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Original article

A preliminary study on ticks and equine Babesiosisin rural areas (North-east of Algeria), Algeria

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ABSTRACT

During the year 2015-2016, a study was conducted to identify ticks that infect horses in rural areas in North-east of Algeria. Five parasitic species were observed, *Rhipicephalus bursa* predominates with 45%, *Rhipicephalussanguineus*: 10%, *Rhipicephalusannulatus* 15%, *Hyalommamarginatum* 13% and *Hyalommaanatolicum:* 17%. Moreover, the prevalence of babesiosis in local horse breeds was determined. A global prevalence of 70% was found for equine babesiosis in the two areas (Sedrata and Lake of birds). The horses were found positive (Giemsa coloration) for *Theileriaequi* and *Babesiacaballi*. No significant differences were encountered concerning sex and age of infected animals. Removal of ticks manually does not prevent for ticks infestation and the exposure of horses to piroplasmosis. So, apart from favorable climatic factors, the presence of horses with other animals (ruminants and dogs) appears to be a factor in the infestation of horses by ticks. There were no significant differences between the positivity to *Babesia* with the factors sex and age of the horses (p>0, 0.5). After this preliminary work, a wider study of ticks of the equine population in Algeria is essential for the establishment of a plan of prevention against these mites and the diseases that they transmit.

1. Introduction

Ticks are arthropod pests of horses and other animals. In addition to theist blood sucking activity they might cause skin irritation and allergy, additionally; some species are known to transmit pathogens to the equine species (Magnarelli et al., 2000; Soulsby, 1982). Along with the recent reconnaissance of the species of these arthropods on different animals in Algeria (Boozer et Macintire, 2003; Matallah et al., 2013; Meddour Bouderda et Meddour, 2006), there is a growing interest in the importance of equine infections with pathogens such as that: *Babesiacaballi*, *Theileriaequi*. Equine babesiosis is a disease frequently observed by our veterinary practitioners. It is a tick-borne protozoan disease of horses caused by *Babesiacaballi* and / or *Theileriaequi* (Barbosa et al., 1995). It has been estimated that only 10% of the world's horses live in areas considered free from babesiosis (Rothschild, 2013).

The occurrence of equine babesiosis is closely linked with the geographic distribution and seasonal activity of its biological vectors: *Dermacentor*, *Rhipicephalus* and *Hyalomma* species of ticks (Ibrahim, 2011; Rothschild, 2013), However, the severity of clinical signs is variable. Furthermore, horses affected with babesiosis remain carriers for long periods and may act as a source of infection for ticks (Barbosa et al., 1995; Mantran et al., 2004). *Rhipicephalus bursa* and *Rhipicephalussanguineus* are the two main vectors of this disease in North Africa (Bourdeau, 1993).

Little information is available on this subject in North-east Algeria; therefore the current study aimed to determine the most common tick species in horses and the prevalence of exposure of horses to infection with transmitted pathogens.

2. Materials and methods

2.1. Areas of study (Fig. 1)

Two different bioclimatic areas were selected for this study. The climate of the first area (Sedrata) is influenced by factors that give its specific characteristics. Distancing 120 km from the Mediterranean Sea, the penetration of marine and wet currents is easy. The town of Sedrata is located in a basin, surrounded by a mountainous relief. This area is characterized by a *semi-humid* climate. Sedrata is distinguished by a warm summer and a cold and humid winter and the rainfall reaches an average of 640 mm per year (Marre, 1987).

The Mediterranean climate of the second area (Lake of birds) shows a wet winter between October and April, and a dry summer with an annual average of 800 mm per year. The winds are fairly common but weak (Marre, 1987).

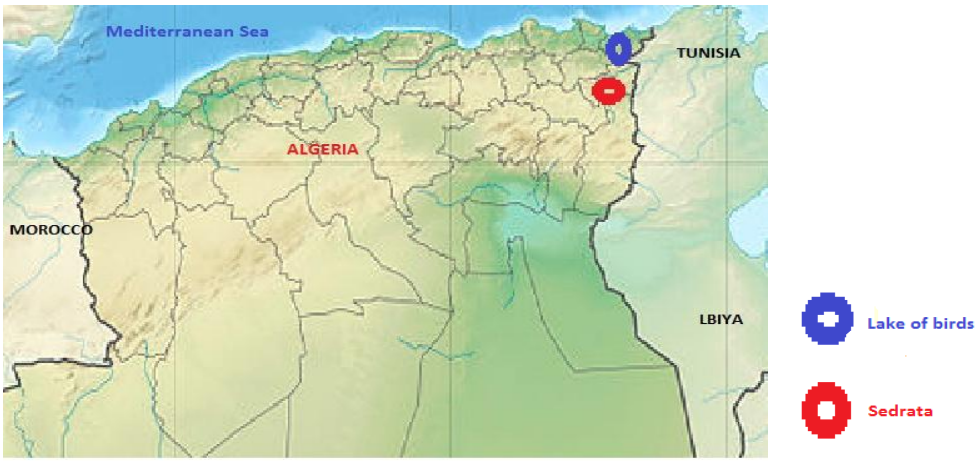

Fig. 1. Geographical location of the study areas.

2.2. Animal population and period of study

Thirty horses (12 females and 13 males and 5 foals) of the local race “barbe” distributed in the two areas (fifteen for each) were included in the study. These horses were kept under an extensive management in a rural

environment. The horses (different sex and age) lived with large, small ruminants and herding dogs. The animals' owner does not use any kind of medication against ectoparasites but they practice manual ticking (removal by hands). The study took place from November 2015 to June 2016.

2.3. Collection, observation and identification of ticks

The collection of ticks was carried out on accessible anatomical parts because of the difficulties found at the pasture (animals free). The ticks were preserved in 70° ethanol. Tick identification was performed at the Laboratory of Parasitology (Veterinary Sciences Department (Faculty of Science and Life, Chadli Ben jdid University El Tarf) using a binocular. Determination of the genus and species was based on the observation of the morphological characteristics established by Hillyard (1996) and Estrada-Peña et al. (2004) (Estrada-Peña et al., 2004; Hylliard, 1996).

2.4. Equine piroplasmosis

Complete clinical examination was carried out in all horses. Moreover, blood smears were made using a blood sample taken from the ear of each horse after clipping, shaving and disinfection of the area. Smears were fixed in methanol and then stained with Giemsa and examined under light microscope.

The original grinding technique of May Grunwald Giemsa (MGG) consists of covering the smear with a solution of May Grunwald and allowing to act for 3 minutes. Wash immediately with buffered water. Cover with Giemsa diluted to 3% in phosphate buffer at pH 7.2 and allow standing for 15 minutes. Wash with tap water and dry (Benchikh-Elfegoun et al., 2007; Levine, 1971).

This method is reliable when parasitaemia is moderate or elevated. However, the severity of the clinical signs is not always correlated with high parasitaemia (Benchikh-Elfegoun et al., 2007).

Our study is based on clinical signs (Anemia, abatement) as well as factors favoring animal infestation, humid climate, and external parasitism of horses by ticks. The coloring method of Giemsa remains useful in the acute phase of the disease (with hyperthermia) (Hendrix et Robinson, 2006).

Under microscopic examination, *Babesiacaballi* was identified as large paired pyriform parasites, while the *Theileriaequi* parasites were identified as Maltese cross arrangement of merozoites (Hendrix et Robinson, 2006; Levine, 1971).

3. Results and discussion

3.1. Ticks

Throughout the study period a total of 47 ticks were collected on horses in the two areas with a majority of males (60%). Eighteen ticks (38%) were collected and identified in Sedrata, while the number was 29 ticks (62%) in Lake of birds. Five species of ticks belonging to two genera. *Rhipicephalus* and *Hyalomma* were identified: Rhipicephalus *bursa*, *Rhipicephalussanguineus*, *Rhipicephalusannulatus*, *Hyalommaanatolicum* and *Hyalommamar ginatum*. Rhipicephalus *bursa* is the predominant tick in the two areas with a global proportion of 45% the genus *Rhipicephalu*s (*bursa*, *sanguineus* and *annulatus*) accounted for 70% of the ticks (Table 1). A maximum parasitic load of *Rhipicephalus bursa* is 1, 5 tick / horse in June whereas it is 3 ticks / horse in the same month in the area of Lake of birds, Furthermore, In November, in each area, 10 horses were infected with ticks (67% of all horses), 33 ticks were collected in this month. Four species of ticks were identified in area of Sedrata; *Rhipicephalus bursa*- *Rhipicephalusannulatus- Hyalommamarginatum* and *Hyalommaanatolicum* whereas an additional tick species was found in the area of Lake of birds, *R. sanguineus*. The genera of *Rhipicephalus* presented 63% (genera of *Hyalomma*: 37%). 4 females and 6 males of horses were infected by ticks in Sedrata while 3 females and 7 males were infected in Lake of birds, Moreover, no ticks were found during the period from December to May, in addition that in June, 2 horses (females) in each area were infected with ticks, However We were able to collect 14 ticks belonging to the same previous genera and *Rhipicephalus* predominates with 60% (*Hyalomma*: 40%). The most important regions for tick attachment were at limbs 36%. Furthermore, ticks were also found attached at neck, ears and inguinal part with rates of 28%, 19% and 17% respectively (Table 2).

Table 1
Distribution of ticks collected on horses in the two study areas.

Areas	Sedrata			Lake of birds		
Ticks	♀	♂	Total (%)	♀	♂	Total (%)
Rhipicephalusbursa	3	5	8(44)	4	9	13(45)
Rhipicephalussanguineus	0	1	1(5)	2	2	4(14)
Rhipicephalusannulatus	0	3	3(17)	0	4	4(14)
Hyalommamarginatum	3	0	3(17)	3	0	3(10)
Hyalommaanatolicum	2	1	3(17)	2	3	5(18)
Total	8(44%)	10(56%)	18(100%)	11(38%)	18(62%)	29(100%)

Table 2
Attachment sites of ticks.

Anatomical placement					
Ticks	Neck	Ears	Limbs	Inguinal region	Total
Rhipicephalus bursa	5	3	9	4	21
Rhipicephalussanguineus	2	0	1	2	5
Rhipicephalusannulatus	2	1	3	1	7
Hyalommamarginatum	0	4	2	1	6
Hyalommaanatolicum	4	1	2	1	8
Total	13(28%)	8(17%)	17(36%)	9(19%)	47(100%)

3.2. Study of piroplasmosis

Examination of blood smears stained with Giemsa of suspected horses revealed that animals were infected with Botha small cross (Maltese cross) indicated the *Theileriaequi* and the large paired pyriform parasites which indicated *Babesiacaballi*. Seven (7) horses out of 10 (70%) were infected with both type of babesia. The detection of *babesia* takes place in the months of November, April, May and June, respectively (Photo 1), On the other hand *Theileriaequi* is observed alone in three horses at the area of Lake of birds. *Babesiacaballi* is observed just in the area of Sedrata with a double infestation (+ *Theileriaequi*) in four horses.

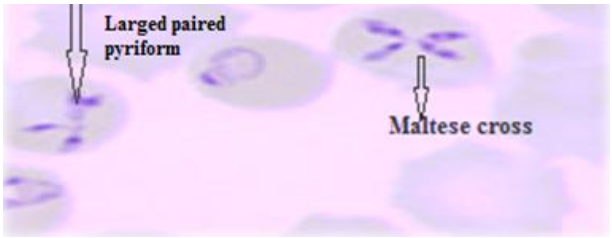

Photo 1. Giemsa stain blood smear show both type of *babesia*, light microscope 1000.

Despite the apparently low number of 30 horses and a global search for ticks (Accessible anatomical parts) and the difficulties of the environment where the horses lived, we were able to find fives spices belonging to the two genera (*Rhipicephalus* and *Hyalomma*) of ticks which shows an affinity of these arthropods for this host (horse) (Filipe Dantas-Torres, 2013; Goddard, 2000; Wall et Shearer, 2001). So, the genera of *Hyalomma* and *Rhipicephalus* predominate in southern Europe, North Africa and the Middle East while species of the genus *Dermacentor* are more common in northern Europe (Gummow et al., 1996). The low number of ticks collected without the advice of any veterinary medical interfusion is explained by the fact that the owners practiced the removal of the parasites manually. *Rhipicephalus bursa* were found as a predominance ectoparasite which were parasite the horses environment in this area these result were in sporting data with (Goddard, 2000; Perez-Eid, 2007), Since, this tick parasites cattle and sheep also which makes a favorable environment in the presence of these animals with our horses (Soulsby, 1982), Moreover, In the north of Algeria, The activity of this tick was reported by Benchikh-Elfegoun et al. (2007), which he was able to find a percentage of 9% of *Rhipicephalus bursa*

on all ticks infesting the cattle, occupying the second position after *Rhipicephalus (Boophilus) annulatus* (80%). *Rhipicephalusannulatus* was recorded with 17% (second position after *Rhipicephalus bursa*) in Sedrata and 14% in Lake of birds (third position after *Rhipicephalus bursa* and *Hyalommaanatolicum*); a percentage that gives it an affinity for horses.

Other tick species, including *Hyalommaanatolicum* and *Hyalommamarginatum,* are also cited as ticks infesting the horse (Goddard, 2000; Hylliard, 1996). *Rhipicephalussanguineus* is a dogs tick by excellence (Solano-Gallegoet and Baneth, 2011; Wall et Shearer, 2001), and its presence has shown this animal as a source of infestation of horses as well. *Hyalommaanatolicum* and *Rhipicephalussanguineus* were also found on dogs in Punjab, Pakistan (Ul-Hasan et al., 2012). Climatic conditions, in particular humidity (Matallah et al., 2013) and the mixed breeding of animals at pasture seems to be an important factor in the infestation of horses by these tick species. In the current study ticks were found attached to different body parts, since animals limbs were indicated the most prominent area for attachment, this difference can be explained by the external morphology of the horse of which members are the first part to be met for ticks (fine skin) (Boozer et Macintire, 2003; Matallah et al., 2013).

Microscopic examination of the blood smears of the suspected horses detected the two forms of *babesia* (paired large merozoites and the cross of Malta), and consequently *Theileriaequi* and *babesiacaballi* which are responsible for equine piroplasmosis. 70% of suspected horses were carrying pathogens of piroplasmosis (7 / 10), This rate is important when comparing with that reported is reported by Salim et al. (2007) which they showed the presence of piroplasmosis in all regions of Sudan with a prevalence of 35.95% and an absence of *Babesiacaballi* (Salim et al., 2007). The piroplasmosis also led to the death of two mares in Jordan (Hailat et al., 1997). In France, a thesis carried out by Le Matayer (2007), reports a prevalence of 18.92%, of which 13.78% are equine sera positive for *Theileriaequi* and 9.24% positive for *Babesiacaballi* (Le Matayer, 2007). This rate should be taken into account for more in-depth studies on a higher number of horses.

In our study, *Theileriaequi* is the predominant piroplasmid infecting horses. This fact is in agreement with previous reports by other authors studying horses from the Mediterranean region (Bashiruddin et al., 1999). Also, the predominance of this pathogen was reported in Spain with a prevalence of 18, 92% (Garcia-Bocanegra et al., 2013), South Africa (18, 4%) (Gummow et al., 1996). And in Belgium one study of which showed 16 positive cases of piroplasmosis out of 23 suspected (Mantran et al., 2004). So, *Babesiacaballi* was present with low frequency in various studies (Bashiruddin et al., 1999; Le Maytayer, 2007; Salim et al., 2007).

It is important to note that Criado-Fornelio et al. (2003), did not found this piroplasmid, although they found *babesiacaniscanis* instead. For them, the main reason explaining why nobody ever found *babesiacaniscanis* to be a parasite of horses is simply that it is morphologically very similar to *babesiacaballi* (Purnell, 1981). The same author has reported in Spain (CriadoFornelio et al., 2006), that *babesiabovis* is an occasional parasite of equines, since it was detected in two symptomatic horses and according to him the ability of some piroplasms to infect multiple hosts. These results must be taken into account, particularly in relation to our method of diagnosis, which remains less sensitive to confirm the presence of certain piroplasms.

4. Conclusion

The predominance of *Rhipicephalus bursa* and the frequency of *Theileriaequi* reported by our work remain very close to the different results entailing this subject. Despite the little sampling and the classic method used in this research, the results gave us an image on this research theme for ticks and diseases transmissible to horses in northeastern of Algeria. The study of ticks and vectorial diseases in horses makes a very important research axis that must be extended for the equine population in Algeria in order to identify the different species of these mites, their seasonal activities and the diseases they transmit. The use of more precise techniques notably the PCR (polymerase chain reaction) is indispensible for more reliable studies in order to draw a preventive plan to minimize the infestation of our horses.

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