NOTE *Parasitology*

Seroprevalence of *Toxoplasma gondii* in Donkeys (*Equus asinus*) in Italy

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ABSTRACT. Toxoplasmosis, an important zoonosis, can be transmitted by eating meat or drinking milk of animals infected with *Toxoplasma gondii*. Samples were collected from 238 donkeys in the year 2010 in Italy, which included 207 females and 31 males of five breeds and crossbreeds with the average age 9 years (1 month−24 years). Sera were tested for *T. gondii* antibodies using a latex agglutination test and the indirect fluorescent antibody test; 5 and 8% seropositivity were recorded, respectively. We found significant correlation between the presence of *T. gondii* antibodies and sex, age, grazing and presence of cats on the farms and their access to donkey feed. This is the first detection of *T. gondii* antibodies in donkeys in Italy.

KEY WORDS: *Equus asinus,* Italy, risk factors, serology, toxoplasmosis.

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The donkey (*Equus asinus*) is one of the ancient domestic animals used as working animal for breeding or for meat and milk production. Recently, in some European countries including Italy, there is an increasing interest in donkeys due to their use as pet, for onotherapy and for the rediscovery of donkey milk as a feed source for children affected with cow milk allergy. Toxoplasmosis is a zoonotic infection transmissible by ingestion of infected uncooked meat or raw milk [4]. Little is known concerning on *T. gondii* infection in donkeys. The aim of study is to investigate seroprevalence of *T. gondii* in donkeys from Italy and associated risk factors for *T. gondii* infection.

Between September and October 2010, blood samples were collected by venipuncture from 238 apparently healthy donkeys born and raised on 20 farms in southern Italy. This sample size was calculated using the formula proposed by Thrusfield [11] inserting the following values: study population in South Italy (9,991 donkeys, data supplied by Italian Association of Breeders, 2010), expected prevalence of toxoplasmosis (20%, data reviewed by Tassi [10] in horses tested in Italy), confidence interval (95%) and desired absolute precision (5%). The donkey owners participated voluntarily in this study, and background data on donkeys were obtained through a questionnaire filled during sample collection (Table 1). The average age of donkeys was 8 years and 11 month (1 month − 24 years). A complete clinical examination was performed on each donkey.

Blood samples were centrifuged, and serum was removed and stored at −20 °C. The presence of antibodies to *T. gondii* was detected by a latex agglutination test (Pastorex TM TOXO, BIO-RAD, Marnes-la-Coquette, France) according to the manufacturer's instructions and by indirect fluorescence antibody test (IFAT) using a commercially available *T. gondii* antigen IFR and anti-horse IgG FITC conjugate (VMRD, Pullman, WA, U.S.A.). The sera were diluted with physiological solution two-fold starting at 1:50; a titer of 50 was considered positive for both tests. Procedure briefly: *T. gondii* antigen fixed on glass slide was overlaid with 15 μl of the examined serum and incubated in a humid chamber for 30 min at 37°C followed by washing (2 × 10 min), drying and applying 15 μl of specific conjugate. Then, the slides were incubated for 30 min at 37°C in a humid chamber. After washing (2 × 10 min) and drying, the smear was overlaid with 80% glycerol (pH 7.4) and covered with cover glass, and the smears were examined by fluorescence microscope OLYMPUS BX 41 at 1,000 × magnification with oil immersion. Continuous peripheral fluorescence was considered specific. Positive and negative control sera were included in each slide. Sera from domestic horse screened by LAT and IFAT served as *T. gondii* positive and negative controls.

The prevalence of antibodies to *T. gondii* and corre-

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Table 1. Factors associated with seropositivity to *Toxoplasma gondii* by univariate analysis in donkeys from southern Italy

Variables	No. tested	Positive in LAT (%)	Positive in IFAT (%)	Range of titres in IFAT	*P* value*
Gender					
Female	207	12 (6%)	18 (9%)	1:50–1:1600	
Male	31	0	1 (3%)	1:50	0.559
Age categories					
< 1year	32	0	2 (6%)	1:50	
1–4 years	44	2 (5%)	3 (7%)	1:50; 1:200	
5–9 years	60	5 (8%)	3 (5%)	1:50	
>10 years	102	5 (5%)	11 (11%)	1:50–1:1600	0.044
Breed					
Crossbreeds	110	7 (6%)	10 (9%)	1:50–1:1600	
Martina-Franca	53	2 (4%)	5 (9%)	1:50–1:200	
Amiata	46	2 (4%)	3 (7%)	1:50	
Sicilian-Grey	14	1 (7%)	1 (7%)	1:50	
Ragusano	8	0	0	–	
Sardinian	7	0	0	–	0.222
Use					
Milk	183	9 (5%)	16 (9%)	1:50–1:1600	
Pet	27	3 (11%)	2 (7%)	1:50; 1:200	
Meat	16	0	1 (6%)	–	
Breeding	12	0	0	–	0.738
Cats in farms					
Yes	166	8 (5%)	14 (8%)	1:50–1:1600	
No	72	4 (6%)	5 (7%)	1:50	0.422
Access of cats to food					
Yes	141	8 (6%)	12 (9%)	1:50–1:1600	
No	97	4 (0.4%)	7 (7%)	1:50	0.312
Domestic ruminants in farms					
Yes	160	6 (4%)	12 (8%)	1:50–1:1600	
No	78	6 (8%)	7 (9%)	1:50–1:200	0.572
Grazing whole year					
Yes	187	9 (5%)	16 (9%)	1:50–1:1600	
No	51	3 (6%)	3 (6%)	1:50–1:200	0.589
Size of farm					
>20 animals	196	9 (5%)	17 (9%)	1:50–1:1600	
<20 animals	42	3 (7%)	2 (5%)	1:50–1:200	0.396

**P* values are set for the results in IFAT, as it is reference method.

sponding to 95% confidence intervals were estimated using exact binomial test. Statistical analyses were performed on the basis of the individual animal as the unit. Association between the serological results and independent variables were analyzed using Pearson's χ^2 test and Fisher's exact test. Difference was considered statistically significant when *P*-value<0.05; *P*-values were set for the results in IFAT, as it is reference method. Moreover, a multivariate analysis was used to evaluate the contribution of each variable involved in infection risk. A logistic regression (general linear models, GLM) was used to predict seropositivity according to additive and linear relationship between variables. Statistical analysis was performed using GraphPad Prism version 6.00 for Mac OS X, GraphPad Software, La Jolla, CA, U.S.A.

A complete clinical examination confirmed that all donkeys surveyed were apparently healthy. Antibodies to *T. gondii* were detected in 12 (5%) of 238 donkeys by LAT and in 19 (8%) donkeys by IFAT (15 with titer 50, one with titer 100, two with titer 200 and one with titer 1,600); eight sera were positive in both tests (3.4%). The results of serological examination in donkeys based to their gender, age category, breed, use and risk factors are summarized in Table 1. Seropositivity increased with age. Through the statistical analysis of the data obtained from questionnaire, the following risk factors revealed a significant correlation between the presence of *T. gondii* antibodies with sex, age, grazing and presence of cats on the farms and their access to donkey feed (Table 2). The results showed that the positivity was higher in females (9%) compared to males (3%), particularly in adult donkeys (>10-year-old) irrespective to breed (Table 1). The percentage of time spent with grazing seemed to be a positive factor influencing *T. gondii* infection. Similarly, the presence of cats on the farm and their possible contamination of donkey feed with oocyst of *T. gondii* were also considerable factors.

There are only few reports of *T. gondii* infection in donkeys worldwide with seroprevalences ranging from 11 to 62%. Some of the differences in seroprevalences are prob-

Table 2. Risk factors for *Toxoplasma gondii* infection in donkeys as a result of the logistic regression multivariate analysis

	Coefficient	Std Error	*P*
Intercept	–17.8	–0.037	
Gender (female)	–1.30	–8.872	*
Age	0.01	44.102	*
Cat in the farm	2.33	15.601	*
Cat access to food	0.51	7.814	*
Grazing whole year	–0.62	–4.126	*
Purebreed	12.75	0.032	0.452
Crossbreed	10.56	0.025	0.574
Cat acces to water	16.70	0.035	0.972
Ruminants in the farm	–18.30	–0.023	0.982
Size of farm	11.77	0.029	0.5667

$P<0.001$.

ably related to different serological tests and the cut-off titer, and the number of donkeys sampled. The serological test and the cut-off titer that should be considered specific for the diagnosis of *T. gondii* antibodies in donkeys are unknown, and there is no report of attempts to isolate viable *T. gondii* from donkey tissues. In our study, seropositivity varied from 5% by LAT to 8% by IFAT using the same cut off titer (50). Using a titer of 64, seropositivity varied from 1.5% of 197 [8], 28.6% of 7 [9] and 43.2% of 88 [3] donkeys from Brazil. Among the three reports from Europe, seroprevalences were 25.6% (MAT, titer 25) of 25 donkeys from Spain [6], 11% (LAT, titer 64) of 100 donkeys from Turkey in one study [12] and 62% (dye test, titer 16) of 92 donkeys from Turkey in another report [1]. Based on an in-house ELISA, seroprevalences were 45% of 100 donkeys in one study [7] and 65.6% of 121 donkeys in another report [5]. Thus, it is apparent that our study provides the most balanced data on toxoplasmosis in donkeys.

There is little information with respect to epidemiology of toxoplasmosis in donkeys. Analysis of our limited data revealed higher seroprevalence in females than males, similar to the observations by Zeybek *et al.* [12] and Haridy *et al.* [7]. This higher seroprevalence could be due to the fact that females are raised outdoors as grazing animals and thus could have more contact with oocysts shed by cats in the environment. For more than half of the donkeys (n=166) examined, the cats were present in the farms, and this presence could represent a significant risk factor of toxoplasmosis. Moreover, the cat could play an important role in spreading of *T. gondii* infection also during the indoor confinement. These cats had an easy access to feed administered to donkeys (such as hay, bales and sacks of concentrates) that sometimes were used as a litter box for defecation. Moreover, this feline behavior could explain why the cat access to donkey's water was not confirmed as a risk factor to *T. gondii* infection (*P*-value=0.972). In the present study, the seropositivity to *T. gondii* infection increased significantly with the age of donkeys that could be explained by a greater exposure time; the seropositivity in the 1-month-old donkey likely was colostrally-derived.

This is the first epidemiological survey on *T. gondii* in donkeys and first report from Italy. We did not test milk or meat from donkeys, but viable *T. gondii* was recently isolated from samples of milk from asymptomatic cattle, sheep, goats, buffalo and camel milk samples [2]. That is why although seroprevalence was low, the risk of human infection should not be dismissed, since these animals are bred also for human consumption.

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