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Research Article
Prevalence of *Theileria equi* Infection in Algiers Urban Area Using cELISA and Microscopic Examination

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Abstract

Background: Equine piroplasmosis are emerging diseases caused by *Babesia caballi* and/or *Theileria equi*. These parasites are transmitted by ticks and cause anemia, jaundice and hemoglobinuria. **Objective:** The objective of this study was to determine the prevalence of *Theileria equi* infection among horses residing on farms in the Algiers region to know the level of exposure to equine piroplasmosis and to investigate risk factors associated with this infection. **Methodology:** A total of 182 horses were tested. All sera were tested using a commercial kit of competitive ELISA and all blood samples were observed by microscopy. After microscopic examination of thin blood smears, parasites were detected in 29 of 182 horses (15.9%). The overall seroprevalence rate of *Theileria equi* infection was 29.1% (53 of 182 horses). **Results:** The results showed that 10.1% (20 horses) of the horses tested positive only by microscopic examination, 24.2% (44 horses) tested positive exclusively by competitive ELISA and 4.9% (9 horses) tested positive for both tests. These results demonstrates that equine piroplasmosis is present in Algiers region and showed that most of the positive horses are carriers ones. The study of risk factors showed that age, sex and activity had significant effects on the prevalence of *Theileria equi*. The absence of clinical signs in this study confirms the state of high endemicity. **Conclusion:** The results suggest that Algiers and surroundings is an endemic country and the deplacement of horses must be controlled to reduce risk of infection.

Key words: *Theileria equi*, piroplasmosis, prevalence, equine, cELISA

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The equine piroplasomosis is protozoal infection caused by *Babesia caballi* and *Theileria equi* which are tick-borne haemoprotozoan parasites[1].

The transmission of this pathology is caused by ticks of the genera *Dermacentor*, *Hyalomma* and *Rhipicephalus* of the Ixodidae family[2,3]. The disease is endemic in tropical and subtropical regions and can also be found in temperate areas[4].

This disease has high economic repercussions in the equine industry worldwide and have negative effects on the trade and transport of animals. It also causes losses in breedings ranging from a drop in performance until death[5,2].

The infection can be acute or sub-acute, it is characterized by fever, anemia, jaundice and hemoglobinuria[6,7] which can cause serious health effects on horses[8]. Some cases may lead to death[2] but most animals survive the acute phase and become asymptomatic carriers, which constitute the reservoirs of infection[2,4,9].

The parasite can be identified by microscopic examination of thin blood smears after their coloration by Giemsa stain[5]. This method is not very efficient in subacute forms, the parasite is difficult to find in blood smears of asymptomatic animals carriers.

Other methods of diagnosis are used for detection of chronic and inapparent forms of piroplasmosis, the most used are serological tests which include ELISA tests[5]. These methods are often used in epidemiological studies.

Up to now, few studies have been made in Africa, where piroplasmosis is hyperendemic[10,4,2] and no study has been made in Algeria. The aim of this study is to determine the prevalence of *T. equi* in Algiers by using microscopic examination and cELISA test.

MATERIALS AND METHODS

Horses and samples: Samples were collected from each animal between July, 2013 and April, 2014, a total of 182 horses of both sexes were tested for detection of equine piroplasmosis. The horses belong to 20 different stables which are distributed in different areas of Algiers (Algiers is located in Northern Algeria).

The age range was from 1-24 years. At the time of sampling, animals were examined for clinical disease and presence of ticks which were not found in our population of horses.

The horses of this study were used for different activities such as racing, recreation and breeding. Different information on the horses and their environment were collected through a questionnaire and transcribed in an excel table for statistical analysis.

From each animal, it was collected that blood sample from the jugular vein into sterile vacuum tube and a peripheral puncture for preparation of thin blood smears. Sera obtained from these blood samples were stored at -20°C until needed.

Microscopic observation: After Giemsa stain coloration, the observation of blood smears under the objective 100 with oil immersion allows to search intraerythrocytic piroplasms.

The differentiation of *T. equi* and *B. caballi* is based on the size of parasite and the number of cells formed by division[11]. *Theileria equi* is generally described as a group of four small piroplasms of 1-2 µm, forming a tetrad called "Maltese cross" (Fig. 1)[12,11], large pairs of piroplasms of 2×3 µm that are linked by their finest extremities are the characteristic form of *B. caballi*[12].

Competitive enzyme-linked immunosorbent assay (cELISA): Serum samples were tested for the presence of *T. equi* antibodies using commercial cELISA according to the manufacturer's instructions (*Babesia equi* antibody test kit, cELISA, référence catalogue No. 274-5, Veterinary Medical Research and Development). This test is a competitive, enzyme-linked immunosorbent assay (cELISA) and is intended for detection of *T. equi* antibodies in equine serum samples by using recombinant proteins of *T. equi* merozoites.

Statistical analysis: Chi-square test was used to see the potential impact that can have the variables concerning the

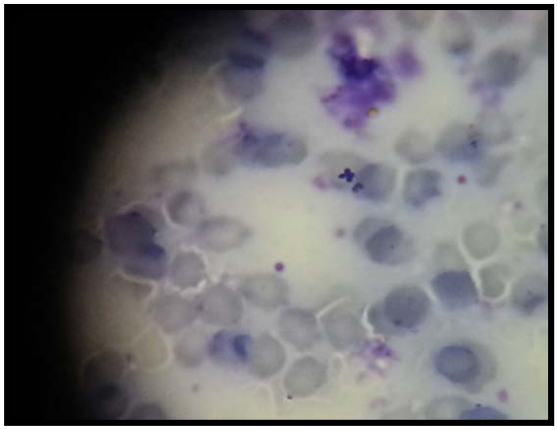

Fig. 1: *Theileria equi* "Maltese cross" observed under optical microscope

horses (age, gender and activity) and their environment (presence of herbs, management team) on the overall prevalence. Variables showing a significant difference ($p<0.05$) were selected to identify risk factors. For this, the Odds Ratio (OR) and their Confidence Intervals (CI) with a 95% confidence level was calculated. Analysis were done with XLSTAT 2014.

RESULTS

Of the 182 samples tested by cELISA, 53 horses (29.12%) were positive for *T. equi*. Microscopic examination reveals that 29 horses (15.9%) were positive to *T. equi*. An association with *B. caballi* was found on 11 horses (37.9%).

The study of serological results demonstrates that environmental parameters like of season, humidity, presence of herbs, water and horses surroundings, management team and type of housing show no significant differences of positivity rates.

Concerning horse-related parameters, only gender, age and ability show significant differences of positivity rates. The horses used for breeding showed higher seropositivity (47.9%) than horses used for recreation (31.4%) and those used for competition (18.7%).

The Odds Ratio (OR) confirms that a breeding activity is a risk factor for a seropositivity (OR = 2.57, 95% CI, 1.21-5.43, p = 0.0133) (Table 1).

Prevalence of antibodies against *T. equi* shows significant differences between males and females. Indeed the seropositivity rate in females (39.3%) is lower than seropositivity in males (24.6%). The horses aged between 10 and 14 years also show a higher seropositivity regarding the others age groups. The OR confirms that the category "female" (OR = 0.98, 95% CI, 1.012-3.88, p = 0.046) that is in the scope of an age between 10 and 14 years (OR = 2.11, 95% CI, 1.08-4.14, p = 0.028) are both risk factors for seropositivity (Table 1).

The other parameters like race vaccination and vermifugation don't show correlation statistically significant with positivity of results.

The results of combined tests show that 109 (59.89%) horses are negative for both tests. Nine horses (4.94%) are simultaneously positive by serology and microscopy, 20 horses (10.98%) are positive by microscopy and negative by serology and 44 horses (24.17%) are negative by microscopy and positive by serology (Table 2).

DISCUSSION

This study is the first research aimed to estimate the prevalence of equine piroplasmosis in North of Algeria using serological and microscopic tests. The equine piroplasmosis is an important disease with repercussions on horse market and equestrian sports events.

The microscopic observation of thin blood smears for diagnosis of *B. caballi* and *T. equi* may give false negative results because it doesn't have enough sensitivity to give an accurate result in case of low parasitemia.

Babesia and *Theileria* organisms may be carried by clinically healthy horses for several years[2]. The movement of asymptomatic carrier horses may contribute to the transmission and propagation of this pathology. The absence of clinical signs means that horses are asymptomatic chronic carriers and demonstrates that Algiers and its surroundings are an endemic area.

A higher positivity of clinically suspect horses to *T. equi* by microscopy may be due to an increased virulence of

Table 1: Risk factors associated to Equine piroplasmosis in Algiers urban area, Algeria

	Serological results (positive)				
Characteristics	No.	%	p	OR	IC
Ability					
Competition	14	18.7	0.0105	0.40	0.19-0.800
Recreation	22	31.4	0.5882		
Breeding	17	47.9	0.0133	2.57	1.21-5.430
Gender					
Male	31	24.6	0.0460	0.50	0.25-0.980
Female	22	39.3	0.0460	0.98	1.012-3.88
Age					
1-4	1	8.3	0.1356		
5-9	19	25.3	0.3472		
10-14	23	40.4	0.0280	2.11	1.08-4.140
15-19	10	30.3	0.8688		
> = 20	0	0.0	0.2959		

Table 2: Results of piroplasm detection by qualitative assessment of blood smears and cELISA analysis from asymptomatic horses in Algiers urban area, Algeria

	Serology	
	Positive (%)	Negative (%)
Parasitology		
Positive	9 (4.94)	20 (10.98)
Negative	44 (24.17)	109 (59.89)

this pathogen in comparison with *B. caballi*[1]. Sensitivity of the *T. equi* and *B. caballi* cELISAs is higher than that of the complement fixation test; specificity of the cELISAs lie between 99.2 and 99.5%[13].

Previous studies using cELISA test in different countries of Turkey and Portugal showed positivity rates ranging between 19.3 and 48.6%[14,5,15]. In this study, it was found that a seroprevalence of *T. equi* 29.12% which joined the prevalence found in a study conducted by Garcia-Bocanegra *et al.*[14] on imported horses from Spain to Switzerland (27.3%).

Different studies performed in Turkey using microscopic examination of Giemsa-colored thin blood smears showed a prevalence between 0-58.18%[5]. In Venezuela, one horse 0.3% had a positive result to *T. equi*[16].

In this study, piroplasms having a *T. equi* shape are found in 29 blood smears (15.9%). This prevalence is surprisingly high despite the fact that these horses had no clinical signs. That results suggest that these animals were in early infection or that the region of sampling is an endemic region, where the horses always have a basic rate of parasites in their blood because of the repeated infections. Microscopic examination has a low sensitivity[17], that makes us think that this prevalence could be higher.

In the present study, 44 horses (24.17%) are negative by microscopy and positive by serology, these horses are carrier horses, they represent the principal disease reservoir. On the other hand, the horses that are seronegative and positive by microscopy are those newly infected.

The results showed that microscopic examination of blood smears is less sensitive diagnostic method than antibodies detection, especially in cases of low parasitemia. The microscopic examination can be effective in acute piroplasmosis[2].

In this study, differences are found between seropositivity in competition horses (18.7%) and breeding horses (45.9%). This could be attributed to the fact that competition horses are better cared than breeding ones.

Seropisitivity rates in mares and stallions are different. Of the 126 stallions 31 (24.6%) are seropositive for *T. equi* and the seropositivity in mares is 22 of 56 mares (39.3%). The statistical study shows that the female sex was a risk factor for seropositivity that could be attributed to the gravid state of females which could cause immunodepression.

CONCLUSION

The seroprevalence of *T. equi* piroplasmosis in horses is evaluated at 29.1% in this set. The region is therefore, considered as an endemic area. This prevalence doesn't represent the global one but it allows to give an idea by extrapolation.

Predisposing risk factors to equine piroplasmosis are individualised in this study, the majority can be compared to those found through other studies on the subject. Other factors remain to be demonstrated through larger studies.

The results of this study allow to have an idea of the prevalence of affection particularly that of *T. equi* in Algiers and surroundings, as they permit to know physical, sanitary and environmental characteristics of seropositive horses. These results though very modest can assist veterinary doctor in his daily practice.

This study is in our knowledge, the first about equine piroplasmosis in Algeria. It highlights the importance of knowing epidemiology, distribution and prevalence of equine piroplasmosis for implementation of control measures.

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