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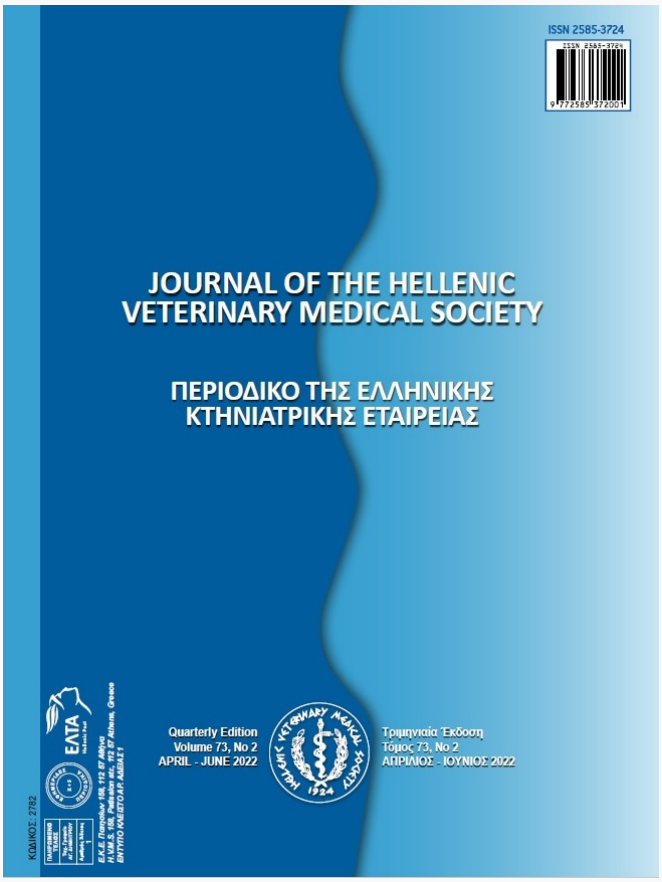


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Research article
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# Equine piroplasmosis in northern Algeria: Haematological and serological parameters

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**ABSTRACT:** Equine piroplasmosis is an acute, subacute or chronic tick-borne disease due to *Babesia caballi* and/or *Theileria equi*, affecting Equidae and causing economic losses to horse breeders and poor performances during tournaments. The objectives were fourfold: first to determine the seroprevalence of piroplasmosis in horses via cELISA, second to establish the haematological profile of piroplasmosis in horses of different Algerian areas through optical microscopy, third to identify the risk factors associated with the infection, and forth to try to elucidate any eventual correlation between piroplasmosis and anaemia. The study was carried out in different regions of northern Algeria. A total of 182 horses of both sexes were blood tested to estimate the prevalence of *Theileria equi* and *Babesia caballi* via competitive ELISA and to examine microscopically thin stained blood smears looking for haematological alterations using a standard cell counter. Parasites were detected in 42.9% of horses after microscopic examination of thin blood smears. The seroprevalence of equine piroplasmosis infection using competitive ELISA was 39% and 1,1% for *Theileria equi* and *Babesia caballi* respectively. Therefore, equine piroplasmosis is present in different regions of Algeria with a predominance of positivity in the central region (54,5%). Season (winter), region (central) and intended activity are the risk factors significantly associated with the prevalence of the disease. Anaemia was observed in 34,61% of individuals but there was no significant differences between positive and negative populations. Piroplasmosis is endemic in Algeria. Measures such as limiting horses' mobility should be taken to reduce/prevent dissemination.

***Keywords:*** Equine piroplasmosis, cELISA, *Theileria equi*, *Babesia caballi,* Haematological parameters, Algeria

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## INTRODUCTION

Equine piroplasmosis (EP) is one of the most common tick-borne diseases caused by hemoprotozoan parasites (Camacho et al., 2005) either *Babesia caballi* (*B. caballi*) or *Theileria equi* (*T. equi*). Ticks of the genera *Dermacentor, Hyalomma* and *Rhipicephalus*, part of the Ixodidae family are its biological vectors (Baptista et al., 2013; Ribeiro et al., 2013).

The distribution of equine piroplasmosis is worldwide; it is closely linked to the presence and proliferation of ticks causing a high prevalence of the acute form in autumn and spring. All horses are susceptible to infection but it is more severe in older ones (Maurin, 2017). The disease can cause economic losses due to reduced performances of infected horses, international requirements for exports or involvements in equestrian sports (Aziz & Al-Barwary, 2019).

The disease appears in acute (potentially fatal), subacute or chronic form. The acute form, easily diagnosed by suggestive clinical symptoms, is characterised by fever, haemolytic anaemia, haemoglobinuria and icterus due to lysis of erythrocytes (Le Metayer, 2007; Maslin et al., 2004; Van der Kolk & Veldhuis Kroeze, 2013). Some symptoms may characterize the chronic form, like deterioration of the general condition with inappetance, chronic fatigue, persistent anaemia and icterus, this form can set up immediately or follow an acute form unnoticed (Le Metayer, 2007; Maurin, 2017).

Piroplasmosis causes serious economic losses to farmers and has serious effects on horses' health (Ribeiro et al., 2013; Sevinc et al., 2008; Wang et al., 2014). Some cases may lead to death in absence of treatment but most animals recover and become asymptomatic carriers for several years and reservoirs for vector ticks (Alhassan et al., 2005; Aziz & Al-Barwary, 2019; Silva et al., 2013).

Several methods are used to diagnose equine piroplasmosis. Identification of the parasite by microscopic examination of stained thin blood smears is effective only during the acute stage of infection, but does not identify chronic carriers (Camacho et al., 2005; Maurin, 2017; Sevinc et al., 2008). Serological methods are recommended for the detection of asymptomatic animals especially those moved to areas free from the disease (Aziz & Al-Barwary, 2019; Baptista et al., 2013). Competitive inhibition enzyme-linked immunosorbent assay (cELISA) and indirect fluorescent antibody test (IFAT) have been approved by OIE for the detection of antibodies against *T. equi* and *B. caballi* and as a specified test for global horse activity (OIE, 2018).Serological tests are also used in epidemiological studies for detecting chronic and inapparent forms of piroplasmosis needed for evaluating infection risks; direct tests are not very efficient in subacute forms since the parasite is difficult to find in blood smears of asymptomatic carriers (Aziz & Al-Barwary, 2019). Molecular tools can also be used for direct detection of parasites in the blood such as PCR, reverse line bloat (RLB) hybridization assay or direct immunofluorescence.

In a previous study (Benfenatki et al., 2016), EP was shown to be endemic in Algiers. The aim of the present study were fourfold: first to determine the seroprevalence of piroplasmosis in horses via cELISA, second to establish the haematological profile of piroplasmosis in horses of different Algerian areas through optical microscopy after smearing and staining (throphozoites within erythrocytes in equine populations), third to identify the risk factors associated with the infection, and forth to try to understand any correlation between piroplasmosis and anaemia.

## MATERIALS AND METHODS

### Study area

The study was carried out between May 2018 and April 2019. It concerned 3 northern regions of Algeria (East, West and Centre) subdivided into 12 sub-locations (figure 1).The western and central regions have a Mediterranean climate characterised by mild-rainy winters and hot summers. Rainfall is, however, scarcer in the western region. The eastern region is several hundred meters above sea level and has a continental climate characterised by cold winters and hot summers (*climates to travel*, n.d.).

### Sample collection and data recording

A total of 182 horses of both sexes, aged 1 to 26 years, were blood sampled. Data on each animal such as sex, age, and type of activity were recorded. The living conditions of horses and characteristics of the region such as temperature, hygrometry and presence of other animals were noted. The animals were thoroughly examined for the presence of ticks and any signs of illness were recorded. Sampling occurred during the four seasons of the year. Blood was collected via jugular vein from each animal into two separate tubes (with and without EDTA).

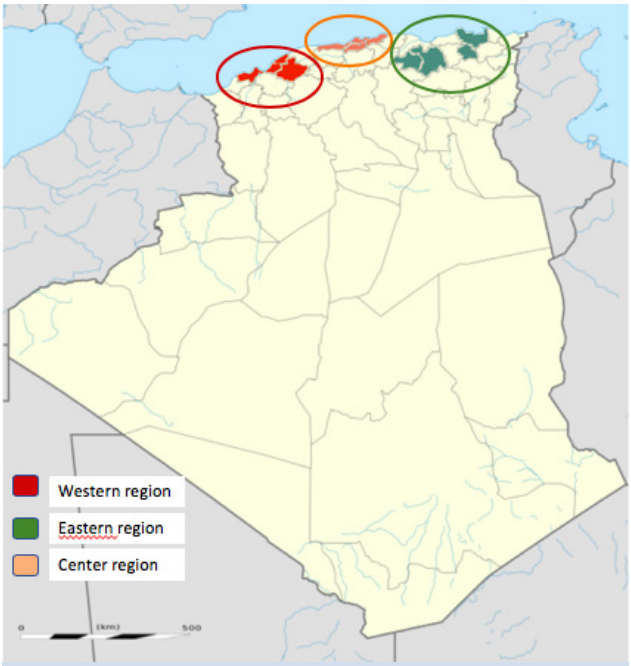


**Figure 1:** Geographic location of sampling areas (Gaba, 2020)

### Blood cell count

A fully automatic haematology analyser (MINDRAY 2800) served for blood cell count (BCC) of each sample. The BCC allowed the quantitative analysis of blood elements such as red blood cells (RBC), white blood cells (WBC), and platelets. It also helped assessing the mean corpuscular volume (MCV) and the mean corpuscular haemoglobin concentration (MCHC) that orientate the mechanism of anaemia (Thrall et al., 2004).

### Microscopic examination

Thin blood smears were prepared from blood samples stored in EDTA tubes and stained with Giemsa (10%) technique using a Hematek SlideStainer. Once dried, they were examined under optical microscopy through the oil immersion lens (× 100) to detect intraerythrocytic parasites (Aziz & Al-Barwary, 2019). To differentiate *T. equi* from *B. caballi* (fig. 2) the size of parasites and the number of cells formed by division were considered. *T. equi* is generally described as oval, rod, single pear and/or double pear shaped of 1-2 µm and sometimes a characteristic group of four small piroplasms, forming a tetrad called "Maltese cross" can be seen (Van der Kolk & Veldhuis Kroeze, 2013). *B. caballi* appeared as a single pear or double large pear shaped piroplasms of 2×3 µm attached by their thinnest extremities which is a feature characterizing it (Aziz & Al-Barwary, 2019; Maurin, 2017; Schnittger et al., 2012).

### Serological detection of antibodies anti B.*caballi* and *T. equi*

Collected samples were tested for the presence of antibodies against *T. equi* and *B. caballi* using a commercial cELISA kit (VMRD, Inc., Pullman, and WA99163 USA) according to the manufacturer's instructions. This test is a competitive, enzyme-linked immunosorbent assay (cELISA) used to detect antibodies anti Equine Merozoite Antigen1 (EMA-1) surface protein of *T. equi*, and rhoptry-associated protein (RAP-1) of *B. caballi* (Aziz & Al-Barwary, 2019).

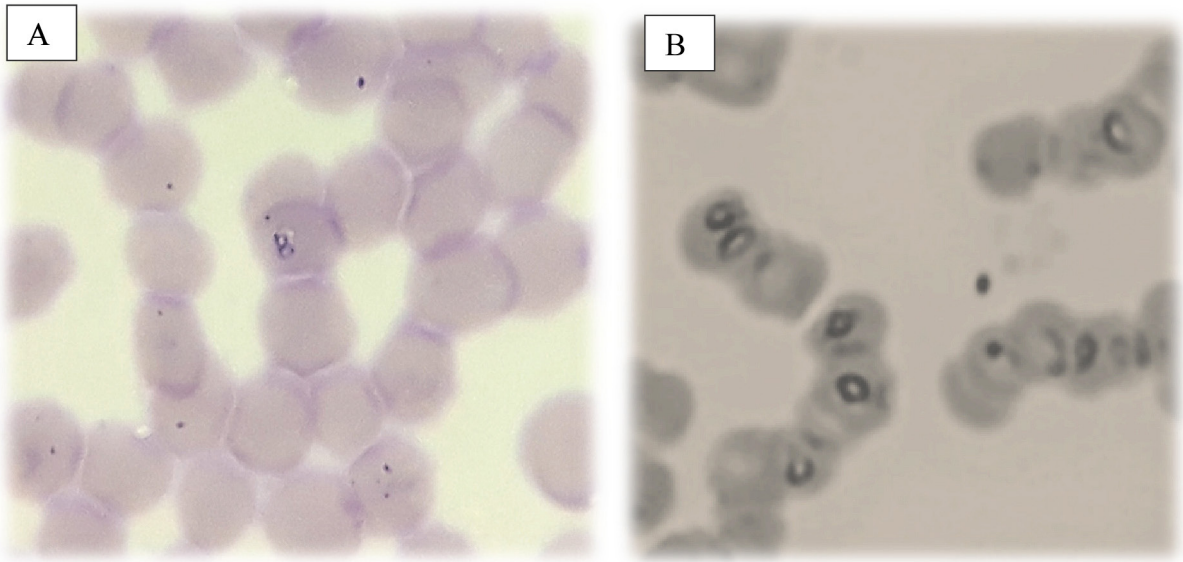


**Figure 2:** photomicrograph of blood smears stained with Giemsa, A) *T. equi*: infected cell with small, pair pyriform and single pyriform parasites. B) *B. caballi*: single pear or double large pear piroplasms (10x100)

### Statistical analysis

The univariate and multivariate statistical analyses were performed using the SPSS software. Chi-square ($\chi^2$)test was applied at a significance probability level of $P<0.05$ to compare infection rates between different groups and determine the potential impact of the dependent variables (age, gender and intended activity) and that of the environment (presence of grass, management practices) on the overall prevalence. Odds ratios were calculated to determine the effect of risk factors on the incidence of the disease.

## RESULTS

### Prevalence of equine piroplasmosis (EP)

Direct microscopic examination of 182 horses' samples detected 78 (42.9 %) positive samples. A 42.9% (n=78) rate of infection was recorded (table1). The overall seroprevalence of EP using cELISA attained 39% (71/182) and the rate of infection was 2 (1.1%) and 71 (39%) of *B. caballi* and *T. Equi* respectively. The detection of *parasites* in blood smears was positive in 43.7% (31/71) in seropositive animals, and 42.3% (47/111) in seronegative ones. These rates were similar to those found in the overall horse's population followed up (42.9%) (table1).The infection rate via optical microscopic examination did not significantly differ between seropositive and seronegative populations (p=0.861).

**Table 1:** Results of EP detected by cELISA and microscopy

| | | Microscopy | | | | | |
|---|---|---|---|---|---|---|---|
| | | + | | - | | Total | P |
| | | N | % | N | % | N | |
| **Serology** | + | 31 | 43,7 | 40 | 56,3 | 71 | 0,861 |
| | - | 47 | 42,3 | 64 | 57,7 | 111 | |
| | Total | 78 | 42,9 | 104 | 57,1 | 182 | |

### Multivariable analysis of seroprevalence of EP

Variable conditions related to horses and their environments were considered. A khi2 test was applied to each parameter to indicate any difference in EP's seroprevalence between sub-categories. The results are summarized in table 2.

Seroprevalence was not significantly different between females and males (p=0.156) and between age groups (p= 0.233). No difference in clinical signs was noticed between the animals. Horse activity, however, had a significant influence on seropositivity (p= 0.045); the latter was significantly lower in competition horses (p= 0.013) than in other activities. The OR suggests that competition activity may play a role in

**Table 2:** Relative risk factors associated with seropositivity of T. equi

| Parameters | | Total N | Seroprevalence of *T. equi* (%) | | Negative serology | | P | Odds Ratio | 95% Confidence Interval |
|---|---|---|---|---|---|---|---|---|---|
| | | | N | % | N | % | | | |
| **Sex** | **Male** | 88 | 39 | 44,3 | 49 | 55,7 | 0,156 | 1,542 | 0,847-2,807 |
| | **Female** | 94 | 32 | 34,0 | 62 | 66,0 | | 0,648 | 0,356-1,181 |
| **Age** | **0-5** | 45 | 17 | 37,8 | 28 | 62,2 | 0,845 | 0,933 | 0,467-1,867 |
| | **6-9** | 47 | 13 | 27,7 | 34 | 72,3 | 0,063 | 0,508 | 0,246-1,047 |
| | **10-14** | 41 | 18 | 43,9 | 23 | 56,1 | 0,465 | 1,299 | 0,642-2,629 |
| | **>=15** | 49 | 23 | 46,9 | 26 | 53,1 | 0,183 | 1,567 | 0,807-3,041 |
| **Ability** | **Competition** | 55 | 14 | 25,5 | 41 | 74,5 | 0,013 | 0,419 | 0,208-0,845 |
| | **Recreation** | 60 | 26 | 43,3 | 34 | 56,7 | 0,401 | 1,308 | 0,697-2,455 |
| | **Breeding** | 67 | 31 | 46,3 | 36 | 53,7 | 0,125 | 1,615 | 0,873-2,986 |
| **Symptoms** | **Presence** | 31 | 14 | 45,2 | 17 | 54,8 | 0,441 | 1,358 | 0,622-2,963 |
| | **Absence** | 151 | 57 | 37,7 | 94 | 62,3 | | 0,736 | 0,337-1,606 |
| **Season** | **Spring** | 44 | 9 | 20,5 | 35 | 79,5 | 0,003 | 0,315 | 0,141-0,705 |
| | **Summer** | 62 | 28 | 45,2 | 34 | 54,8 | 0,22 | 1,475 | 0,790-2,752 |
| | **Autumn** | 36 | 7 | 19,4 | 29 | 80,6 | 0,007 | 0,309 | 0,127-0,751 |
| | **Winter** | 40 | 27 | 67,5 | 13 | 32,5 | 0,000 | 4,626 | 2,183-9,805 |
| **Region** | **East** | 63 | 25 | 39,7 | 38 | 60,3 | 0,893 | 1,044 | 0,559-1,951 |
| | **Centre** | 55 | 30 | 54,5 | 25 | 45,5 | 0,004 | 2,517 | 1,316-4,813 |
| | **West** | 64 | 16 | 25,0 | 48 | 75,0 | 0,004 | 0,382 | 0,195-0,747 |
| **Global** | | 182 | 71 | 39,0 | 111 | 61,0 | | | |

N: number of horses

reducing seropositivity (OR= 0.419).

Analysis of environmental parameters showed that, apart from season (p= 0.000) and sampling area (p= 0.004) that impacted significantly the prevalence rate, the other parameters (humidity, presence of grass, water sources and animals surroundings, management and type of housing) did not influence EP's positivity rates. The prevalence of EP was significantly higher in winter (p=0.000). The odds infection in winter was 4.626 times greater than those recorded during the other seasons. The prevalence was significantly the highest in the central region (p=0.004), where the odds infection of EP was 2.517 times greater than in other areas (table 2).

**Haematological parameters**

The hematological parameters analysis showed average values within standard limits proving no influence of piroplasmosis' positivity on them. When comparing hematological parameters between seropositive and seronegative populations no significant difference in terms of average value of each parameter studied was noticed (p> 0.05).

The impact study of EP's seroprevalence and parasitaemia on the occurrence of anaemia revealed that 63 (34.6%) of the sampled horses had a degree of anemia leading to significant pathological variations of the red line blood cells (RBC, HCT, WBC).Anaemia was related to the presence of parasitemia in 46% and to its absence in 54% (p = 0.529). Its prevalence was similar to that of the overall population (34.6%). One can suggest that the serological status and presence of parasitemia have no influence on the occurrence of anaemia and therefore, anaemia is not a marker for equine piroplasmosis.

**Mechanism of anaemia based on parasitaemia findings**

The mechanism of anaemia was studied in a population of 56 horses, and the results were compared according to parasitaemia status. In the current study, anaemia was normocytic in almost all cases regardless of parasite status (54/56; 96.4%). Normocytosis was observed in 92.6% and 100% of the cases in the population with positive and negative parasitaemia respectively. Normocytosis is associated in 04 cases with hypochromia in horses affected with positive parasitaemia (CCMH <31%). Macrocytosis was found in two horses with positive parasitemia and has not been noted in the population with negative parasitaemia.

## DISCUSSION

Equine Piroplasmosis (EP) is an important disease that causes health problems and economic losses which impinge on horse trade and equestrian sports events. In Algeria, EP has already been investigated in horses but only in Algiers' province (Benfenatki et al., 2016).

Although optical microscopic examination lacks sensitivity and specificity particularly in low parasitemia carriers horses (Aziz & Al-Barwary, 2019), the rate of infection obtained via this method was 42.9 % (n=78). The latter is rather high in comparison with the previous rate (15.9%) reported in the same species in Algeria (Benfenatki et al., 2016). This prevalence is surprisingly high despite the fact that most of these horses had no specific clinical signs which suggest that they were either in an early infection state or that the investigated region is endemic and its horses developed protective immunity (Oduori et al., 2015). Similar prevalence rates were recorded in Egypt by Salib et al. in 2013 (41.61%) and by Farah et al. in 2003 (38.8%) but less than that obtained in Turkey (58.18%) by Sevinc et al. in 2008.

Through use of cELISA rates of 39% (n=71) and 1.1% (n=2) for *T. equi* and *B. caballi* were respectively obtained. This is higher than that observed earlier for *T. equi* (29.12%) in Algiers (Benfenatki et al., 2016), suggesting persistance of specific antibodies post contamination over the years even in the absence of new exposures (Le Metayer, 2007). The difference in infection rates between the 2 species could be due to the period of surviving in RBC, which is much longer for *T. equi* than for *B. caballi.* The former persists in red blood cells in a very limited number characterizing the healthy carrier horse (Onyiche et al., 2019).

Other countries have reported a wide prevalence range of *T. equi* and B. *caballi* using cELISA. In Malaysia, Al-Obaidi et al., (2016) reported infection rates of 19.59% and 25% for *T. equi* and *B. caballi* respectively, and in Spain, *T. equi* counted for 50.3% and *B. caballi*for11.4% (AL-OBAIDI et al., 2016; García-Bocanegra et al., 2013). This difference between countries in the infection rates could be related to prophylactic measures taken by different countries such as mobility restriction (Aziz & Al-Barwary, 2019), distribution of ticks, climatic factors (humidity, temperature) and management conditions (Al-Obaidi et al., 2016).

*Babesia* and *Theileria* may be carried by clinically

healthy horses for several years (Ribeiro et al., 2013). Few symptoms were noticed, mostly not specific to piroplasmosis including loss of condition, poor exercise tolerance and slow recovery in the horse population followed up. Chronic forms were the most encountered remaining generally asymptomatic (Alhassan et al., 2005; Ribeiro et al., 2013). Anaemia, resulting from an eventual hemoparasite infection (Maslin et al., 2004) was usually the nonspecific sign encountered.

The absence of correlation between seropositivity and the presence of clinical signs may be due to the endemic status of the region sampled whose horses are repeatedly infected (Oduori et al., 2015).

Epidemiological studies identified several risk factors related to high EP's prevalence such as equidae species, age, sex, breed, presence of ticks, type of activity and region (Aziz & Al-Barwary, 2019). In the current study, there were no significant differences between sexes and age groups, observation made by other authors (Aziz & Al-Barwary, 2019).

A significant difference between the type of activity groups ($p=0.045$) suggests that competition is a protecting factor for seropositivity. This could be the result of a better caring attributed to the fact that competition horses are better looked after and subject to a regular checking up during.

Although ticks are reported to be usually active in autumn and spring, some species such as *Dermacentor marginatus* and *Rhipicephalus bursa* are active during dry and wet seasons (Le Metayer, 2007); this may partly explain why *T. equi* infection was higher in winter than in other seasons in the current study. The minimum temperature for tick activity is evaluated at around 4.5 degrees on the ground (Kraemer, 2018) but with the increasing temperatures (17°C on average) ticks remain active. Piroplasmosis is endemic in temperate climates where the competent tick vectors prevail (Onyiche et al., 2019). The central region of Algeria was significantly the most affected ($p=0.004$). It can be considered as a risk factor for seropositivity since the variation in climatic conditions can lead to differences in prevalence and distribution of ticks.

In EP, a decrease of erythrocytic indicators (RBC count, haematocrit and haemoglobin levels) together with an increase in the MCV and MCHC were observed in most studies, but the existence of thrombocytopenia was also mentioned in several studies (Onyiche et al., 2019; Zobba et al., 2008).In the present study, unlike what was mentioned above, there were no significant differences in haematological parameters between the EP positive and the negative populations. Seropositivity did not modify the haematological parameters or haematological constants.

Anaemia was detected in only 63 horses (34.6%) may be as a result of parasitaemia in 29 cases (46%). It is the presence of antibodies that prevents massive infestations to occur as seen through the absence of clinical signs and presence of moderate anaemia. Low levels of parasitaemia maintain a chronic haemolysis state that is partially compensated by spinal regeneration as evidenced by moderate anaemia, absence of clinical signs and normocytosis. Studying more biological parameters would be useful to assess haemolysis (Knowles et al., 1994).In seropositive horses anaemia is moderate and normocytosis is present in nearly all cases. The latter can be associated with parasitaemia in the initial stage then macrocytosis appears and the number of reticulocytes increases in blood circulation (Onyiche et al., 2019).In 6 horses affected with positive parasitaemia, normocytosis was associated with hypochromia in 4, signalling an iron deficiency that can mask an initial macrocytosis, and macrocytosis was present in 2 resulting from hyperhemolysis. In all 6 cases, macrocytosis is probably related to haemolysis due to the presence of parasitaemia, although a deficiency in antipernicious factors (folates, B12) cannot be excluded. Other indicators (reticulocyte count, vitamin B12 and/or folic acid blood test) would have brought more clues on the mechanism of anaemia.

## CONCLUSION

Epidemiological studies carried out around the world have made it possible to determine the prevalence of EP, via various tests, to determine the characteristics of the affected horses as well as the strategies to fight the disease.

In Algeria, the results of a previous study has given information on the prevalence of *T.equi* in Algiers and its surroundings, and by extrapolation, on the physical and environmental characteristics of the horses' seropositivity and the risk factors.

EP is endemic in Algeria since all the regions sampled were affected with a predominance of positivity in central region. Season (winter), region (central) and intended activity are the risk factors associated with

the prevalence of the disease. Serological status and presence of parasitaemia have no influence on haematological parameters and on occurrence of anaemia.

EP has considerable consequences on health, economic and sports, owing to the very long lifespan of *T. equi* in the host and its intraerythrocytic multiplication and erythrocyte lysis causing anaemia. These facts remain unknown by breeders, as well as, by the veterinary authorities, the only actors able to control the spread of the disease.

## ACKNOWLEDGMENTS

The authors thank the Algerian Ministry of Higher Teaching and Scientific Research for contributing to her PhD training.

## ETHICAL STATEMENT

All animals' owners declared their oral consent before blood sampling which was performed by a qualified veterinarian according to the guidelines for the care and use of animal.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest related to this work.

## REFERENCES

1. Al-Obaidi, Q. T., Arshad, M. M., Al-Sultan, I. I., Azlinda, A. . B., Mohd Azam, K. G. K. 2016. Comparison between microscopic examination and competitive ELISA for diagnosis of equine piroplasmosis in Kelantan, Malaysia. Mal J of Vet Res, *7* (2), 23-29.
2. Alhassan, A., Pumidonming, W., Okamura, M., Hirata, H., Battsetseg, B., Fujisaki, K., Yokoyama, N., Igarashi, I., 2005. Development of a single-round and multiplex PCR method for the simultaneous detection of Babesia caballi and Babesia equi in horse blood. Vet. Parasitol. 129, 43-9. https://doi.org/10.1016/j.vetpar.2004.12.018
3. Aziz, K.J., Al-Barwary, L.T.O., 2019. Epidemiological study of equine piroplasmosis (Theileria equi and Babesia caballi) by microscopic examination and competitive-ELISA in Erbil Province North-Iraq. I*ran. J. Parasitol. 14, 404-412. https://doi.org/10.18502/ijpa.v14i3.1479*
4. Baptista, C., Lopes, M.S., Tavares, A.C., Rojer, H., Kappmeyer, L., Mendonça, D., Da Câmara Machado, A., 2013. Diagnosis of Theileria equi infections in horses in the Azores using cELISA and nested PCR. Ticks Tick. Borne. Dis. 4, 242-5. https://doi.org/10.1016/j.ttbdis.2012.11.008
5. Benchikh Elfegoun, M.C., Gharbi, M., Merzekani, Z., Kohil, K., 2018. Bovine piroplasmosis in the provinces of Skikda and Oum El Bouaghi (Northeastern Algeria): Epidemiological study and estimation of milk yield losses. Rev. d'Elev. Méd. Vét. des Pays Trop. https://doi.org/10.19182/remvt.31519
6. Benfenatki, A., Younes Bouacida, N.S., Ait Oudhia, K., Khelef, D., 2016. Prevalence of Theileria equi infection in Algiers urban area using cELISA and microscopic examination. Asian J. Anim. Vet. Adv. 11, 511-515. https://doi.org/10.3923/ajava.2016.511.515
7. Camacho, A.T., Guitian, F.J., Pallas, E., Gestal, J.J., Olmeda, A.S., Habela, M.A., Telford, S.R., Spielman, A., 2005. *Theileria (Babesia) equi* and *Babesia caballi* infections in horses in Galicia, Spain. Trop. Anim. Health Prod. 37, 293-302. https://doi.org/10.1007/s11250-005-5691-
8. Climates to travel [WWW Document], n.d. URL : https://www.climatestotravel.com/climate/algeria (accessed 4.1.20).
9. Farah, A.W., Hegazy, N.A., Romany, M.M., Soliman, Y.A., Daoud, A.M., 2003. Molecular detection of *Babesia equi* in infected and carrier horses by polymerase chain reaction. Egypt. J. Immunol. 10, 73-9.
10. Gaba, E., 2020. Wikipedia [WWW Document]. URL https://fr.m.wikipedia.org/wiki/Fichier:Algeria_location_map.svg (accessed 4.12.20).
11. García-Bocanegra, I., Arenas-Montes, A., Hernández, E., Adaszek, Ł., Carbonero, A., Almería, S., Jaén-Téllez, J.A., Gutiérrez-Palomino, P., Arenas, A., 2013. Seroprevalence and risk factors associated with *Babesia caballi* and *Theileria equi* infection in equids. Vet. J. 195, 172-8. https://doi.org/10.1016/j.tvjl.2012.06.012
12. Gray, J.S., Dautel, H., Estrada-Peña, A., Kahl, O., Lindgren, E., 2009. Effects of Climate Change on Ticks and Tick-Borne Diseases in Europe. Interdiscip. Perspect. Infect. Dis.https://doi.org/10.1155/2009/593232
13. Hinchcliff, K.W., Kaneps, A.J., Geor, R.J., 2004. Equine Sports Medicine and Surgery, Equine Sports Medicine and Surgery.https://doi.org/10.1016/B978-0-7020-2671-3.X5001-6
14. Kerario, I.I., Simuunza, M., Laisser, E.L.K., Chenyambuga, S., 2018. Exploring knowledge and management practices on ticks and tick-borne diseases among agro-pastoral communities in Southern Highlands, Tanzania. Vet. World. https://doi.org/10.14202/vetworld.2018.48-57
15. Knowles, D.P., Kappmeyer, L.S., Perryman, L.E., 1994. Specific immune responses are required to control parasitemia in *Babesia equi* infection. Infect. Immun. 62, 1909-13. https://doi.org/10.1128/iai.62.5.1909-1913.1994
16. Kraemer, D., 2018. Variation intra-journalière de l'activité des tiques Ixodes ricinus en fonction des données météorologiques. DVM thesis.
17. Le Metayer, A., 2007. Seroprevalence des piroplasmoses equines en france entre 1997 et 2005. Ecole nationale veterinaire d'alfort.
18. Maslin, J., Beugnet, F., Davoust, B., Klotz, F., 2004. Babésioses. EMC - Mal. Infect. https://doi.org/10.1016/j.emcmi.2004.07.003
19. Maurin, E., 2017. Guide pratique de médecine équine, MED'COM. ed.
20. Oduori, D.O., Onyango, S.C., Kimari, J.N., MacLeod, E.T., 2015. A field survey for the seroprevalence of *Theileria equi* and *Babesia caballi* in donkeys from Nuu Division, Kenya. Ticks Tick. Borne. Dis. 6, 683-688. https://doi.org/10.1016/j.ttbdis.2015.05.015
21. OIE, 2018. Equine piroplasmosis, in: Terrestrial Manuel. pp. 1310-1319.
22. Onyiche, T.E., Suganuma, K., Igarashi, I., Yokoyama, N., Xuan, X., Thekisoe, O., 2019. A review on equine piroplasmosis: Epidemiology, vector ecology, risk factors, host immunity, diagnosis and control.Int. J. Environ. Res. Public Health. https://doi.org/10.3390/ijerph16101736
23. Ribeiro, I.B., Câmara, A.C.L., Bittencourt, M. V., Marçola, T.G., Paludo, G.R., Soto-Blanco, B., 2013. Detection of *Theileria equi* in spleen and blood of asymptomatic piroplasm carrier horses. Acta Parasitol.58, 218-22. https://doi.org/10.2478/s11686-013-0127-9
24. Salib, F.A., Youssef, R.R., Rizk, L.G., Said, S.F., 2013. Epidemiology, diagnosis and therapy of *Theileriaequi* infection in giza, egypt. Vet. World 6, 76-82. https://doi.org/10.5455/vetworld.2013.76-82
25. Schnittger, L., Rodriguez, A., Florin-Christensen, M., Morrison, D., 2012. *Babesia*: A world emerging. Infect. Genet. Evol. 12, 1788-809.
26. Sevinc, F., Maden, M., Kumas, C., Sevinc, M., Ekici, O.D., 2008. A comparative study on the prevalence of *Theileria equi and Babesia*

*caballi* infections in horse sub-populations in Turkey. Vet. Parasitol.156, 173-7. https://doi.org/10.1016/j.vetpar.2008.06.006
27. Silva, M.G., Graça, T., Suarez, C.E., Knowles, D.P., 2013. Repertoire of *Theileria equi* immunodominant antigens bound by equine antibody. Mol. Biochem. Parasitol. 182, 109-15. https://doi.org/10.1016/j.molbiopara.2013.03.002
28. Thrall, M.A., Baker, D.C., Campbell, terry W., DeNicola, D., Fettman, M.J., Duane Lassen, E., Rebar, A., Weiser, G., 2004. Vet Hemato and Clin Chemist.
29. Van der Kolk, J.H., Veldhuis Kroeze, E.J.B., 2013. Infectious Diseases of the Horse : Diagnosis, pathology, management, and public health.
30. Wang, M., Guo, W., Igarashi, I., Xuan, X., Wang, X., Xiang, W., Jia, H., 2014. Epidemiological investigation of equine piroplasmosis in China by enzyme-linked immunosorbent assays. J. Vet. Med. Sci.76, 549-52. https://doi.org/10.1292/jvms.13-0477
31. Wilson, D., 2011. Clinical veterinary advisor: The horse, Clinical Veterinary Advisor: The Horse. https://doi.org/10.1016/C2009-0-38641-9
32. Ziam, H., Ababou, A., Kazadi, J.M., Harhoura, K.H., Aissi, M., Geysen, D., Berkvens, D., 2016. Prévalences et signes cliniques associés des piroplasmoses bovines dans les Wilayates d'Annaba et El Tarf, Algérie. Rev. Med. Vet. (Toulouse).
33. Zobba, R., Ardu, M., Niccolini, S., Chessa, B., Manna, L., Cocco, R., Pinna Parpaglia, M.L., 2008. Clinical and Laboratory Findings in Equine Piroplasmosis. J. Eq Vet. Sci. 28, 301-8.