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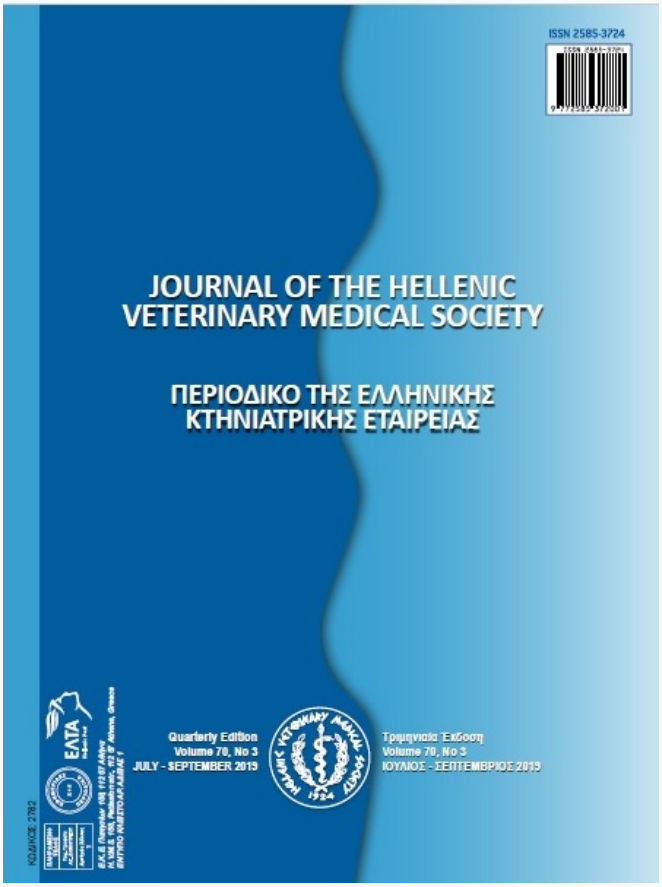

Prevalence of Trypanosoma evansi in horses (Equus caballus) and donkeys (Equus asinus) in El-Bayadh district, southwestern Algeria

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Research article
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Prevalence of *Trypanosoma evansi* in horses (*Equus caballus*) and donkeys (*Equus asinus*) in El-Bayadh district, southwestern Algeria

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ABSTRACT. *Trypanosoma evansi* is a parasite that causes surra in a variety of wild and domestic animals and is mainly transmitted by biting flies in Africa, Asia and Latin-America. Horses infected by *Trypanosoma evansi* present a chronic weight loss, icterus, oedema, anemia, abortions and neurological troubles. Due to this parasite, cases of human trypanosomiosis have been reported in different countries by contacting with infected animals. In this study, 206 healthy equines (177 horses and 29 donkeys) from El-Bayadh district, located in southwest Algeria, were tested for the presence of parasites in blood using Giemsa-stained blood films and for the presence of antibodies against *T. evansi* using CATT /*T. evansi*. While none of the equines showed detectable parasites in the blood, the individual seroprevalence of *T. evansi* was found to be 46.6% (CI 95%, 40.7-54.4%). Out of 98 positives samples, 56.1% (55/98) were shown at level 1 (+), 27.5% (27/98) at level 2 (++) and 16.3% (16/98) at level 3 (+++). The results show that out of 177 tested horses, 80 were seropositive to *T. evansi*, 45.2% (CI 95%, 37.8-52.5%) and out of 29 tested donkeys, 18 were seropositive to *T. evansi,* 62.1% (CI 95%, 44.4-79.7%). A questionnaire for the owners, targeted to associate risk factors for surra in horses, showed that environmental factors that are favorable for Tabanids, such as water and vegetation, but also promiscuity with dromedaries were positively associated with the seroprevalence rate in the horses. El-Bayadh district is a highly endemic region for surra in Algeria.

Keywords: *Trypanosoma evansi*, Prevalence, Horses, Donkeys, Algeria

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INTRODUCTION

Trypanosoma evansi was first identified in India where it caused an endemic disease known as "surra" in equines and camels (Evans, 1880-1881). Since then, this parasite has been found to infect many more domestic and wild animals, such as bovines, small ruminants and dogs (Fernandez et al., 2009, Desquesnes et al., 2013). Surra is widespread throughout Africa (Hilali et al., 2004, Birhanu et al., 2015, Fikru et al., 2015); Asia (Tuntasuvan et al., 2003, Hasan et al., 2006); and Latin-America (Hoare, 1965) and even shows occasional outbreaks in Europe (Tamarit et al., 2010). The disease is transmitted mechanically by insect vectors such as *Tabanus* and *Stomoxys* (Luckins, 1988, Brun et al., 1998) and also by vampire bats (*Desmondus rotundus)* in South America (Desquesnes et al., 2013).

Surra is a neglected tropical disease with devastating clinical impacts for the affected animals. Infected Horses with *T. evansi* have fever usually associated with parasitaemia, chronic weight loss, icterus, oedema, anemia, abortions and neurological deficits (Gardiner and Mahmoud, 1992). The parasite can cross the blood brain barrier and cause ataxia, hyperexitability and progressive paralysis of hind quarters (Rodrigues et al., 2009). In contrast, *T. evansi* infected donkeys present in general a subclinical infection (Mahmoud and Gray, 1980). In Brazil, losses due to mortality and treatment in equines infected by *Trypanosoma evansi* have been estimated to amount to 2.4 million dollars per year (Herrera et al., 2004). Human trypanosomiosis due to *T. evansi* has been reported in different countries (Joshi et al., 2005, Powar et al., 2006, haridy et al., 2011). A transmission by ingestion of meat from infected animals has been demonstrated in Vietnam (Van Vinh Chau et al., 2016).

In Algeria, there is little information about the prevalence of surra in most animals and districts. One previous article reported a 14% prevalence of the parasite in blood smears of camels in southwest Algeria (Bennoune et al., 2013).

The objectives of this study were twofold: first, to determine the prevalence of *T. evansi* parasites and antibodies in equines in El-Bayadh region and second, to associate physical and environmental risk factors for contracting surra in equines using a questionnaire.

MATERIALS AND METHODS

Study design and sample collection

EL-Bayadh district is located on the southwest of Algeria. It covers a total area of 71 697 km² (3 % of national territory) between 33°40′49″ N and 1°01′13″ E. The district consists of three distinct agro-climatic zones: high plains, near saharan and saharan atlas.

The population of horses and donkeys in El-Bayadh region is about 1250 and 1897 heads, respectively (MADR, 2016).

The study is achieved during March 2016. A number of 206 equines have been collected from the three agro-climatic zones.

A total of 177 horses and 29 donkeys were sampled, 139 males (121 horses and 18 donkeys) and 67 females (56 horses and 11 donkeys). These animals are divided into 3 different classes according to their age: < 5 years (104 horses and 17 donkeys); 6 to 11 years (58 horses and 12 donkeys) and 15 horses older than 11 years old. Different breeds of horses were sampled: Barb (n=155), Arab/barb (n=22). The chosen horses were used either for racing (n=10), or for pleasure riding (n=167). Donkeys were used for working.

Blood samples were obtained via a jugular vein by using 10 ml vacutainer tubes (which contained no anti-coagulants or preservatives), and then centrifuged at 3000 rpm for 10 min to get serum. The serum was collected in 1.5 ml Eppendorf tubes and kept at −20 °C until analysis by CATT/*T. evansi*.

Questionnaire

A case study has been done for each sampled animal. It contains information for the physical characteristics (gender, age, breed, purpose and herd structure), the environmental characteristics (promiscuity with dromedaries, contact with watering points and vegetation) and health care (vaccination, vermifugation). Each animal was examined for clinical signs of trypanosomiosis (anemia, icterus, oedema, weight loss and neurological signs).

Blood smears

Blood samples were collected into sterile vacuum tubes from the jugular vein of each animal. A blood film was prepared for each animal sampled. The smear was fixed by May-Grunwald stain for 3 minutes. It was washed with distilled water and then covered by

Giemsa stain for 30 minutes and again washed with distilled water to eliminate excess of stain. The slides were left to dry and examined with a light microscope by immersion oil at objective X100.

Serology

The CATT /*T. evansi*™ (Card agglutination test for trypanosomiosis due to *Trypanosoma evansi*) (Prince Leopold Institute of Tropical Medicine, Antwerp. Belgium) is a direct agglutination test used for detection of specific antibodies in blood, plasma and sera. It is included in the terrestrial manual of the word organization for animal health. The performance of the CATT /*T. evansi*™ was evaluated with two complement fixation tests in horses in Kazakhstan, the results determined the sensitivity of the kit at 80.2% and the specificity at 98.5% (Claes et al., 2005).

The test was used for serodiagnostic of trypanosomiosis due to *T. evansi* according to the manufacturer's instructions. Briefly, the agglutination reaction is done on card composed of 10 circles. In each circle, 25µl of diluted sera and one drop of Ag (about 45µl) are deposited and then mixed. Once the ten mixtures were made, the card was placed on an electric rotator at 70 rpm for 5 minutes. Each card had two circles for the positive and negative controls delivered by the manufacturer. Samples were considered positives when they have a blue granular agglutination. Agglutination levels were scored as: -, ± (negatives) or +, ++, +++ (positives).

Statistical analysis

The seroprevalence of *Trypanosoma evansi* in horses and donkeys were calculated as the number of seropositive animals divided by the total of animals sampled. For risk factors analysis, the independent variables were subjected to univariate analysis which was performed using the chi^2 test ($n>5$) or Fisher's exact test ($n<5$). The P value < 0.05 was considered statistically significant. Associations between individual animal serostatus and independent variables were assessed using logistic regression. A backwards stepwise approach was used to find the best fitting model to describe the dataset. Multivariate model selection was based on the Akaike information criterion (AIC) and the best model was selected using the lowest AIC. The P value, odds ratio with 95% CI for explanatory variables were also calculated. All analyses were carried out using RStudio (version 1.1.383, RStudio Inc., Boston, MA).

RESULTS

Blood smears

All the examined blood smears (177 horses and 29 donkeys) were negative for the presence of *Trypanosoma evansi*.

Serology

Out of 206 samples (177 horses and 29 donkeys) tested by CATT /*T. evansi*, 98 were found to be seropositive. Among the 98 seropositive samples, agglutination reactions were scored level 1 (+) in 56.1% (55/98), level 2 (++) in 27.5% (27/98) and level 3 (+++) in 16.3% (16/98). Out of 177 tested horses, 80 (45, 2% [CI 95%, 37.8-52.5%]) were seropositive for *T. evansi:* 27% (47/177) scored level 1 (+), 11% (20/177) level 2 (++) and 8% (14/177) level 3 (+++).

Out of 29 tested donkeys, 18 (62.1% [CI 95%, 44.4-79.7%]) were seropositive for *T. evansi:* 28% (8/29) scored level 1 (+), 24 % (7/29) at level 2 (++) and 7% (2/29) at level 3 (+++).

Risk factors

Horses

Results of the univariate analysis of physical and environmental variables compared to the prevalence of *Trypanosoma.evansi* infection in horses (*Equus caballus*) in El-Bayadh region are shown in table 1. Seropositivity for *T. evansi* in horses was detected in all three agroclimatic zones of EL-Bayadh district. The highest seroprevalence rate was founded in the high plains area: 71.8% (CI 95%, 65.6-78.09%), followed by the near-Saharan area: 41.67% (CI 95%, 34.8-48.5%), while the lowest rate was observed in the Saharan Atlas: 26.9% (CI 95%, 20.8-33.1%). The area's in the agro-climatic zones showed a significant association with seropositivity (P= 0.00001).Female horses had higher seropositive rates than male horses, with 57.1% (CI 95%, 44.2-70.1%) versus 39.66% (CI 95%, 30.9-48.4%) respectively. There was a significant association between gender and seropositivity (P = 0.029).

The horses that avoided promiscuity with dromedaries had a lower seropositivity rate 33.3% (CI 95%, 21.1 – 45.6%) than horses that had contact with dromedaries 50.8% (CI 95%, 41.9-58.9%) . The association between promiscuity with dromedaries and seropositivity was significant (P = 0.028).

Table 1. Univariate analysis of *Trypanosoma.evansi* infection in horses (Equus caballus) compared to physical and environmental variables

Risk factors	Category	No	Positive No	Seroprevalence % (95% CI)	P-value
Age	≤ 5	106	46	43.4 (34 – 52.87)	0.258
	6 - 11	58	29	50 (37.1 – 62.9)	
	> 11	15	4	26.7 (4.3 – 49)	
Gender	Male	121	48	39.7 (30.9 – 48.4)	
	Female	56	32	57.1 (44.2 – 70.1)	0.029
Breed	Barbe	155	69	44.5 (36.7 – 52.4)	0.628
	Arabe-Barbe	22	11	50 (29.1 – 70.9)	
Housing	Box	10	2	20 (23.6 – 28.1)	
	Stable	167	78	46.7 (16.1 – 26.2)	0.099
Purpose	Racis	10	2	20 (4.8 – 44.8)	
	Hobies	167	78	46.7 (39.1 – 54.3)	0.099
Promiscuity with Dromedaries	Yes	120	61	50.8 (41.9 – 59.8)	0.028
	No	57	19	33.3 (21.1 – 45.6)	
Watering points	Yes	133	66	49.6 (41.1 – 58.1)	
	No	44	14	31.8 (18 – 45.6)	0.039
Vegetation	Yes	157	76	48.4 (40.6 – 56.2)	
	No	20	4	20 (2.5 – 37.5)	0.000000
Zone	High Plains	64	46	71.8 (65,6-78,1)	
	Saharan Atlas	89	24	26,9 (20.8 – 33.1)	0.00001
	Near Sahara	24	10	41.7 (34.8 – 48.5)	

Univariate analyses ($\chi 2$ test for significance)

Table 2: Univariate analysis of *Trypanosoma.evansi* infection in donkeys (Equus asinus) compared to physical and environmental variables

Risk factors	Category	No	Positive No	Seroprevalence % (95% CI)	P-value
Age	< 5	17	12	70.6 (48,9-92,2)	0,438
	6 - 11	12	6	50 (21,7-78,3)	
Gender	Male	18	10	55.5 (32,6-78,5)	0,448
	Female	11	8	72.7 (32,9-93,9)	
Promiscuity with Dromedaries	Yes	20	12	60 (38,5-81,5)	1
	No	9	6	66.7 (35,9-97,5)	
Watering points	Yes	23	15	65.2 (45,7-84,7)	0,645
	No	6	3	50 (9,1-90)	
Zone	High Plains	15	11	73,3 (39,4-92,2)	0,371
	Saharan Atlas	8	4	50 (17,2-84,3)	
	Near Sahara	6	3	50 (13,9-88,2)	

Univariate analyses ($\chi 2$ test for significance)

Horses that did not live near water points had a lower seroprevalence rate 31.8% (CI 95%, 18 – 45.6%) than those who did 49.62% (CI 95%, 41.1 – 58.1%). Similarly, horses that are not surrounded by dense vegetation had a lower seroprevalence 20% (CI 95%, 2.5 – 37.5%) than those who did 49.03% (CI 95%, 40.6 – 56.2%). Both environmental characteristics: water points (P=0.039) and vegetation (P=0.000000) were associated with seropositivity (P< 0.005).

The age of animals, horse breed and housing conditions were not statistically significantly associated with seropositivity (p > 0.05).

Results of the multivariate logistic regression are summarized in table 3. Two risk factors were associated with *T.evansi* infection: sex and promiscuity with dromedaries (P<0.005). In this study, the risk of males for being seropostive was decreased by 67% than in femals (P=0.006, OR=0.37, 95% CI= 0.17-0.74%). Horses living in promiscuity with dromedaries were 2.49 times more likely to be infected by *T.evansi* (P=0.013, OR=2.49, 95% CI= 1.23-5.25%).

Table3: Factors influencing the risk of *Trypanosoma.evansi* infection in horses (Equus caballus)

Risk factors	Category	Odds ratio	95% confidence interval (OR)	P-value
Gender	Male Female	0.37 Ref	0.17-0.74	0.006
Age	≤ 5 6 – 11 > 11	Ref 1.35 0.28	 0.68-2.69 0.07-0.95	 0.386 0.054
Watering points	Yes No	2.00 Ref	0.94-4.36	0.074
Promiscuity with Dromedaries	Yes No	2.49 Ref	1.23-5.25	0.013

Donkeys

No significant differences were found in the study of the influence of physical and environmental characteristics on the seroprevalence of donkeys' samples (Table 2).

DISCUSSION

Trypanosoma evansi is the most widely distributed pathogenic salivarian trypanosome in animals. This parasite is cosmopolitan, affects a wide range of hosts (Desquesnes et al., 2013). In this paper, we have studied the prevalence of *T. evansi* parasites and antibodies in equines and the risk factors for surra in El-Bayadh district, southwestern Algeria.

A general weakness of this study is the fact that we were not able to microscopically observe the infecting parasite. The prevalence of parasites with the blood smear technique was 0%. This result is in agreement with a study on horses in Jordan (Abo-Shehada et al., 1999). Direct examination of the parasite in blood films usually fails in detecting the parasite if their concentration is less than 2.5 x 10^6 parasites per ml of blood (Chappuis et al., 2005). The sensitivity of detecting parasites in stained blood smears is thus very low. Concentration techniques such as the micro-haematocrite centrifugation technique (MHCT) or the mini-anion-exchange centrifugation technique (MAECT) are recommended to increase the sensitivity of direct observation of the parasite (Tehseen et al., 2015). Alternatively, PCR is the most sensitive diagnostic tool for the molecular confirmation of *T. evansi* infection (Ramírez-Iglesias et al., 2011). PCR has been demonstrated to be a method of choice to detect infection by *T. evansi* in horses (Clausen et al., 2003); this technique was not available in our study.

To our knowledge, this study is the first report of the detection of *T. evansi* antibodies in equines in Algeria. The total CATT/*T. evansi* seroprevalence in equines in El-Bayadh district was 47.6% (CI 95%, 40.7-54.4%). The majority of the tested animals (56.1%) scored the lowest agglutination score (+). This result confirms that trypanosomiosis due to *Trypanosoma evansi* is endemic in Algeria.

The seroprevalence obtained in horses alone was 45.2% (CI 95%, 37.8-52.5%), similar rates were found in Egypt and Jordan (Abo-Shehada et al., 1999; Zayed et al., 2010). Other publications reported higher seroprevalence rates, 73% were found in Brazil using IFAT (Herrera et al., 2004) and 92% were demonstrated in Philippine (Dargantes et al., 2009). Some studies report lower seroprevalence, as is the case in Malaysia (13%) (ELshafie et al., 2013) and India (27%) (Laha and Sasmal, 2008).

For donkeys, the seroprevalence was 62.1%. This rate is much higher than the seroprevalence reported in other countries; Egypt (44%) (Zayed et al., 21010) and India (11.53%) (Kumar et al., 2013).

The serological test used in this study, CATT/ *T. evansi* is a rapid and easy test to use but it cannot differentiate between past and present infection (Tehseen et al., 2015).

In this study, female horses are more exposed to the infection than males (P=0.006, OR=0.37, 95% CI= 0.17-0.74%). That result may be explained by the immunosuppression due to the gestation and to the difference of activities between females and males. This result agrees with some studies in horses, Elshafie et al (2013) were reported that female horses were 2.1 times more likely to be infected by *T.evansi*. In

contrast, a study in camels demonstrated that higher rates of male camels were infected with *T. evansi* than females (Njiru et al., 2004).

The seroprevalence of *T. evansi* varied in relation to the 3 agroclimatic zones. The highest rate was detected in the high plains zone (P=0.00001). Here, Tabanids encounter favorable conditions (water, vegetation and woodland) that allow proliferation of these vectors.

Several studies have showed that the Tabanids in North Africa are implicated as the main vectors of *T.evansi* transmission (Baldacchino et al., 2014). Also, Animals that live near watering points (P=0.039) and dense vegetation (P=0.000000) are the most infected. Watering points and vegetation constitute a favorable environment to the survival of vectors.

The dromedaries' population in EL-Bayadh district is about 1450 heads (MADR, 2016). In this study, the contact of horses with dromedaries was considered to be a real risk factor, horses in contact with dromedaries were 2.49 times more likely to be positive for serology. Camels are known to reach high parasitemia in their blood, and therefore there mere proximity to horses in an area with vectors already likely increase risk for transmission. A study realized in El-Oued province, southeast Algeria, reported parasites in 14% of the Giemsa-stained blood smears of dromedaries (Bennoune et al., 2013).

No significant association was found between seropositivity and age, breed, housing and purpose of horses. Age was not significantly associated with the infection by *T.evansi* but horses aged between 6 and 11 years had higher rates of seroprevalance. A previous study revealed an augmentation of seropositivity to *T. evansi* in camels aged 5-11 years (Dia et al., 1997).

Horses that are living in individual boxes are less exposed to the infection by *T. evansi* because the contact with vectors and other sick animals is decreased. In this study, the breed and the purpose of horses were not significantly associated with the seropositivity. In the contrary, a significant difference was been reported in Malaysia (Elshafie et al., 2013).

No physical or environmental characteristic was significantly associated in this study for donkeys. Donkeys are less susceptible to the infection by *Trypanosoma evansi* than horses (Desquesnes et al., 2013).

CONCLUSION

This is the first study of seroprevalence of *T. evansi* in horses and donkeys in Algeria. It has shown that the seroprevalence of *Trypanosoma evansi* in horses was 45.2% (CI 95%, 37.8-52.5%) and 62.1% (CI 95%, 44.4-79.7%) in donkeys. The results indicate that El-Bayadh district is a highly endemic area. Gender, high plains zone, promiscuity with dromedaries, presence of animals near than watering points and vegetation are risk factors to be infected by *Trypanosoma evansi*. Other characteristics like breed, purpose and housing are not revealed as risk factors for the infection by trypanosomiosis. However, physicals and environmental characteristics were not significantly associated with the infection by *Trypanosoma evansi* in donkeys.

Despite this first detection of *Trypanosoma evansi* in equines, in Algeria, and the real risks that may be caused by its spread in many areas of the country, it's essential to increase the epidemiological surveillance of animals in infected and unaffected areas. Therefore, the scientific community and health authorities must pay attention to this parasite, which can be opportunities for new developments in its geographical distribution, especially to the North.

CONFLICT OF INTEREST

None declared.

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