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

Tereza MACHACOVÁ¹, Eva BARTOVA¹*, Antonio DI LORIA², Kamil SEDLAK³, Ugo MARIANI⁴, Giovanna FUSCO⁴, Domenico FULLGIONE⁵, Vincenzo VENEZIANO⁶ and Jitender P. DUBEY⁷

¹Department of Biology and Wildlife Diseases, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences, Palackého tr. 1/3, Brno, Czech Republic
²Department of Health Science, University of Magna Gracia, Catanzaro, Italy
³Department of Virology and Serology, State Veterinary Institute Prague, Sídliště 136/24, Prague 6, Czech Republic
⁴Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici, Napoli, Italy
⁵Department of Biology, University of Naples, Federico II, Naples, Italy
⁶Department of Veterinary Medicine and Animal Production, University of Naples, Federico II, Naples, Italy
⁷Animal Parasitic Diseases Laboratory, Beltsville Agricultural Research Center, Agricultural Research Service, United States Department of Agriculture, BARC-E, Building 1001, Beltsville, MD, U.S.A.

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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.

KEYWORDS: 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 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-
responding 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 \( \chi^2 \) 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.

| 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             | 0.559         |
| Male                              | 31         | 0                   | 1 (3%)               | 1:50                    |               |
| Age categories                    |            |                     |                      |                         |               |
| < 1 year                          | 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             | 0.738         |
| Pet                               | 27         | 3 (11%)             | 2 (7%)               | 1:50; 1:200             |               |
| Meat                              | 16         | 0                   | 1 (6%)               | –                       |               |
| Breeding                          | 12         | 0                   | 0                    | –                       |               |
| Cats in farms                     |            |                     |                      |                         |               |
| Yes                               | 166        | 8 (5%)              | 14 (8%)              | 1:50–1:1600             | 0.422         |
| No                                | 72         | 4 (6%)              | 5 (7%)               | 1:50                    |               |
| Access of cats to food            |            |                     |                      |                         |               |
| Yes                               | 141        | 8 (6%)              | 12 (9%)              | 1:50–1:1600             | 0.312         |
| No                                | 97         | 4 (0.4%)            | 7 (7%)               | 1:50                    |               |
| Domestic ruminants in farms       |            |                     |                      |                         |               |
| Yes                               | 160        | 6 (4%)              | 12 (8%)              | 1:50–1:1600             | 0.572         |
| No                                | 78         | 6 (8%)              | 7 (9%)               | 1:50–1:200              |               |
| Grazing whole year                |            |                     |                      |                         |               |
| Yes                               | 187        | 9 (5%)              | 16 (9%)              | 1:50–1:1600             | 0.589         |
| No                                | 51         | 3 (6%)              | 3 (6%)               | 1:50–1:200              |               |
| Size of farm                      |            |                     |                      |                         |               |
| >20 animals                       | 196        | 9 (5%)              | 17 (9%)              | 1:50–1:1600             | 0.396         |
| <20 animals                       | 42         | 3 (7%)              | 2 (5%)               | 1:50–1:200              |               |

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

1. Balkaya, I., Babur, C., Celebi, B. and Utuk, A. E. 2011. Seroprevalence of toxoplasmosis in donkeys in Eastern Turkey. Israel J. Vet. Med. 66: 39–42.
2. Dehkordi, F. S., Boroujeni, M. R. H., Rahimi, E. and Abdizadeh, R. 2013. Detection of Toxoplasma gondii in raw caprine, ovine, buffalo, bovine and camel milk using cell cultivation, cat bioassay, capture ELISA and PCR methods in Iran. Foodborne Pathog. Dis. 10: 120–125. [Medline] [CrossRef]
3. de Oliveira, E. P. P. F., De Albuquerque, O. L., Neto, E. B., Faria, J. W. and Pinheiro Júnior Mota, R. A. 2013. Occurrence of antibodies to Toxoplasma gondii in mules and donkeys in the northeast of Brazil. J. Parasitol. 99: 343–345. [Medline] [CrossRef]
4. Dubey, J. P. 2010. Toxoplasmosis of Animals and Humans, 2nd ed., CRC Press, Boca Raton.
5. El-Ghaysh, A. 1998. Seroprevalence of Toxoplasma gondii in Egyptian donkeys using ELISA. Vet. Parasitol. 80: 71–73. [Medline] [CrossRef]
6. Garcia-Bocanegra, I., Cabezón, O., Arenas-Montes, A., Carbonero, A., Dubey, J. P., Perea, A. and Almeria, S. 2012. Seroprevalence of Toxoplasma gondii in equids from Southern Spain. Parasitology Int. 61: 421–424. [Medline] [CrossRef]
7. Haridy, F. M., Saleh, N. M. K., Khalil, H. H. M. and Morsy, T. A. 2010. Anti-Toxoplasma gondii antibodies in working donkeys and donkey’s milk in Greater Cairo, Egypt. J. Egypt. Soc. Parasitol. 40: 459–464. [Medline]
8. Mendonça, A. O., Cerqueira, E. J. L., Araujo, W. N., Silva, E. M., Shimabukuro, F. H., Sarkis, D. T., Sherlock, I. and Langoni, H. 2001. Serological survey to toxoplasmosis in equids from two regions of Bahia State, Brazil. Semina: Ci. Agrárias 22: 115–118.
9. Oliveira Filho, R. B., Malta, K. C., Oliveira, J. M. B., Albuquerque, P. P. F., Mota, R. A., Assis Santana, V. L., Alves, L. C. and Pinheiro Jr, J. W. 2012. Epidemiological situation of Toxoplasma gondii infection in equids from Brejo Paraibano microregion. Brazil Pesq. Vet. Bras. 32: 995–1000. [CrossRef]
10. Tassi, P. 2007. Toxoplasma gondii infection in horses: a review. Parasitologia 49: 7–15. [Medline]
11. Thrusfield, M. 2007. Surveys. pp. 228–242. In: Veterinary Epidemiology, Blackwell Science, London.
12. Zeybek, H., Dündar, B., Altintas, K. and Güngör, C. 1998. The seroprevalence of toxoplasmosis in equids. Türk. Parazitol. Derg. 22: 424–427.

| Table 2. Risk factors for Toxoplasma gondii infection in donkeys as a result of the logistic regression multivariate analysis |
|-------------------------------------------------------------|
| Factor | Coefficient | Std Error | P     |
| --- | --- | --- | --- |
| Intercept | –17.8 | –0.037 | 0.001 |
| Gender (female) | –1.30 | –8.872 | 0.972 |
| Age | 0.01 | 44.102 | 0.972 |
| Cat in the farm | 2.33 | 15.601 | 0.972 |
| Cat access to food | 0.51 | 7.814 | 0.972 |
| Grazing whole year | –0.62 | –4.126 | 0.972 |
| Purebreed | 12.75 | 0.032 | 0.972 |
| Crossbreed | 10.56 | 0.025 | 0.972 |
| Cat acces to water | 16.70 | 0.035 | 0.972 |
| Ruminants in the farm | –18.30 | –0.023 | 0.972 |
| Size of farm | 11.77 | 0.029 | 0.567 |

P<0.001.