

Detection of anti-*Toxoplasma gondii* antibodies among horses (*Equus caballus*) and donkeys (*Equus asinus*) in Tiaret province, northwestern Algeria

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SUMMARY

Toxoplasma gondii is an important zoonotic pathogen infecting humans and almost all warm-blooded animals. One of the most common source of human *T. gondii* infection is ingestion of tissue cysts in raw or undercooked meat. In the present study, antibodies to *T. gondii* in sera of 293 horses and 30 donkeys in Tiaret province, northwestern Algeria were determined using the modified agglutination test (MAT). Seventy-five of 293 (26%) horses were seropositive, with titers of 1:25 in 43, 1:50 in 19, 1:100 in 11, and 1:200 in 2. Antibodies to *T. gondii* were found in 9 of 30 (30%) donkeys with titers of 1:25 in 3, 1:50 in 3, 1:100 in 3. Seroprevalence varied in 3 different regions, ranging from 18% to 33.5%. The results of the present study indicated that the rate of infection with *T. gondii* in horses and donkeys is a little high in Tiaret province, northwestern Algeria in comparison to other surveys in Algeria, which suggests that consumption of horse meat in this area may represent a potential source for human infection with *T. gondii*.

Keywords: *Toxoplasma gondii*, Seroprevalence, Horses (*Equus caballus*), Donkeys (*Equus asinus*), MAT, Tiaret province, Algeria.

RESUME

Détection des anticorps anti-*Toxoplasma gondii* chez les chevaux (*Equus caballus*) et les ânes (*Equus asinus*) dans la région de Tiaret, au Nord-Ouest d'Algérie

Toxoplasma gondii est un important agent pathogène zoonotique infectant l'homme, et presque tous les animaux à sang chaud. Les sources les plus courantes d'infection humaine sont l'ingestion de kystes dans la viande crue ou insuffisamment cuite. Dans la présente étude, les anticorps de *T. gondii* ont été recherchés par le test d'agglutination modifié (MAT), chez 293 et 30 échantillons de sérum de chevaux et d'ânes, dans trois régions de la province de Tiaret Nord-Ouest d'Algérie. 75 des 293 chevaux (26%) étaient sérologiquement positifs à *T. gondii* avec des titres de 1:25 chez 43 chevaux, 1:50 chez 19, 1: 100 chez 11 et 1: 200 chez 2. Les anticorps à *T. gondii* ont été trouvés chez 9 ânes des 30 échantillonnés (30%) avec des titres 1 :25 chez 3 ânes ; 1:50 chez 3 et 1: 100 chez 3. La séroprévalence dans les 3 régions d'étude, variait de 18% à 33,5%. Les résultats de la présente étude ont indiqué que le taux d'infection par *T. gondii* chez les chevaux et les ânes est un peu élevé dans la province de Tiaret, Nord-Ouest d'Algérie, ce qui suggère que la consommation de viande de cheval dans cette région peut représenter une source d'infection toxoplasmique importante pour l'homme.

Mots-clés: *Toxoplasma gondii*, Seroprevalence, Chevaux (*Equus caballus*), Anes (*Equus asinus*), MAT, Tiaret, Algérie.

Introduction

Toxoplasma gondii is an important zoonotic parasite, which can infect humans and almost all warm-blood animals, with a worldwide distribution (6). Toxoplasmosis is not only of great importance for livestock and causes economic loss to the livestock industry, it is also a public health problem owing to its transmission to humans by ingestion of uncooked meat containing tissue cysts, or consuming food or drink contaminated with oocysts, or accidental ingestion of sporulated oocysts from the environment (6). Although *T. gondii* infection rarely causes clinical symptoms in adults, it may lead to severe consequences in an immunodeficient person such as an AIDS patient, and infection in pregnant women may lead to abortion, stillbirth, or other serious congenital consequences in newborns (6; 14). Consumption of undercooked meat has been well established as a major risk factor for human *T. gondii* infection worldwide (14). Horses (*Equus caballus*) and donkeys (*Equus asinus*) are important

and useful animals to humans in many ways, such as sport competitions, police work, carriage, and so on. Horse and donkey meat are also the popular and delicate food for people worldwide (12, 13). Moreover, traditionally in some regions of Algeria, the undercooked meat of horse is recommended for pregnant women leading thus to infection of mothers. No data are available on the prevalence of the parasite in horses in Algeria. Therefore we investigated the determination of its serological survey with different correlations between the geographic location, the gender, the age and the breed of the animal.

Materials and methods

ANIMALS SURVEYED

Blood samples were obtained from 293 horses and 30 donkeys from different farms in 3 districts: in the north, Tiaret City (n = 161, 140 horses and 21 donkeys), the south, Ain Dheb (n = 78, 73 horses and 5 donkeys) and the middle,

Sougueur (n = 84, 80 horses and 4 donkeys) of Tiaret province, Northwestern Algeria (North of Africa) between June and November 2013. Different breeds of horse were tested: Arabian horse (n = 141), Arab/Barb (n = 36), Arab Pur Sang (n = 9), Barb (n = 97), Breton (n = 2), Barb/Breton. (n = 2). The different ages of animals were pooled into three groups: one group with an age between 1 and 5 years old (n = 161; 152 horses and 9 donkeys), the second group with horses between 6 and 11 years old (n = 101, 94 horses and 7 donkeys) and the third group with horses older than 11 years old (n = 61, 47 horses and 14 donkeys). 163 females (146 horses and 17 donkeys) and 160 males (147 horses and 13 donkeys) were screened. These equids fed on forage, and most of these equids had outdoor access. The information was obtained via personal interviews with the veterinarians and owners. Domestic and stray cats are in free circulation in all farms of the present study.

BLOOD SAMPLING AND SEROLOGICAL EXAMINATION

Blood samples were obtained via a jugular vein, as approved by the National consultative ethnical committee for life sciences and health, centrifuged at 2500 rpm for 10 min and sera were stored at -20°C until use. Antibodies to *T. gondii* were determined using an in house Modified Agglutination Test (MAT) as described previously (4).

Briefly, *Toxoplasma* antigen was made by growing the parasite in mice intra-peritoneally followed by treatment with trypsin and fixation with formaldehyde. The whole antigen was used to coat 96 well U bottomed polystyrene plates. The sera were screened first at two dilutions 1:20 and 1:200 in 2-Mercaptoethanol/PBS buffer. The plates were shaken for 1 min and then covered and incubated at room temperature for at least 5 hours free of any vibrations. The test was considered positive when a layer of agglutinated antigen/serum was formed covering at least 50% of the bottom of the wells at one dilution at least. In negative wells, antigen precipitation

is observed. The positive samples were then titrated by two fold dilution.

STATISTICAL ANALYSIS

Differences in the seroprevalence of *T. gondii* infected horses between the different regions, male and female, and different age groups were analyzed using a Chi square test calculated with SPSS, version 13 (SPSS Inc, Chicago, Illinois, USA). The P value < 0.05 was considered statistically significant.

Results

Among the 293 horses and 30 donkeys tested for the presence of anti- antibodies, and 9 (30%) were found to be seropositive, respectively. Antibodies to *T. gondii* were found in 75 (26%) of 293 horses with a 1:25 dilution in 43 (57.3%), 1:50 in 19 (25.3%), 1:100 in 11 (14.6%), and 1:200 in 2 (2.6%). Antibodies to *T. gondii* were found in 9 of 30 donkeys with titers of 1:25 dilution in 3, 1:50 in 3 and 1:100 in 3.

The 75 positive horses consisted of 35 Arabian horses out of 141, 7 Arab/Barb breed out of 36, 6 Arab PS out of 9, 23 Barb out of 97, 1 Breton out of 2 and 3 Thoroughbred out of 8 horses tested. The statistical correlation analysis showed no statistically significant difference observed between different breed (Table I).

Horses from the three cities were positive with higher prevalence in the Tiaret City 47 (33.5%) from 140, followed by the Central City: Sougueur 16 (19%) from 80 equids. The City in South: Ain Dheb, showed the lowest prevalence with 12 positive sample (16.34%) from 78. Seroprevalence in animals of three regions was significant (Yates corrected chi-square:0.275; p: 0.008) (Table I).

Factor	Category	Sample size	Positive no	Seroprevalence % (95% CI)	p-value
Age	1 – 5	152	42	27,63 (20.6 - 34.2)	0.724
	6 – 11	94	22	23,4 (15.1 - 32)	
	> 11	47	11	23,4 (16.2 – 30.1)	
Gender	Female	146	38	26,02 (20.2 – 32.2)	0.683
	Male	147	37	25,17 (17.2 – 32.1)	
Race	Arabian	141	35	24,82 (17.7 – 32)	0.132
	Arab/barb	36	07	19,44 (6.5 – 32.4)	
	Arab PS	09	06	66,66 (35.9 – 97.5)	
	Breton	02	01	50,00 (/ – /)	
	Barb	97	23	23,71 (15.2 – 32.2)	
	Thoroughbred	08	03	37,50 (4 – 71)	
Region	Tiaret	140	47	33,5 (25 – 39.8)	0.008
	Sougueur	80	16	19,04 (10.6 – 27.4)	
	Ain Dheb	73	12	16,34 (11.4 – 24.2)	
TOTAL		293	75	25.59 (20.6 – 30.6)	0.599

TABLE I: Epidemiological results of *Toxoplasma gondii* infection in horses by age, gender, race and region in Tiaret Province, Northwestern Algeria

Discussion

Worldwide seroprevalence of *T. gondii* in horses (0.36-27%) and donkeys (11-65%) were summarized by others. In these reports seroprevalence varies tremendously (9). The overall seroprevalence of 26% in the present study is higher than other reports from other countries in Africa. The serological survey of this parasite has previously been conducted only in three countries on the African continent: Nigeria, Egypt and Tunisia (1, 3, 8). In the first country, the overall rate of anti-toxoplasma antibodies, exceeds 30% among Polo horses, local breed and the Argentine breed (1). In Egypt a first study reported an upper rate of 40% (8) but the sera samples were collected from horses with neurological clinical manifestations. Recently a prevalence rate of 25% has been reported in this country among draught horses.

Worldwide seroprevalence of *T. gondii* infection in horses has been summarised prior to 2010 by Dubey, ranging from 0.4% to 48.1% (3) ; and since then, it has been reported between 11% and 65% (2, 3, 7, 9, 11)

The different seroprevalence results may due to differences in hygiene conditions, climates, and the prevalence of *T. gondii* in cats, as well as the sensitivity of the serological methods. In the present study, we used MAT to detect antibodies to *T. gondii* in horse and donkey serum samples because MAT is considered one of the most sensitive and specific serological methods for detecting *T. gondii* antibodies in equids, other animals and humans, and it have been extensively used in the world (6). This method is cheaper, easier than other tests and does not need special sophisticated equipment.

Results of the present study indicated that *T. gondii* infection is common in horses and donkeys, and the parasite will remain present in their tissues for life. Equid meats were mostly consumed by local people. Therefore, animals such as horse are at a high risk of infection and act as a transmission route to humans. Further research on the role of equid meat in human infections and the pathogenesis of *T. gondii* is needed.

Despite the low number of donkeys sampled in this study, it is important to note that the seroprevalence for donkeys was relatively high (30%). This higher seroprevalence could be due to the fact that donkeys are raised outdoors and have more contact with oocysts shed by cats in the environment. These cats had an easy access to feed administered to donkeys that sometimes were used as a litter box for defecation (9). The present serological survey of donkeys suggests that donkeys should be considered as potential sources for human infection in Algeria. Dubey et al (2014) suggest that donkey milk consumption for people allergic to cow's milk has a high risk of human contamination (5)

Concerning geographic location, the north city had the highest antibody prevalence in horses tested, more than the middle and south cities. It is known that north climatic

conditions are more likely favorable to the infection survey. Moreover, cats could freely be present and the water supplied to the horses was not controlled. However, in some farms, very strict hygienic measures are applied in breed farming which could explain why this has the lowest prevalence. In addition, the south part is almost desert with climatic conditions not favorable at all to the survey of the parasite *Toxoplasma gondii*. Climatic conditions can influence the ecology of hosts and the pathogen survival which can easily spread throughout the environment (10).

In conclusion, toxoplasmic infection seems to be not rare in Algeria. The difference could be, thus, associated with ecological/climatic conditions, type of farming, the presence of cats and the quality of water. The results of the present study indicated that the rate of infection with *T. gondii* in horses and donkeys is a little high in Tiaret province, northwestern Algeria, which suggests that consumption of equids meat in this area may represent a potential source for human infection with *T. gondii*.

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