Seroprevalence of *Toxoplasma gondii* infection and variables associated with seropositivity in donkeys in eastern China

Qing-Feng Meng1,2,3, Dan Li1, Gui-Zhe Yao2, Yang Zou3, Wei Cong4, and Xiao-Feng Shan3,*

1 Engineering Research Centre of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University, Changchun 130118, PR China
2 Inspection and Quarantine Technology Center of Jilin Entry-Exit Inspection and Quarantine Bureau, Changchun, Jilin Province 130062, PR China
3 College of Animal Science and Technology, Jilin Agricultural University, Changchun, Jilin Province 130118, PR China
4 Marine College, Shandong University at Weihai, Weihai, Shandong Province 264209, PR China

Received 22 October 2018, Accepted 28 November 2018, Published online 11 December 2018

**Abstract** – Donkeys (*Equus asinus*) are widely distributed throughout China; they are used for their meat, as food, and certain donkey-derived items are also important for traditional Chinese medicinal purposes. However, only limited information is available on *Toxoplasma gondii* infection in donkeys in China, especially in the eastern region, which is one of the largest production areas. Thus, the present study was conducted to detect specific anti-*T. gondii* antibodies using a commercially available indirect hemagglutination test (IHA) kit and to evaluate the risk factors that are associated with seroprevalence in the Shandong province of eastern China. A total of 213/1278 (17%) donkeys tested from Shandong province were positive for *T. gondii* antibodies. Statistical analysis revealed that gender and feeding habits of the animal are associated with *T. gondii* infection. These results provide information for the prevention and control of toxoplasmosis in donkeys, other animals, and humans in this region and elsewhere.

**Key words:** *Toxoplasma gondii*, donkeys, seroprevalence, eastern China.

**Introduction**

*Toxoplasma gondii* is a foodborne parasite that causes zoonotic toxoplasmosis and can infect the humans and nearly all the warm-blooded animals [15]. Currently, one third of the global population is estimated to be infected with this parasite [21]. Interestingly, the definitive hosts of *T. gondii* include wild and domestic felids, which can discharge millions of oocysts of the parasites into the environment following primary infection, thereby posing a major public health concern [15]. As a result, toxoplasmosis remains an unsolved public health issue worldwide [8]. The primary routes of *T. gondii* infection in humans are by ingesting raw or undercooked meat from infected animals and consuming food or water contaminated with infected cat feces containing *T. gondii* oocysts [4]. Thus, consumption of meat from *T. gondii*-infected animals (including poultry, pigs, donkeys, sheep, and cattle) plays a major role in the transmission of the parasite [4].

The donkey (*Equus asinus*), a member of the Equine family, has been identified as an intermediate host of *T. gondii* [1–3, 5, 7, 11–13]. According to a previous study, the output of donkey meat is 182,900 tons in China, accounting for about one third of global donkey meat production [20]. In recent years, the demand for donkey meat has risen sharply.
A number of studies have been conducted worldwide to detect the prevalence of *T. gondii* infection in donkeys; however, the surveys conducted in China were done several years ago. Although Shandong province is a major donkey breeding area in China, only limited information on *T. gondii* infection in this species is available for this part of eastern China. In recent years, the provincial government has begun to focus on the development of the donkey industry. However, large gaps exist in the prevention and control of parasitic diseases, especially for food-borne zoonotic diseases. Therefore, the present study was conducted to detect the seroprevalence of *T. gondii* infection and to evaluate the variables associated with seropositivity in donkeys in Shandong province. These data would provide valuable information for the prevention and control of toxoplasmosis in donkeys and humans in this region and elsewhere.

**Materials and methods**

**Ethics statement**

This study was approved by the Animal Ethics Committee of Jilin Agricultural University. Serum samples were collected and handled in accordance with the requirements of the Animal Ethics Procedures and Guidelines of the People’s Republic of China.

**Collection and preparation of serum samples**

Serum samples were randomly collected from the jugular vein of 1278 donkeys from various regions in Shandong province (4°23′~38°24′ N, 114°48′~122°42′ E) between July 2015 and December 2016 by local veterinary practitioners. The animals from each farm were selected randomly using a table of random digits. Several large-scale farms (>500 animals) were not included because the owner was not available to obtain permission. Finally, a total of 17 farms were selected, and approximately 30% of donkeys were sampled on each farm. All the sampled animals were clinically healthy. For backyard donkeys, we randomly visited local individual farmers, obtained permission, and collected the blood samples from the animal. Finally, a total of 623 Dezhou donkeys (257 from Heze, 142 from Jining, 224 from Liaocheng), 354 Sanfen donkeys (53 from Heze, 152 from Jining, 149 from Liaocheng), and 301 Wutou donkeys (66 from Heze, 110 from Jining, 125 from Liaocheng) were sampled (Table 1). Donkeys from farms and backyards were commonly used for slaughtering and agriculture, respectively. The serum was obtained by centrifugation of the blood samples at 1000 *g* for 5 min and stored at −20 °C until analysis.

**Serological examination**

Antibodies against *T. gondii* were detected using a commercially available indirect hemagglutination test (IHA) kit (Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, Gansu Province, China) (http://lvr.caas.cn/kjpt/zdzczx/index.htm), according to the manufacturer’s recommendations. The method is a national standard (NY/T 573-2002) of China for the detection of animal toxoplasmosis. Briefly, sera were added to 96-well V-bottomed polystyrene plates and diluted two-fold from 1:4 to 1:2048. IHA titers ≥1:64 (manufacturer’s recommendation) were considered positive when forming a layer of agglutinated erythrocytes; sera with dubious results were re-tested. Positive and negative controls, supplied by the Lanzhou Veterinary Research Institute, were included and tested at dilutions identical to those of the serum samples. Consequently, IHA showed 89.8% sensitivity and 96.6% specificity in detecting *Toxoplasma* IgG antibody [18].

**Statistical analysis**

The current data were analyzed by the SPSS 18.0 software package (IBM, Armonk, NY, USA). *P*-values <0.05 were considered to reflect a significant difference. Logistic regression was used to analyze the association between *T. gondii* infection and the potential risk factors. The multivariate logistic analysis was performed with the full model including all potential risk factors.

**Results and discussion**

In the present study, 214/1278 donkeys (16.75%, 95% confidence intervals (CIs): 14.70–18.79) were seropositive for *T. gondii* (Table 1). The *T. gondii* seroprevalence in Dezhou Donkeys, Wutou Donkeys, and Sanfen Donkeys was 17.98%, 13.56%, and 17.61%, respectively. The highest seroprevalence was found in Liaocheng (17.47%), followed by Jining (16.58%) and Heze (15.96%). The seroprevalence in female and male donkeys was 19.10% and 14.81%, respectively. The seroprevalence among different age groups ranged from 14.01% in the Age > 5 years group to 18.50% in the 1 < Age ≤ 3 years group. Moreover, donkeys bred in the backyard (30.15%) showed a significantly higher seroprevalence than those on the farm (14.27%) (*P* < 0.001) (Table 2). Further analysis using multivariate logistic regression revealed that gender (odds ratio (OR) = 1.350, 95% CI: 1.00–1.82, *P* = 0.049) and feeding habits (OR = 2.572, 95% CI: 1.80–3.67, *P* < 0.001) were risk factors for *T. gondii* seroprevalence.

Donkeys are an important animal for traditional Chinese medicinal and comestible purposes and are widely distributed in China [19]. Previous surveys have reported the prevalence of *T. gondii* infection in donkeys in China [14, 17, 20]. However, the present results cannot be compared with those from other studies due to the differences in factors such as the breed of the donkeys, the number of tested animals, age classes, and animal hygiene standards. Furthermore, it is difficult to compare the current and the previous studies due to different serological tests employed.

In the present study, the feeding habits of donkeys were the only variable significantly associated with seroprevalence (*P* < 0.001). Interestingly, the donkeys bred in backyards were more easily infected with *T. gondii* as compared to those bred on farms. During this survey, cats were commonly found in the backyards. Although the infection status of cats was not tested, breeding cats at home might be the primary cause of high seroprevalence in donkeys bred in backyards. Also, other risk factors such as water, food, or pastures contaminated with
T. gondii oocysts should be considered. Thus, additional studies are essential to substantiate the current findings and identify the optimal method for reducing Toxoplasma infection in donkeys.

The seropositivity of T. gondii appears to be related to age [6, 16]; however, T. gondii seroprevalence in donkeys was not significantly influenced by age. The analysis of age distribution in donkeys demonstrated that most of the backyard donkeys could be categorized in the 1 < Age ≤ 3 years group, which might influence seropositivity. However, further targeted studies are essential to explore the effect of age on T. gondii seropositivity with respect to different feeding habits in donkeys. Strikingly, female donkeys showed a significantly higher seroprevalence than male donkeys (P = 0.041), and thus, future studies should focus on the impact of T. gondii infection in the offspring of donkeys.

In the present study, all farms were positive for the presence of T. gondii, rendering it impossible to evaluate the role of farms as a risk factor. Thus, further studies should be conducted to explore the role of toxoplasmosis in reproductive and economic losses in donkey breeding in these regions.

Several laboratory tests have been used for the detection of T. gondii antibodies in donkeys, including the modified agglutination test, ELISA, IHA, and PCR [11–14, 17]. Herein, we selected IHA to detect T. gondii antibodies in donkeys because this kit has high sensitivity and specificity and is easily available [18]. Moreover, the IHA kit has been extensively used for detecting specific antibodies to T. gondii in horses, pigs, sheep, and other mammals in China for several years [9, 10]. In the present study, we used this kit to detect specific antibodies to T. gondii in donkeys. Thus, further studies should be conducted, using different techniques, to confirm the present results.

The current results showed that T. gondii infection is common in donkeys in Shandong province, and the parasite is likely to prevail in the tissues of the animals lifelong. Thus, the donkey, at high risk of infection, could act as a transmission route to humans because donkey meat is a standard food in China. Therefore, additional studies are essential to investigate the role of donkey meat in human infections and the pathogenesis of toxoplasmosis in the animal.

**Conclusions**

The present survey revealed a 16.75% seroprevalence of T. gondii infection in donkeys in Shandong province, eastern China. Feeding habits were identified as the primary risk factor for T. gondii infection in donkeys. The present results provide baseline data for designing and evaluating tools for prevention and control, as well as for future studies on T. gondii infection in a large population of donkeys in China.

**Competing interests**

The authors declare that they have no competing interests.

**Acknowledgements.** This work was supported by a grant from the Technology Development Program of Jilin Province (20180520034JH).
References

1. Alvarado-Esquivel C, Alvarado-Esquivel D, Dubey JP. 2015. Prevalence of Toxoplasma gondii antibodies in domestic donkeys (Equus asinus) in Durango, Mexico slaughtered for human consumption. BMC Veterinary Research, 11, 6.

2. Bártová E, Sedlák K, Kobédová K, Budíková M, Joel Atuman Y, Kamani J. 2017. Seroprevalence and risk factors of Neospora spp. and Toxoplasma gondii infections among horses and donkeys in Nigeria, West Africa. Acta Parasitologica, 62(3), 606–609.

3. de Oliveira E, de Albuquerque PP, de Souza Neto OL, Faria EB, Júnior JW, Mota RA. 2013. Occurrence of antibodies to Toxoplasma gondii in mules and donkeys in the northeast of Brazil. The Journal of Parasitology, 99(2), 343–345.

4. Dubey JP. 2010. Toxoplasmosis of animals and humans, 2nd edn. CRC Press: Boca Raton, FL. p. 1–313.

5. Dubey JP, Ness SL, Kwok OC, Choudhary S, Mittel LD, Divers TJ. 2014. Seropositivity of Toxoplasma gondii in domestic donkeys (Equus asinus) and isolation of T. gondii from farm cats. Veterinary Parasitology, 199(1–2), 18–23.

6. Falusi O, French AL, Seaberg EC, Tien PC, Watts DH, Minkoff H, Piessens E, Kovacs A, Anastos K, Cohen MH. 2002. Prevalence and predictors of Toxoplasma seropositivity in women with and at risk for human immunodeficiency virus infection. Clinical Infectious Diseases, 35(11), 1414–1417.

7. Gennari SM, Esmerini Pde O, Lopes MG, Soares HS, Vitaliano SN, Cabral AD, Pena HF, Horta MC, Cavalcante PH, Fortes KP, Vilalobos EM. 2015. Occurrence of antibodies against Toxoplasma gondii and its isolation and genotyping in donkeys, mules, and horses in Brazil. Veterinary Parasitology, 209(1–2), 129–132.

8. Innes EA. 2010. A brief history and overview of Toxoplasma gondii. Zoonoses and Public Health, 57(1), 1–7.

9. Li F, Wang SP, Wang CJ, He SC, Wu X, Liu GH. 2016. Seroprevalence of Toxoplasma gondii in goats in Hunan province, China. Parasite, 23, 44.

10. Luo H, Li K, Zhang H, Gan P, Shahzad M, Wu X, Lan Y, Wang J. 2017. Seroprevalence of Toxoplasma gondii infection in zoo and domestic animals in Jiangxi Province, China. Parasite, 24, 7.

11. Machacova T, Bartova E, Di Loria A, Sedlak K, Mariani U, Fusco G, Fulgione D, Veneziano V, Dubey JP. 2014. Seroprevalence of Toxoplasma gondii in donkeys (Equus asinus) in Italy. The Journal of Veterinary Medical Science, 76(2), 265–267.

12. Mancianti F, Nardoni S, Papini R, Mugnaini L, Martini M, Altomonte I, Salari F, D’Ascenzi C, Dubey JP. 2014. Detection and genotyping of Toxoplasma gondii DNA in the blood and milk of naturally infected donkeys (Equus asinus). Parasites & Vectors, 7, 165.

13. Martini M, Altomonte I, Mancianti F, Nardoni S, Mugnaini L, Salari F. 2014. A preliminary study on the quality and safety of milk in donkeys positive for Toxoplasma gondii. Animal, 8(12), 1996–1998.

14. Miao Q, Wang X, She LN, Fan YT, Yuan FZ, Yang JF, Zhu XQ, Zou FC. 2013. Seroprevalence of Toxoplasma gondii in horses and donkeys in Yunnan Province, Southwestern China. Parasites & Vectors, 6, 168.

15. Montoya JG, Liesenfeld O. 2004. Toxoplasmosis. Lancet, 363, 1965–1976.

16. Nowakowska D, Wujcicka W, Sobala W, Spiewak E, Gaj Z, Wilczyński J. 2014. Age-associated prevalence of Toxoplasma gondii in 8281 pregnant women in Poland between 2004 and 2012. Epidemiology and Infection, 142(3), 656–661.

17. Yang N, Mu MY, Yuan GM, Zhang GX, Li HK, He JB. 2013. Seroprevalence of Toxoplasma gondii in slaughtered horses and donkeys in Liaoning province, northeastern China. Parasites & Vectors, 6, 140.

18. Yang YX, Chen YK, Wei SJ, Song RH. 2014. Efficiency of three methods for detecting Toxoplasma IgG antibody. Chinese Journal of Schistosomiasis Control, 26, 109–110.

19. Zhang P, Ma J, Han BY, Liang RQ. 2013. Analysis of the status quo and trend of donkey industry in China. Chinese Abstract Animal Husbandry and Veterinary Medicine, 2013(99), 36–38 (In Chinese).

20. Zhang XX, Shi W, Zhang NZ, Shi K, Li JM, Xu P, Zhao Q, Du R. 2017. Prevalence and genetic characterization of Toxoplasma gondii in donkeys in northeastern China. Infection, Genetics and Evolution, 54, 455–457.

21. Zhou P, Chen Z, Li HL, Zheng H, He S, Lin RQ, Zhu XQ. 2011. Toxoplasma gondii infection in humans in China. Parasites & Vectors, 4, 165.