

# Bovine piroplasmosis in the provinces of Skikda and Oum El Bouaghi (Northeastern Algeria): Epidemiological study and estimation of milk yield losses

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## Keywords

Cattle, *Babesia bigemina*, *Babesia bovis*, *Theileria annulata*, piroplasmosis, Algeria

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## Summary

During a survey carried out in 88 farms located in two provinces of Northeastern Algeria – Oum El Bouaghi and Skikda –, 89 clinical cases of piroplasmosis (i.e. an 11.6% infection rate in the examined cattle) were reported between May and September 2011 in 86.4% of the visited farms. Three species of piroplasms were identified in the blood films stained with Giemsa: *Theileria annulata* (in 94.0% of the sick animals), *Babesia bovis* (33.7%) and *Babesia bigemina* (3.4%), with 31% of co-infections. No significant difference was observed in the prevalence between the two provinces, nor between cattle breeds. Tropical theileriosis caused a significant drop in milk production estimated at 319 liters per infected cow during the two months following the disease. Seven tick species were collected in the farms during the study period. The infestation peak was observed in July for *Hyalomma scupense* (syn. *H. detritum*), *H. lusitanicum*, *H. anatolicum*, *Rhipicephalus bursa* and *R. turanicus*, and in August for *H. marginatum* and *H. excavatum*. A positive correlation was observed between clinical cases of tropical theileriosis and *H. scupense* infestation on one hand, and clinical cases of babesiosis and *R. bursa* infestation on the other hand.

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## ■ INTRODUCTION

Piroplasmosis are tick-borne diseases due to protozoa belonging to two genera, *Babesia* and *Theileria*. These infections cause high losses in many countries (Minjauw and McLeod, 2003) and have an important impact, particularly since a high proportion occurs in

farms with limited resources. Moreover, the infections are frequently asymptomatic leading to low but persistent losses for many years. In North Africa, four species of piroplasms have been described, namely *Theileria annulata*, *Babesia bovis*, *B. bigemina* and *B. divergens*, of which the first three species cause high losses in cattle (Flach and Ouhelli, 1992; Flach et al., 1995; Gharbi et al., 2006; Darghouth, 2008; Gharbi et al., 2011). In Algeria, numerous epidemiological and parasitological studies have been carried out more than 70 years ago (Sergent et al., 1945) and led to the development of the first live attenuated vaccine, using the *Theileria annulata* strain 'Kouba'. This vaccine has been used to immunize thousands of animals in Morocco, Algeria and Tunisia.

Since this period, few studies have been performed in Algeria despite the probable high socio-economic impact of these infections and the high incidence of clinical cases. There is a gap in recent knowledge

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relative to the epidemiology of these infections and the biology of their vector ticks. Indeed, the implementation of a control program and the ranking of the different health problems require a deep knowledge of the local epidemiology of these diseases. The present study focused on the estimation of the prevalence of bovine piroplasmosis (babesiosis and tropical theileriosis) and on the characterization of tick species in two enzootic provinces of Algeria. Milk yield losses caused by *T. annulata* infection have also been estimated in a sample of the diseased cows.

MATERIALS AND METHODS

Study region

The study was carried out in two different bioclimatic regions of Northeastern Algeria, one semiarid (province of Oum El Bouaghi), the other subhumid (province of Skikda), between May and September 2011 (Figure 1; Table I).

Table I

Characteristics of Oum El Bouaghi and Skikda provinces, Algeria

Characteristics	Oum El Bouaghi	Skikda
Location	From 35° 42' to 36° 16' N From 6° 19' to 7° 56' E	From 36° 40' to 37° 01' N From 6° 24' to 7° 56' E
Surface (km <sup>2</sup> )	7638	4118
Altitude (m)	700–1700	0–300
Mean yearly rainfall (mm)	North: 350–400 South: 200–250	800–1200
Mean temperature (°C)	Summer: 32.6 Winter: 9.3	Summer: 27.0 Winter: 13.5
Mean monthly pluviometry (mm)	Summer: 11.3 Winter: 49.7	Summer: 2.7 Winter: 118.0

Source: Directions des services agricoles, Wilaya de Skikda, Wilaya d'Oum El Bouaghi; 2012

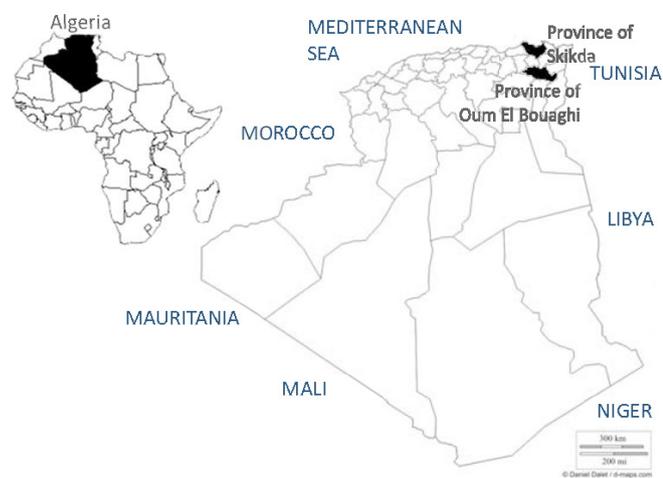


Figure 1: Geographical location of Oum El Bouaghi and Skikda provinces, Algeria.

The province of Oum El Bouaghi is located west of Constantine plains. It consists of three topographic domains: the south Tell in the North, highlands, and Saharan Atlas in the South. The plains are large, including depressions forming endorheic basins (sebkha). Mountains (between 800 and 1700 m altitude) represent 25% of the province total area. The climate is continental: cold and wet in winter, hot and dry in summer. The forest is mainly constituted of reforestation trees, 'maquis', shrubland and oak. Cattle farms are predominantly semi-intensive. Animals pasture only during the spring (Direction des services agricoles, Wilaya d'Oum El Bouaghi, 2012).

The province of Skikda is located 70 meters above sea level on average. It is characterized in its western and eastern sides by rugged mountains carved by rivers. The climate is Mediterranean: temperate and rainy in winter, hot and dry in summer. The vegetation is varied. Forests cover 1984 square kilometers (48% of the total surface), consisting mainly of cork oak (*Quercus suber* L.), maritime pine (*Pinus pinaster* Aiton), Algerian oak (*Quercus canariensis* Willd.) and eucalyptus (*Eucalyptus* spp.). Although cattle breeding is intensive, farms contain a limited number of cows (eight on average), mainly of exotic dairy breeds (Direction des services agricoles, Wilaya de Skikda, 2012).

Farms, piroplasms and ticks

Eighty-eight farms, randomly chosen from the list of farmers in the districts, were included in the present study. Sixty-nine were located in the province of Oum El Bouaghi (48 had exotic animal breeds and 21 local ones) and 19 in the province of Skikda (12 had exotic animal breeds and seven local ones), keeping in total 770 cattle head.

Each farm was visited once during our investigation. Thorough clinical examination of all the animals was performed. Clinical signs of piroplasmosis (pyrexia, anorexia, anemia and enlargement of lymph nodes) were recorded. Five milliliters of EDTA blood samples were collected from the jugular vein of the sick animals, and a Giemsa-stained blood smear was made for each sample at the Laboratory of Parasitology, Institute of Veterinary Sciences (University of Mentouri, Constantine, Algeria). Slides were examined for hemoparasites under microscope (x 100 objective).

Ticks infesting all the cattle of the farms were collected in tubes containing 70% ethanol and further identified at the laboratory according to Bouattour's keys (2002). For each province, parasitological indicators were estimated (Table II).

Table II

Epidemiological indicators determined in Oum El Bouaghi and Skikda provinces, Algeria

Epidemiological indicator	Formula
Infection prevalence (%)	100 x num. of infected animals / num. of examined animals
Infection frequency of piroplasm species (%)	100 x num. of infected animals by species / num. of infected animals
Tick infestation prevalence (%)	100 x num. infested animals / num. of examined animals
Tick intensity	Num. of collected ticks / num. of infested animals
Tick abundance	Num. of collected ticks / num. of examined animals

**Estimation of milk yield losses caused by tropical theileriosis**

All the diseased cows were treated against tropical theileriosis with buparvaquone at the conventional dose of 2.5 mg/kg intramuscularly. Fifteen milking cows with typical symptoms of tropical theileriosis and positive blood smears were randomly included in this part of the survey. Their milk yield production was assessed once a week by the National Inter-Professional Office Council of Milk who paid the farmer 42 Algerian dinars (AD) (1 AD ≈ 0.01 €) per liter of milk. Milk yield losses of these cows were quantified by estimating the variations in milk yields, considering the difference between the production for the week before the occurrence of the disease and the production for each week until day 56 (week 8) after the occurrence of clinical tropical theileriosis, when normal production was apparently restored. The loss for each day between two successive weekly production assessments was estimated linearly.

**Statistical analyses**

All the data were processed with Excel software. The comparison of different indicators was performed with a cut-off value of 0.05 (Schwartz, 1993) and the comparison of the percentages was carried out with the Chi-square test with EpiInfo 6 software. The correlation study was performed with Spearman test to compare the monthly prevalence of tropical theileriosis with *H. scupense* abundance or that of babesiosis with *R. bursa* abundance (see definition in Table II) in the farms visited during the same month.

■ **RESULTS**

**Epidemiological data**

Out of 770 examined cattle, 89 were clinically infected by piroplasmosis *sensu lato*, as confirmed by blood smears examination, i.e. an overall infection rate of 11.6%. Seventy-six of the farms (86.4%) were infected with piroplasms and only 12 were uninfected. There was no difference between the infection rates in the two provinces ( $p > 0.05$ ) (Table III). Infection rates varied according to the period with a peak in July: 10.5%, 37.3%, 7.8% and 1.3% in June, July, August and September, respectively ( $p < 0.001$ ).

**Parasitological results**

Examination of Giemsa-stained blood smears showed three piroplasm species, *T. annulata*, *B. bovis* and *B. bigemina*, as well as co-infections (Table IV, Figure 2). There was a significant difference between the infection rates by the various piroplasms ( $p < 0.001$ ): *T. annulata* was by far more frequent (observed in 94.4% of the infected

**Table III**

Infection prevalence of cattle from Oum El Bouaghi and Skikda provinces, Algeria

	Num. of cases / total num. of examined animals	Prevalence (CI)
Oum El Bouaghi (semiarid)	70/609 <sup>a</sup>	11.5% (± 2.5%)
Skikda (humid)	19/161 <sup>a</sup>	11.8% (± 5.0%)
Total	89/770	11.6% (± 2.3%)

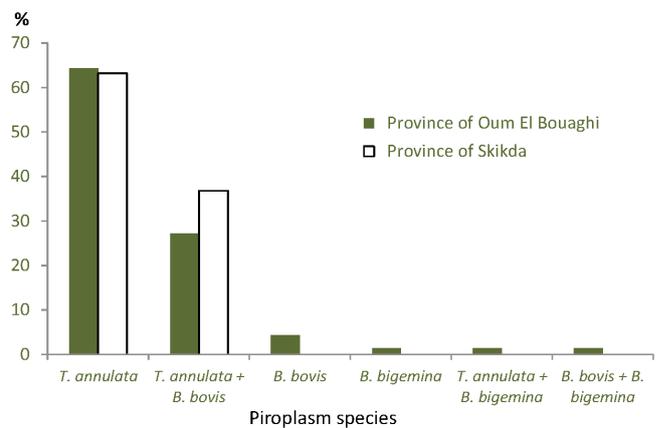
CI: confidence interval; <sup>a</sup> Lines with the same letter indicate the absence of a significant difference.

**Table IV**

Relative importance of the different piroplasmoses in Oum El Bouaghi and Skikda provinces, Algeria

Piroplasm species	Num. of cases / num. of infected animals	% Frequency (CI)
<i>Theileria annulata</i> only	57/89	64.0 (± 10.0%)
<i>Babesia bovis</i> only	3/89	3.4 (± 3.7%)
<i>B. bigemina</i> only	1/89	1.1 (± 2.2%)
<i>T. annulata</i> + <i>B. bovis</i>	26/89	29.2 (± 9.4%)
<i>T. annulata</i> + <i>B. bigemina</i>	1/89	1.1% (± 2.2%)
<i>B. bovis</i> + <i>B. bigemina</i>	1/89	1.1% (± 2.2%)

CI: confidence interval



**Figure 2:** Relative importance of the different piroplasmoses in infected cattle of Oum El Bouaghi and Skikda provinces, Algeria.

cattle) than *B. bovis* (33.7%) and *B. bigemina* (3.4%). Co-infections by *T. annulata* and *B. bovis* were frequent (29.2%) (Table IV). There was a significant positive correlation between prevalence and age. For young animals (< 2 years old), the infection rate was 25.0% (23/92), whereas it was lower for the other categories: 8.9% (35/393) for 2–4-year-old cattle, and 10.9% (31/285) for cattle older than 4 years ( $p < 0.001$ ). The infection rate for the various cattle breeds were 12.6% (60/477) and 9.9% (29/293) for exotic breeds and local breeds, respectively ( $p = 0.26$ , not significant).

**Tick infestation**

In total 459 ticks belonging to the genera *Hyalomma* and *Rhipicephalus* were collected between May and September 2011. Seven species were identified. The three most frequent species were *H. scupense* (56.4%), *H. excavatum* (13.9%) and *H. lusitanicum* (10.9%). The other species (*H. marginatum*, *R. bursa*, *H. anatolicum* and *R. turanicus*) were less frequent (Figure 3). *H. scupense* was the most frequent species in both regions: 56.6% of the collected ticks in Oum El Bouaghi and 52.9% in Skikda (Figure 3). Moreover, all the epidemiological indicators related to *H. scupense* were the highest (Figure 4). The distribution of the ticks was unimodal with a peak in July for *H. scupense*, *H. lusitanicum*, *H. anatolicum* and *R. bursa* (Figure 5), and in August for *H. marginatum* and *H. excavatum* (Figure 6).

There was a significant correlation between the incidence of tropical theileriosis and *H. scupense* intensity ( $R^2 = 0.91$ ;  $p < 0.01$ ) (Figure 7). There was also a strong correlation between babesiosis incidence and *R. bursa* intensity ( $R^2 = 0.99$ ;  $p < 0.01$ ) (Figure 8).

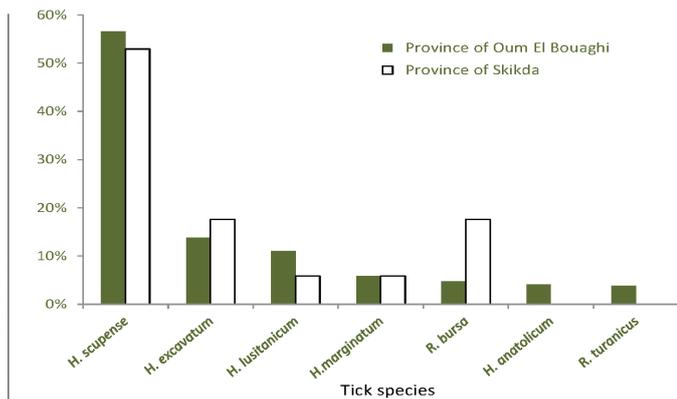


Figure 3: Relative importance of the different tick species in the studied farms of Oum El Bouaghi and Skikda provinces, Algeria.

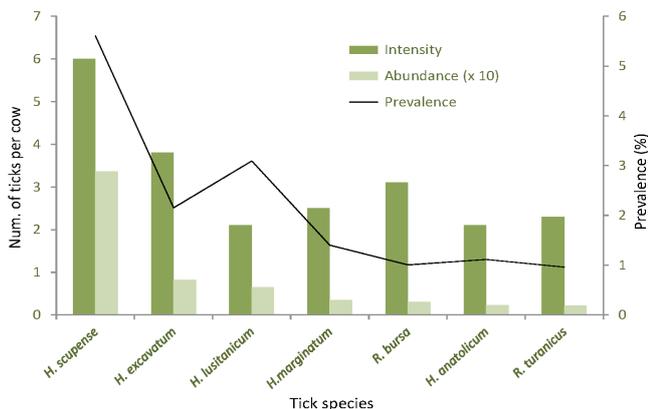


Figure 4: Epidemiological indicators of cattle tick burdens in Oum El Bouaghi and Skikda provinces, Algeria (see Table II for definition of indicators); the abundance was increased tenfold to observe better the differences.

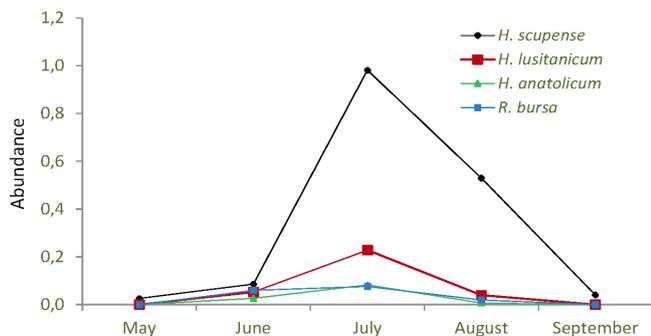


Figure 5: Monthly abundance of four species of adult ticks in the 88 visited farms of Oum El Bouaghi and Skikda provinces, Algeria.

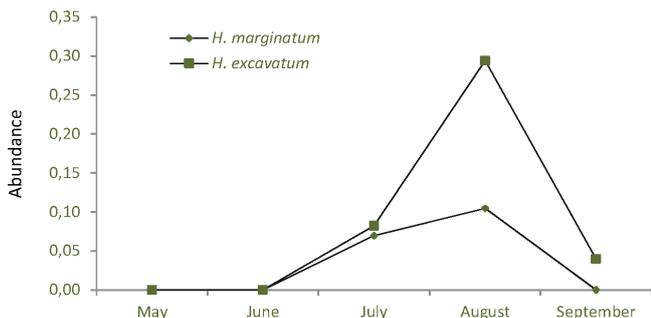


Figure 6: Monthly abundance of two species of adult ticks in the 88 visited farms of Oum El Bouaghi and Skikda provinces, Algeria.

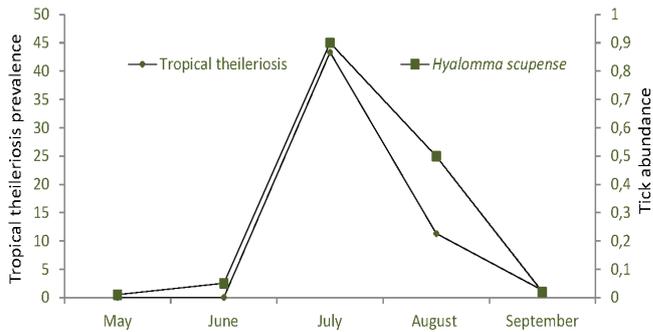


Figure 7: Prevalence of tropical theileriosis and infestation by *Hyalomma scupense* in the infected farms (n = 76) of Oum El Bouaghi and Skikda provinces, Algeria.

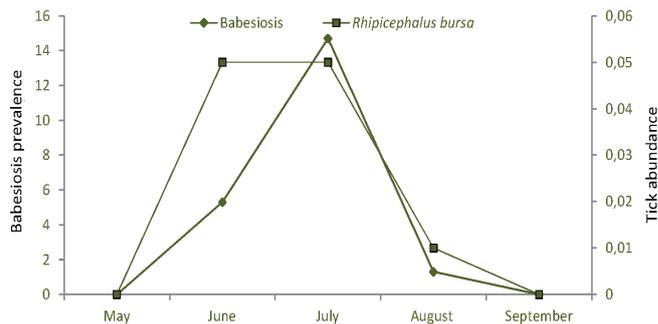


Figure 8: Prevalence of babesiosis and infestation by *Rhipicephalus bursa* in the infected farms (n = 76) of Oum El Bouaghi and Skikda provinces, Algeria.

Milk yield in diseased cows

There was a dramatic drop in milk yield in diseased cows with a maximum mean decrease of 15.5 L per animal at the first production assessment post-disease. The milk yield then slowly increased again and the losses were successively 11.8 L at day 14, 7.5 L at day 21, 6.8 L at day 28, etc. Reestablishment of the milk yield occurred between days 42 and 56 post-disease (Figure 9). The total milk yield loss per diseased cow was estimated at 319 L during the two months following the disease, corresponding to 13,398 AD (133.98 €).

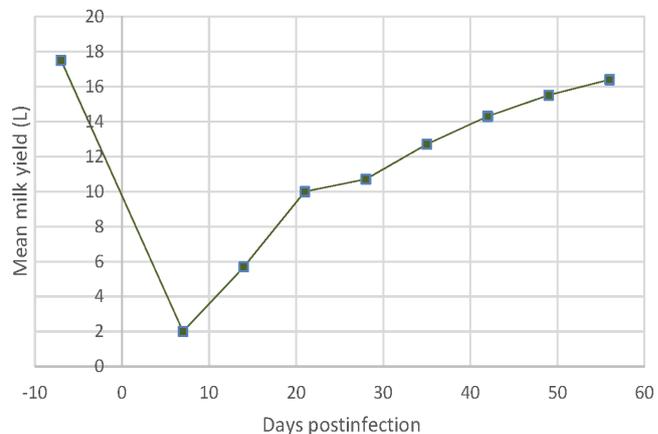


Figure 9: Average weekly assessment of individual milk yield for the 15 surveyed cows of Oum El Bouaghi, and Skikda provinces, Algeria, from one week before tropical theileriosis onset to eight weeks postinfection.

## ■ DISCUSSION

Bovine piroplasmoses are common tick-borne diseases in tropical and subtropical regions with a significant economic impact (Uilenberg, 1995). They represent a real constraint to the development and intensification of cattle breeding in several regions of the world, particularly in North Africa, although recent studies on these diseases are scarce in Algeria. The results of the present study, carried out to provide descriptive epidemiological data, showed that the regions of Oum El Boughi (semiarid region) and Skikda (subhumid region) were endemic for piroplasmosis, especially for bovine tropical theileriosis. Prevalence rates were very similar in the two provinces. In July, during the infection peak, more than one third of the examined cattle were affected by at least one of the piroplasms.

The study showed a high frequency of bovine tropical theileriosis, representing 94% of piroplasmosis cases. Such high infection rates have been reported in Algeria by Sergent et al. (1945) in a longitudinal study performed over 22 years, during which 2094 of 3875 cattle diagnosed with piroplasmosis (i.e. 54%) had been infected with *T. annulata*. More recently, Ziam and Benaouf (2004) have reported a high prevalence (53.7%) of tropical theileriosis in the regions of Annaba and El Tarf (Northeast Algeria, humid climate). The same predominance of tropical theileriosis has also been reported in dairy farms in Morocco: for example, the prevalence of *T. annulata* infection was 41% in the examined animals (vs 13% for *B. bigemina*) in Doukkala, 27% (vs 14% for *B. bigemina*) in Gharb, 66% (vs. 16% for *B. bigemina*) in Haouz (El Haj et al., 2002). A high co-infection prevalence rate was observed in the present survey, especially for the combination *T. annulata* and *B. bovis* (29%). Such high co-infection prevalence has also been reported in Algeria (20.3%; Ziam and Benaouf, 2004) and Tunisia (25%; M'Ghirbi and Bouattour, 2009).

Seven tick species were identified on the animals. *Hyalomma scupense*, the main vector of *T. annulata* in Algeria, was the most frequent species in both provinces with a higher intensity and abundance

than other tick species. The study showed a very high correlation between the incidence of piroplasms and cattle infestation by their vectors, *H. scupense* for *T. annulata* and *R. bursa* for *Babesia* spp.

Tropical theileriosis causes a milk yield drop and in some cases a dry-off. It is even the main symptom for consultation (with hyporexia-anorexia). Total milk yield losses have been estimated in the present survey at 319 liters per infected cow in the two months following the disease. Since all diseased animals during the present study were treated with buparvaquone, this loss assessment may be underestimated as the treatment prevented the occurrence of mortality in cattle. Moreover, losses were calculated only from the first milk production assessment after the infection was observed: the losses between disease onset and this first milk yield estimation were not taken into account, which is another cause of underestimation. On the other hand, it was not possible to take into account the usual evolution of milk production, which slowly and regularly decreased after the production peak occurred approximately on the second month. Losses may have thus been overestimated for some of the surveyed animals: this gap was known but the data needed to overcome it were lacking. This study, however, showed the high impact of milk yield losses in cows affected with tropical theileriosis in Algeria.

The cost of milk loss after two months of disease was estimated at 13,398 DA for each infected animal. If the treatment (5000 DA per diseased cow) was also included, losses due to *T. annulata* infection would be estimated at 18,398 DA (183.98 €) per infected cow. These high losses should motivate the implementation of a national control program against both ticks and tick-borne diseases in Algeria.

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## Résumé

**Benchikh Elfegoun M.C., Gharbi M., Merzekani Z., Kohil K.** Piroplasmoses bovines dans les provinces de Skikda et d'Oum El Bouaghi (nord-est de l'Algérie) : étude épidémiologique et estimation des pertes de production laitière

Au cours d'une enquête menée dans 88 fermes de deux provinces du nord-est de l'Algérie – Oum El Bouaghi et Skikda –, 89 cas cliniques de piroplasmoses, soit un taux d'infection de 11,6 % des bovins examinés, ont été enregistrés entre mai et septembre 2011 dans 86,4 % des fermes visitées. Trois espèces de piroplasmes ont été identifiées dans les étalements sanguins colorés au Giemsa : *Theileria annulata* (chez 94,0 % des bovins malades), *Babesia bovis* (33,7 %) et *Babesia bigemina* (3,4 %), avec 31 % de co-infections. Il n'y avait pas de différence significative de prévalence entre les deux provinces ni entre les races bovines. La theilériose tropicale a provoqué une baisse significative de la production laitière, estimée à 319 litres par vache infectée pendant les deux mois suivant la maladie. Sept espèces de tiques ont été collectées dans les fermes pendant l'étude. Le pic d'infestation a été observé en juillet pour *Hyalomma scupense* (syn. *H. detritum*), *H. lusitanicum*, *H. anatolicum*, *Rhipicephalus bursa* et *R. turanicus*, et en août pour *H. marginatum* et *H. excavatum*. Une corrélation positive a été observée entre les cas cliniques de theilériose tropicale bovine et la charge parasitaire en *H. scupense*, d'une part, et entre les cas cliniques de babesioses et la charge parasitaire en *R. bursa*, d'autre part.

**Mots-clés :** bovin, *Babesia bigemina*, *Babesia bovis*, *Theileria annulata*, piroplasmose, Algérie

## Resumen

**Benchikh Elfegoun M.C., Gharbi M., Merzekani Z., Kohil K.** Piroplasmosis bovina en las provincias de Skikda y Oum El Bouaghi (Argelia del Noreste): Estudio epidemiológico y estimación de las pérdidas de rendimiento de leche

Durante una encuesta llevada a cabo en 88 fincas localizadas en dos provincias del Noreste de Argelia – Oum El Bouaghi y Skikda –, se reportaron 89 casos clínicos de piroplasmosis (o sea una tasa de infección de 11,6% en el ganado examinado), en 86,4% de las fincas visitadas entre mayo y septiembre 2011. Se identificaron tres especies de piroplasmas en los frotis sanguíneos con tinción de Giemsa: *Theileria annulata* (en 94,0% de los animales enfermos), *Babesia bovis* (33,7%) y *Babesia bigemina* (3,4%), con 31% de co-infecciones. No se observó diferencia significativa entre la prevalencia de las dos provincias, ni entre las razas de ganado. La theileriosis tropical causó una caída significativa de la producción estimada de leche de 319 litros por vaca infectada durante los dos meses siguientes a la enfermedad. Durante el periodo de estudio, se colectaron siete especies de garrapatas en las fincas. El pico de infestación fue observado en julio con *Hyalomma scupense* (syn. *H. detritum*), *H. lusitanicum*, *H. anatolicum*, *Rhipicephalus bursa* y *R. turanicus*, y en agosto con *H. marginatum* and *H. excavatum*. Se observó una correlación positiva entre los casos clínicos de la theileriosis tropical y la infección con *H. scupense* por un lado, y los casos clínicos de babesiosis y *R. bursa* por el otro.

**Palabras clave:** ganado bovino, *Babesia bigemina*, *Babesia bovis*, *Theileria annulata*, piroplasmosis, Argelia