfenatki et al. (2016) showed that horses aged between 10 and 14 years are the most affected. However, Salem and El-Sherif (2015) noted a high prevalence of the disease in horses aged 6 to 9 years.

In our investigation, a prevalence of 54.76% was observed in Arabian thoroughbreds while the barb displayed the rate of 45.24% among the horses reached by theileriosis. This could be attributed to the fact that they are breeding horses and are less well cared for than competition horses.

**Collection and identification of Ixodidae ticks.** Ticks were collected from 38.1% (16/42) of horses found positive during microscopic observation of stained blood smears. These ticks were collected from their bodies, especially the tail. All ticks collected belonged to the specie *Hyalomma marginatum marginatum*. Kouidri et al. (2019) reported that the genus *Hyalomma* was predominant genus in the same area of study with 86% and among the genus *Hyalomma*, *H. marginatum* was the predominant.

**Hematological analysis.** Table 1 summarizes the values of the Hemogram in horses with piroplasmosis and healthy horses. During this study we noted a highly

significant change ( $P < 0.001$ ) in the red blood cell count values with a very significant reduction in red blood cells, packed cell volume and hemoglobin. However, a significant increase in erythrocyte index levels (MCV, MCHC, MCH) compared to healthy control. In addition, the white blood cell count was significantly variable associated with very significant neutropenia. Finally, the blood platelets, in number, in rate and in distribution, showed results highly significantly in comparison with the control group.

Table 1

Hemogram in affected piroplasmosis horses and healthy horses

Parameters	Diseased Horses (n=42)	Healthy horses (n = 10)	p
RBC ( $10^6/\mu\text{l}$ )	$3.33 \pm 0.81$	$7.29 \pm 1.02$	$<0.001^{***}$
PCV (%)	$17.89 \pm 2.54$	$32.53 \pm 3.57$	$<0.001^{***}$
Hb (g/dl)	$9.34 \pm 1.27$	$13.01 \pm 3.30$	$<0.001^{***}$
MCV (fl)	$55.44 \pm 8.52$	$44.9 \pm 3.75$	$0.000463^{***}$
MCHC (g/dl)	$52.97 \pm 8.59$	$39.54 \pm 6.33$	$<0.001^{***}$
MCH (pg)	$29.93 \pm 8.65$	$17.72 \pm 2.93$	$<0.001^{***}$
WBC ( $10^3/\mu\text{l}$ )	$3.49 \pm 3.53$	$6.05 \pm 1.40$	$0.0323^*$
Neutrophil ( $10^3/\mu\text{l}$ )	$1.52 \pm 1.54$	$3.14 \pm 0.92$	$0.00291^{**}$
Eosinophil ( $10^3/\mu\text{l}$ )	$0.15 \pm 0.22$	$0.20 \pm 0.13$	NS
Basophil ( $10^3/\mu\text{l}$ )	$0.026 \pm 0.052$	$0.022 \pm 0.036$	NS
Lymphocyte ( $10^3/\mu\text{l}$ )	$1.53 \pm 1.65$	$2.22 \pm 0.60$	NS
Monocyte ( $10^3/\mu\text{l}$ )	$0.27 \pm 0.36$	$0.48 \pm 0.22$	$0.0786$
Platelets ( $10^3/\mu\text{l}$ )	$52.52 \pm 43.19$	$128.9 \pm 34.01$	$<0.001^{***}$
MPV (fl)	$5.56 \pm 1.26$	$7.21 \pm 1.66$	$0.00133^{**}$
THT (%)	$0.030 \pm 0.028$	$0.092 \pm 0.036$	$<0.001^{***}$
PDW (%)	$10.32 \pm 1.61$	$16.97 \pm 5.32$	$<0.001^{***}$

Results are expressed in terms of mean values (M) and standard deviations (SD) at  $P^* < 0.05$ ;  $^{**} < 0.01$ ;  $^{***} < 0.001$ .

Depending on age, the table 2 found a very significant ( $P < 0.01$ ) reduction for red blood cells in the three groups of horses with a very low rate in group C compared to control group. Similarly, very significant leukopenia ( $P < 0.01$ ) was also observed in older and adult horses compared to younger horses. Significant neutropenia ( $P < 0.05$ ) and very significant lymphopenia ( $P < 0.01$ ) were reported in all three age groups compared to control. Significant eosinopenia and monocytopenia ( $P < 0.05$ ) were observed in groups B and C. The number of platelets decreased very significantly ( $P < 0.01$ ) in older horses compared to young horses and adults, as well as in controls, with significant decreases in thrombocrit. However, a significant increase Erythrocyte indices were observed in MCV ( $P < 0.001$ ), MCHC ( $P < 0.05$ ) and MCH ( $P < 0.001$ ) in group C compared to groups A and B as well as group control.

In terms of sex, we notice values of erythrocyte indices were significantly elevated ( $P < 0.05$ ) for MCV. Moreover, very significant decreases in platelets count ( $P < 0.01$ ) were observed in mares than in males compared to control. Likewise, the proportion of platelets contained in the blood relative to the total volume of blood was very significantly decreased ( $P < 0.01$ ) in mares and the PDW was significantly decreased ( $P < 0.05$ ) in both males and females compared to control (Table 3).

Table 2

Hemogram in affected piroplasmosis horses and healthy horses according to age

Parameters	Diseased horses			Healthy horses n = 10	p
	Group A <4 years (n = 22)	Group B >4 years (n = 15)	Group C >10 years (n = 5)		
RBC (10 <sup>6</sup> /μl)	3.54 ± 0.80	3.37 ± 0.68	2.32 ± 0.24	7.29 ± 1.02	0.00588**
PCV (%)	17.59 ± 2.18	19.09 ± 2.71	15.66 ± 1.22	32.53 ± 3.57	0.0835
Hb (g/dl)	9.07 ± 1.42	9.69 ± 0.99	9.52 ± 0.97	13.01 ± 3.30	NS
MCV (fl)	51.15 ± 7.37	57.67 ± 6.35	67.62 ± 2.54	44.9 ± 3.75	<0.001***
MCHC (g/dl)	52.18 ± 9.10	51.56 ± 7.81	60.7 ± 1.91	39.54 ± 6.33	0.0199*
MCH (pg)	27.27 ± 8.20	30.16 ± 7.67	41 ± 1.66	17.72 ± 2.93	<0.001***
WBC (10 <sup>3</sup> /μl)	4.19 ± 3.99	3.52 ± 2.76	3.60 ± 1.00	6.05 ± 1.40	0.00597**
Neutrophil (10 <sup>3</sup> /μl)	1.72 ± 1.69	1.66 ± 1.33	1.83 ± 0.47	3.14 ± 0.92	0.0166*
Eosinophil (10 <sup>3</sup> /μl)	0.20 ± 0.25	0.11 ± 0.18	0.15 ± 0.13	0.20 ± 0.13	0.0237*
Basophil (10 <sup>3</sup> /μl)	0.020 ± 0.050	0.042 ± 0.058	0.022 ± 0.031	0.022 ± 0.036	NS
Lymphocyte (10 <sup>3</sup> /μl)	1.89 ± 1.93	1.46 ± 1.17	1.27 ± 0.55	2.22 ± 0.60	0.00598**
Monocyte (10 <sup>3</sup> /μl)	0.34 ± 0.42	0.23 ± 0.25	0.33 ± 0.10	0.48 ± 0.22	0.0256*
Platelets (10 <sup>3</sup> /μl)	73.27 ± 48.32	30.13 ± 20.45	28.4 ± 12.75	128.9 ± 34.01	0.00155**
MPV (fl)	5.35 ± 0.91	6.21 ± 1.55	4.56 ± 0.25	7.21±1.66	NS
THT (%)	0.04 ± 0.03	0.02 ± 0.01	0.01 ± 0.01	0.092 ± 0.036	0.00537**
PDW (%)	10.69 ± 1.20	9.11 ± 1.84	10.92 ± 1.55	16.97 ± 5.32	NS

Results are expressed in terms of mean values (M) and standard deviations (SD) at P \* < 0.05; \*\* < 0.01; \*\*\* < 0.001.

Table 3

Hemogram in horses with piroplasmosis and healthy horses according to sex

Parameters	Diseased horses		Healthy horses n = 10	p
	Group a Male (n = 16)	Group b Female (n = 26)		
RBC (10 <sup>6</sup> /μl)	3.44 ± 0.83	3.27 ± 0.79	7.29 ± 1.02	NS
PCV (%)	17.57 ± 2.24	18.10 ± 2.68	32.53 ± 3.57	NS
Hb (g/dl)	8.99 ± 1.32	9.56 ± 1.18	13.01 ± 3.30	NS
MCV (fl)	52.72 ± 8.15	57.11 ± 8.32	44.9 ± 3.75	0.0402*
MCHC (g/dl)	51.92 ± 9.59	53.62 ± 7.84	39.54 ± 6.33	0.0695
MCH (pg)	28.06 ± 9.01	31.09 ± 8.21	17.72 ± 2.93	0.0579
WBC (10 <sup>3</sup> /μl)	4.17 ± 4.06	3.08 ± 3.09	6.05 ± 1.40	NS
Neutrophil (10 <sup>3</sup> /μl)	1.72 ± 1.68	1.39 ± 1.43	3.14 ± 0.92	NS
Eosinophil (10 <sup>3</sup> /μl)	0.22 ± 0.28	0.10 ± 0.16	0.20 ± 0.13	0.0776
Basophil (10 <sup>3</sup> /μl)	0.019 ± 0.039	0.03 ± 0.06	0.022 ± 0.036	NS
Lymphocyte (10 <sup>3</sup> /μl)	1.92 ± 1.94	1.29 ± 1.40	2.22 ± 0.60	NS
Monocyte (10 <sup>3</sup> /μl)	0.29 ± 0.33	0.25 ± 0.37	0.48 ± 0.22	NS
Platelets (10 <sup>3</sup> /μl)	71.06 ± 51.78	41.11 ± 31.97	128.9 ± 34.01	0.0038**
MPV (fl)	5.57 ± 1.19	5.56 ± 1.31	7.21±1.66	NS
THT (%)	0.041 ± 0.036	0.022 ± 0.018	0.092 ± 0.036	0.00742**
PDW (%)	10.37 ± 1.13	10.37 ± 1.90	16.97 ± 5.32	0.0223*

Results are expressed in terms of mean values (M) and standard deviations (SD) at P \* < 0.05; \*\* < 0.01.

Table 4

Hemogram in horses with piroplasmosis and healthy horses according to breed

Parameters	Diseased horses		Healthy horses n = 10	p
	Arab thoroughbred (n = 23)	Barb horses (n = 19)		
RBC ( $10^6/\mu\text{l}$ )	3.60 ± 0.60	3.01 ± 0.90	7.29 ± 1.02	NS
PCV (%)	18.56 ± 2.71	17.08 ± 2.03	32.53 ± 3.57	NS
Hb (g/dl)	9.21 ± 1.38	9.51 ± 1.10	13.01 ± 3.30	NS
MCV (fl)	52.02 ± 5.67	59.58 ± 9.50	44.9 ± 3.75	0.0309*
MCHC (g/dl)	50.14 ± 7.74	56.4 ± 8.31	39.54 ± 6.33	NS
MCH (pg)	26.37 ± 6.27	34.25 ± 9.16	17.72 ± 2.93	0.0601
WBC ( $10^3/\mu\text{l}$ )	4.58 ± 3.49	2.18 ± 3.12	6.05 ± 1.40	0.0743
Neutrophil ( $10^3/\mu\text{l}$ )	1.99 ± 1.53	0.95 ± 1.34	3.14 ± 0.92	NS
Eosinophil ( $10^3/\mu\text{l}$ )	0.16 ± 0.21	0.13 ± 0.24	0.20 ± 0.13	NS
Basophil ( $10^3/\mu\text{l}$ )	0.032 ± 0.052	0.018 ± 0.051	0.022 ± 0.036	NS
Lymphocyte ( $10^3/\mu\text{l}$ )	2.08 ± 1.70	0.86 ± 0.13	2.22 ± 0.60	0.019*
Monocyte ( $10^3/\mu\text{l}$ )	0.30 ± 0.36	0.22 ± 0.35	0.48 ± 0.22	NS
Platelets ( $10^3/\mu\text{l}$ )	52.39 ± 41.43	52.68 ± 45.22	128.9 ± 34.01	NS
MPV (fl)	5.97 ± 1.37	5.07 ± 0.90	7.21 ± 1.66	0.0952
THT (%)	0.031 ± 0.025	0.030 ± 0.030	0.092 ± 0.036	NS
PDW (%)	9.91 ± 1.58	10.93 ± 1.55	16.97 ± 5.32	NS

Results are expressed in terms of mean values (M) and standard deviations (SD) at  $P < 0.05$ .

Table 4 summarizes the values of hemogram in horses with piroplasmosis and healthy horses according to breed. Barb horses showed a significant increase ( $P < 0.05$ ) in the level of MCV and likewise a significant decrease ( $P < 0.05$ ) in lymphopenia. Despite the absence of a significant difference for a decrease in their number of red blood cells between the two breeds on the one hand and the two breeds and the control group on the other hand these animals are anemic.

Anemia and weight loss were the most remarkable clinical signs. Equines chronically affected in the absence of signs of acute infection have many disturbances in the hemogram (Mahmoud et al., 2016). In the current study, 33.07% showed clinical signs of emaciation associated with the anemia in these animals. This result coincides with the study of Javed et al. (2014) in Pakistan, Mahdy et al. (2016) in Egypt.

Blood tests for horses at the National Stud Farm in Tiaret region revealed that the average values of red blood cells, packed cell volume and hemoglobin showed a significant decrease, which indicates anemia. Piroplasmosis is known to induce anemia in most horses (Wise et al., 2013). The progression of anemia in equines is a clinical sign of *T. equi* infection (De Waal and Van Heerden, 2004). Moreover, Horses carrying *Theileria*, in stressful situations, can develop exertional rhabdomyolysis (Muñoz et al., 2013). In addition, Piroplasmosis reduces performance causing cramps, abortion, anemia and even death (Fonseca et al., 2015). Horses born and raised in endemic areas generally develop a premonition, characterized by the carrier state, with mild clinical signs (García-Bocanegra et al., 2013; Muñoz et al., 2013). It seems that horses living in Algeria are asymptomatic carriers.

In our investigation we find a disturbance in the hemogram of horses with piroplasmosis. These disturbances presented variations according to sex, age and breed

showing that the sensitivity of each of these categories of horses varies according to their physiological state and influences the values of the hemogram differently with respect to certain hematological parameters. These current results were in agreement with those cited by Zobba et al. (2008), Rashid et al. (2009), Adaszek et al. (2011) and Ibrahim et al. (2011). Horses and donkeys with piroplasmosis have shown a decrease in red blood cells and hemoglobin but a slight increase in MCHC. Donkeys infected by ticks show a decrease in hemoglobin on the 9<sup>th</sup> day and then an increase after the 38<sup>th</sup> day (Javed et al., 2014). Osman (2017) reported reduction in red blood cells, hemoglobin and platelets in infected horses compared to the control. Whereas, Adaszek et al. (2011) showed that hemoglobin and packed cell volume levels have been low associated with thrombocytopenia.

In infected cases, decreases in red blood cells, hemoglobin and thrombocytes have been observed. Nevertheless, hypochromic macrocytic anemia may be due to an increase in MCV (Sumbria et al., 2015 and Al-Obaidi et al., 2016) and increase in MCMH (Ibrahim et al., 2011). In the present study, the levels of red blood cells, packed cell volume and hemoglobin were very significantly decreased, thus the values of the erythrocyte indices were very significantly increased in all the horses of the sick group compared to the healthy control. These characteristics suggested an hemolytic type of anemia. This anemia can be probably the consequence of oxidative damage in red blood cells (Nazifi et al., 2008).

Mierzejewska et al. (2014) described three mechanisms of hemolysis explaining anemia during canine babesiosis. These same mechanisms can be attributed to anemia during equine piroplasmosis: mechanical mechanism by binary fission intra-erythrocytic trophozoite, mechanism immunized by autoantibodies directed against components of infected and uninfected erythrocytes, and toxic mechanism by the hemolytic factor produced by the parasite (Zygner et al., 2007).

## CONCLUSIONS

The prevalence of *T. equi* piroplasmosis in horses is estimated at 33.07%, females are more affected than males while the age of less than 4 years is the most affected. This pathology was associated with significant hematological changes represented by a decrease in GR, Ht and Hb on the one hand. On the other hand, an increase in erythrocyte indices characterizes the red hemogram in horses contaminated with merozoites of *T. equi*. In addition, a decrease in white blood cells and platelets has also been reported. However, age, sex and race have less influence on the variations in the hemogram in horses suffering from theileriosis in stud farms in the Tiaret region in western Algeria. It would be interesting to study the influence of this disease and the hematological and biochemical variations which accompany it on the future breeding and sport horses.

**ACKNOWLEDGMENTS.** The authors would like to thank the director and the technical staff of Chaou-Chaoua National Stud - Tiaret, Algeria- for their precious collaboration. The authors are also grateful to D<sup>r</sup> Benaichata Lazerag for the statistical analyses during this study.



## REFERENCES

1. Adaszek, Ł., M. Górna, M. Krzysiak, M. Adaszek, M. Garbal and S. Winiarczyk. (2011) Identification of the piroplasms isolated from horses with clinical piroplasmosis in Poland. *Wiadomooci Parazytologiczne*, 57(1):21–26.
2. Al-Obaidi Q. T., I. I. Al-Sultan, M. M. Arshad, K. G. K. Mohd Azam and A. M. Mimi. (2015). Clinical case of acute equine piroplasmosis in a Malaysian Mare. *Res Opin Anim Vet Sci*, 5(6): 270-274.
3. Al-Obaidi, Q. T., A. Mohd Mokhtar, I. I. Al-Sultan, A. B. Azlinda and K. G. K. Mohd Azam. (2016). Equine piroplasmosis in Kelantan, Malaysia: clinicohemato-biochemical alterations in subclinically and clinically infected equids. *Tropical Biomedicine*, 33(4): 619–631.
4. Ali, S., M. Ijaz, S. H. Farooqi, A. Z. Durrani, M. I. Rashid, A. Ghaffar, A. Ali, A. Rehman, S. Aslam, I. Khan, A. Masud and K. Mehmood. (2019). Molecular characterisation of *Theileria equi* and risk factors associated with the occurrence of theileriosis in horses of Punjab (Pakistan). *Equine vet Educ*, First published: 11 August 2019 <https://doi.org/10.1111/eve.13161>
5. Allsopp, M. T. E. P., B. D. Lewis and B. L. Penzhorn. (2007). Molecular evidence for transplacental transmission of *Theileria equi* from carrier mares to their apparently healthy foals. *Vet Parasitol*, 48:130-136.
6. Aziz, K. J and L. T. O. Al-Barwary. (2019). Epidemiological Study of Equine Piroplasmosis (*Theileria equi* and *Babesia caballi*) by Microscopic Examination and Competitive-ELISA in Erbil Province North-Iraq. *Iran J Parasitol*, 14(3) :404-412.
7. Bahrami, S., A.R. Ghadrnan, M. Pourmahdi Borujeni and M. Vafayi Salarpur. (2014). Epidemiology of *Theileria equi* in Persian Arab horses from Iran. *Veterinarni Medicina*, 59 (9): 409–414.
8. Benfenatki, A., N. S. Younes Bouacida, K. Ait Oudhia and D. Khelef. (2016). Prevalence of *Theileria equi* Infection in Algiers Urban Area Using cELISA and Microscopic Examination. *Asian J Anim Vet Adv*, 11 (8): 511-515.
9. Dahiya, R., R. K. Salar, K. D. Mandal, R. Kumar, B. N. Tripathi, Y. Pal and S. Kumar. (2018). Risk factor analysis associated with *Theileria equi* infected equines in semiarid and sub-humid ecological enzootic zones of India, *Veterinary Parasitology*, 12:17–21.
10. De Waal, D. T and J. Van Heerden. (2004). Equine piroplasmosis. In: Coetzer JAW, ed. *Equine Babesiosis in Infectious Diseases of Livestock*. 2nd ed. Cape Town, South Africa: Oxford University Press, p 244-245
11. Farah, A. W., N. A. Hegazy, M. M. Romany, Y. A. Soliman and A. M. Daoud. (2003). Molecular detection of *Babesia equi* in infected and carrier horses by polymerase chain reaction. *Egypt J Immunol*, 10: 73-79.
12. Fonseca, L. A., A. R. C. Barreto-Vianna, R. F. Godoy and E. M. M. Lima. (2015). Detection of piroplasmosis in asymptomatic horses by whole and splenic blood PCR or standard splenic, venous and peripheral blood smears. *OJVRTM Online Journal of Veterinary Research*, 19(3): 148-154.
13. Garcia-Bocanegra, I., A. Arenas-Montes, E. Hernandez, L. Adaszek, A. Carbonero, S. Almeria, J. A. Jaen-Tellez, P. Gutierrez-Palomino and A. Arenas. (2013). Seroprevalence and risk factors associated with *Babesia caballi* and *Theileria equi* infection in equids. *Vet J*, 195: 172–178.
14. Guven, E., H. Avcioglu, A. Deniz, I. Balkaya, U. Abay, Ş. Yavuz and M. Akyüz. (2017). Prevalence and molecular characterization of *Theileria equi* and *Babesia caballi* in jereed horses in Erzurum, Turkey. *Acta Parasitologica*, 62(1): 207–213.
15. Ibrahim, A. K., S. Irene, A. A. Gamil, M. Abd-El baky, M. Hussein and A. A. Tohamy. (2011). Comparative molecular and conventional detection methods of *Babesia equi* (*B. Equi*) in Egyptian equine. *Global Veterinaria*, 7 (2):01-210.

16. Javed, K., M. Ijaz, M. A. Muddassir, I. Khan, K. Mehmood and A. Sadaqat. (2014). Prevalence and hematology of tick borne hemoparasitic diseases in equines in and around Lahore. *Pakistan J Zool*, 46(2): 401-408.
17. Kamyngkird, K., S. Yangtara, M. Desquesnes, S. Cao, P.K. Adjou Moumouni, S. Jittapalpong, B. Nimsupan, M.A. Terkawi, T. Masatani, Y. Nishikawa, I. Igarashi and X. Xuan. (2014).<sup>[1]</sup><sup>[2]</sup><sup>[3]</sup><sup>[4]</sup><sup>[5]</sup> Seroprevalence of *Babesia caballi* and *Theileria equi* in horses and mules from Northern Thailand. *J Protozool Res*, 24: 11-17.
18. Kouam, M. K., V. Kantzoura, A. A. Gajadhar, J. H. Theis, E. Papadopolo and G. Theodoropoulos. (2010). Seroprevalence of equine piroplasmosis and host-related factors associated with infection in Greece. *Vet Parasitol*, 169(3-4): 273-278.
19. Kouidri, M., S. M. A. Selles and Z. A-A. Kouider. (2019). First study on the composition species of tick (Ixodidae) infesting horses in Algeria. *Agricultura*, 109 (1 -2) : 215-218
20. Kumar, S., R. Kumar, A. K. Gupta and S. K. Dwivedi. (2008). Passive transfer of *Theileria equi* antibodies to neonate foals of immune tolerant mares. *Vet Parasitol*, 151: 80-85.
21. Mahdy, O. A., M. Ahmed, B. S. Nassar and M. S. Mohamed. (2016). Comparative diagnosis utilizing molecular and serological techniques of *Theileria equi* infection in distinct equine populations in Egypt. *International Journal of Chem Tech Research*, 9(6): 185-197.
22. Mahmoud, M. S., N. T. El-Ezz, S. Abdel-Shafy, S. A. Nassar, A. H. El Namaky, W. K. Khalil, D. Knowles, L. Kappmeyer, M. G. Silva and C. E. Suarez. (2016). Assessment of *Theileria equi* and *Babesia caballi* infections in equine populations in Egypt by molecular, serological and hematological approaches. *Parasites & vectors*, 9: 260.
23. Malekifard, F., M. Tavassoli, M. Yakhchali and R. Darvishzadeh. (2014). Detection of *Theileria equi* and *Babesia caballi* using microscopic and molecular methods in horses in suburb of Urmia, Iran. *Vet Res Forum*, 5(2):129-133.
24. Meddour-Bouderda, K and A. Meddour. (2006). "Clés d'identification des *Ixodina* (*Acarina*) d'Algérie." *Sciences et Technologie C*, 24:32-42.
25. Mierzejewska, E. J., R. Welc-Faleciak, M. Bednarska and A. Rodo. (2014). The first evidence for the vertical transmission of *Babesia canis* in a litter of Central Asian Shepherd dogs. *Ann Agric Environ Med*, 21:500-3.
26. Montes Cortés, M. G., J. L. Fernández-García and M. Á. Habela Martínez-Estélez. (2017). Seroprevalence of *Theileria equi* and *Babesia caballi* in horses in Spain. *Parasite*, 24: 14.
27. Muñoz, A., R. G. M. Rodríguez, C. Riber, P. Trigo, M. Gómez-Díez and F. Castejon. (2013). Subclinical *Theileria equi* infection and rhabdomyolysis in three endurance horses. *Pak Vet J*, 33(2): 256-258.
28. Nazifi, S., S. M. Razavi, M. Mansourian, B. Nikahval and M. Moghaddam. (2008). Studies on correlation among parasitaemia and some hemolytic indices in two tropical diseases (Theileriosis and Anaplasmosis) in Fars province of Iran. *Trop Anim Health Prod*, 40: 47-53.
29. Oliveira, A.R., G.R.G. Pinheiro, T.D. Souza, M.C. Flecher and R.L. Santos. (2019). Abortion in association with transplacental *Theileria equi* infection in a mare from the State of Espírito Santo, southeast Brazil: case report. *Arq Bras Med Vet Zootec*, 71 (2): 369-373.
30. Onyiche, T. E., K. Sukanuma, I. Igarashi, N. Yokoyama, X. Xuan and O. Thekisoe. (2019). A Review on Equine Piroplasmosis: Epidemiology, Vector Ecology, Risk Factors, Host Immunity, Diagnosis and Control. *Int J Environ Res Public Health*, 16(10).
31. Osman, S. A. (2017). Clinical, haematological and therapeutic studies on babesiosis in Arabian horses in the Qassim region, central of Saudi Arabia. *Journal of Applied Animal Research*, 45(1): 118-121.
32. Razi Jalali, M., R. Shahriari, A. A. Ghadrnan Mashadi, H. Hamidinejat, A. Jolodar and M. P. Borujeni. 2015. Hematological parameters and clinical signs associated with equine piroplasmosis in purebred Arabian horses of Ahvaz. *Iranian Veterinary Journal*, 11(1):126

33. Rüegg, S. R., P. Torgerson, P. Deplazes and A. Mathis. (2007). Age-dependent dynamics of *Theileria equi* and *Babesia caballi* infections in southwest Mongolia based on IFAT and/or PCR prevalence data from domestic horses and ticks. *Parasitology*, 134: 939–947.
34. Salem, N. Y and M. A. El-Sherif. (2015). Malondialdehyde status, trace minerals and hematologic results of anemic - *T. equi* infected Egyptian horses. *Inter J Vet Sci*, 4(3): 118-122.
35. Salib, F. A., R. R. Youssef, L. G. Rizk and S. F. Said. (2013). Epidemiology, diagnosis and therapy of *Theileria equi* infection in Giza, Egypt. *Vet World*, 6(2):76–82.
36. Scoles, G. A and M. W. Ueti. (2015). Vector ecology of equine piroplasmosis. *Annu Rev Entomol*, 60: 561–580.
37. Sousa, S. H., G. R. Paludo, C. R. Freschi, R. Z. Machado and M. B. de Castro. 2017. *Theileria equi* infection causing abortion in a mare in Brazil. *Vet Parasitol*, 8: 113-116.
38. Sudan, V., A. K. Jaiswal, A. Srivastava, A. Saxena and D. Shanker. (2015). A rare clinical presentation of transplacental transmission and subsequent abortion by *Babesia* (*Theileria*) *equi* in a mare. *Journal of parasitic diseases*, 39(2): 336–338.
39. Sumbria, D., L. D. Singla, A. Sharma, M. S. Bal and S. Kumar. 2015. Multiplex PCR for detection of *Trypanosoma evansi* and *Theileria equi* in equids of Punjab, India. *Vet. Parasitol*, 211: 293–299.
40. Vianna, A. M., A. P. d-S. S. de Lara, G. B. Weege, R. C. Cunha and F. P. L. Leite. (2018). Equine Theileriosis: Review. *Ann Rev Resear*, 3(4):100-104.
41. Wise, L. N., L. S. Kappmeyer, R. H. Mealey and D. P. Knowles. (2013). Review of equine piroplasmosis. *J Vet Intern Med*, 27: 1334–1346.
42. Zobba, R., M. Ardu, S. Niccolini, B. Chessa, L. Manna, R. Cocco and M. L. Parpaglia. (2008). Clinical and laboratory findings in equine piroplasmosis. *Equine Veterinary Science*, 28:301-308.
43. Zobba, R., M. Ardu, S. Niccolini, B. Chessa, L. Manna, R. Cocco and M. L. Parpaglia. (2008). Clinical and laboratory findings in equine piroplasmosis. *Equine Veterinary Science*, 28: 301-308.
44. Zygner, W., O. Gójska, G. Rapacka, D. Jaros and H. Wêdrychowicz. (2007). Hematological changes during the course of canine babesiosis caused by large *Babesia* in domestic dogs in Warsaw (Poland). *Veterinary Parasitology*, 145: 146-151.