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Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in Tarim Red Deer (*Cervus elaphus yarkandensis*) from Xinjiang Province, Northwest China

[1]Q.L. Meng, [1]J. Qiao, [1]W.S. Wang, [1]C.F. Chen, [1]Z.C. Zhang, [1]K.J. Cai,
[2]X.P. Cai, [2]G.F. Tian, [1]Z.Z. Tian and [1]L.H. Yang
[1]College of Animal Science and Technology, Shihezi University, 832003 Shihezi, China
[2]State Key Lab of Veterinary Etiological Biology, Chinese Academy of Agricultural Sciences, Lanzhou Veterinary Research Institute, 730046 Lanzhou, China

Abstract: *Toxoplasma gondii* (*T. gondii*) and *Neospora caninum* (*N. caninum*) are structurally similar apicomplexan protozoas which can cause neuromuscular disease and abortion in different animal species. Considering the importance of public health and lack of epidemiological data on the seroprevalence of two parasites in Tarim red deer (*Cervus elaphus yarkandensis*), a total of 218 serum samples collected from farmed Tarim red deer in Xinjiang province, Northwest China were detected for antibodies to *T. gondii* by Modified Agglutination Test (MAT) and to *N. caninum* by competitive-inhibition Enzyme-Linked Immunosorbent Assay (c-ELISA). The prevalence of antibodies against *T. gondii* and *N. caninum* were 12.84% (28/218) and 8.00% (17/218) in serum samples examined, respectively. The co-prevalence of antibodies to *T. gondii* and *N. caninum* was also detected in sera (2.75%, 6/218). Statistically significant differences in prevalence were observed among different sampling sites and between the individuals with abortion and no abortion experience ($p<0.05$). The results showed that there exist *T. gondii* and *N. caninum* infection in Tarim red deer. It was the first survey of *T. gondii* and *N. caninum* antibodies in Tarim red deer which suggested that toxoplasmosis and neosporosis may be two important causes of abortive diseases in Tarim red deer.

Key words: Public health, serum, antibodies, abortion, deer, China

INTRODUCTION

Toxoplasma gondii (*T. gondii*) and *Neospora caninum* (*N. caninum*) are two apicomplexan parasites with worldwide distributions which can cause abortions in their intermediate hosts (Dubey and Lindsay, 1996; Dubey *et al*., 2002, 2009; Bonyadian *et al*., 2007; Shaapan and Ghazy, 2007; Kamga-Waladjo *et al*., 2009; Shaapan *et al*., 2012). *T. gondii* infection not only results in significant reproductive losses and hence economic losses but also has implications for public health because consumption of infected meat or milk can facilitate zoonotic transmission in human beings. *N. caninum*, another protozoan closely related to *T. gondii* is now considered as an important cause of bovine abortion (Bartels *et al*., 1999; Ould-Amrouche *et al*., 1999). It can also cause abortion or neonatal mortality in other animal species. The two parasites have indirect life cycles with felids and canids as the definitive hosts, respectively (Montoya and Liesenfeld, 2004; Barber and Trees, 1998). Both can infect a wide range of intermediate hosts including cows, sheep, goats, horses, fox, bison, deer and wildlife animals (Bartova *et al*., 2007; Zhang *et al*., 2000; Wanha *et al*., 2005; Stieve *et al*., 2010; Panadero *et al*., 2010; De Craeye *et al*., 2011). In recent years with the rapid development of the deer industry, emerging infectious diseases such as abortive diseases in Tarim red deer (*Cervus elaphus yarkandensis*) have increased in frequency in Xinjiang province, Northwest China.

The prevalence of *T. gondii* and *N. caninum* infection in deer have been reported in white-tailed deer (*Odocoileus virginianius*), black-tailed deer (*Odocoileus hemionus columbianus*), mule deer (*Odocoileus hemionus hemionus*), red deer (*Cervus elaphus*), sika deer and roe deer (*Capreolus capreolus*) (Williamson and Williams, 1980; Gonzaiez-Zotes *et al*., 2000; Dubey *et al*., 2002; Omata *et al*., 2005; Dubey *et al*., 2008; Dubey *et al*., 2009; Malmsten *et al*., 2011). However, so far, little is known about the prevalence of these parasites in farmed Tarim red deer. In view of serious hazards of the two diseases in reproduction in Tarim red deer and importance of public health, the present study is aimed at determining the seroprevalence of antibodies to *T. gondii* and *N. caninum* in Tarim red deer from Xinjiang and discussing potential implications for public health.

MATERIALS AND METHODS

Locations of sampling sites: The five sampling sites (Hejing, Hesuo, Bohu, Kuche and Shaya) are located

Corresponding Author: J. Qiao, College of Animal Science and Technology, Shihezi University, North Street No. 4, Shihezi City, 832003 Xinjiang Province, The People's Republic of China

in Xinjiang province, Northwest China which is adjacent to the Northern margin of the Tarim basin. Geographical localization and number of blood samples are shown in Fig. 1 and Table 1, respectively. This region belongs to typical temperate continental arid climate in which is dry, arid and semi-arid land. The average annual precipitation ranges from 40-50 mm. The average annual temperature of five sampling sites ranges from 10-15°C.

Serum samples: A total of 218 blood samples were collected randomly from the farmed Tarim red deer. The blood samples were collected by the farms during the harvest of deer velvet, from July to October 2010 and graciously supplied for analysis of anti-*T. gondii* and anti-*N. caninum* antibodies. The blood samples were drawn from the arteries of deer antlers into sterile vacutainer tubes without anticoagulant. The tubes were centrifuged at 2500 r min^{-1} for 10 min and the sera thus obtained were stored at -20°C until assay.

Serological examination: Modified Agglutination Test (MAT) was used for the detection of specific antibody to *T. gondii*. Briefly, serum samples were assayed at the dilutions of 1:20, 1:400, 1:1,600 and 1:6,400 using a commercial kit (Toxo-Screen DA®, bioMerieux, Lyon, France) with whole formalin-fixed tachyzoites as antigen. Positive and negative controls supplied with the kit were included in each testing plate. The results of test were expressed as an antibody titer, namely, the reciprocal of the highest dilution at which agglutination (at least one half of the well's diameter) was still visible after 5-18 h incubation at room temperature. The cut-off titer of 20 (2 IU mL^{-1} in relation to a WHO international reference serum) was chosen to maximize both sensitivity and specificity of the test (Zhang *et al*., 2000).

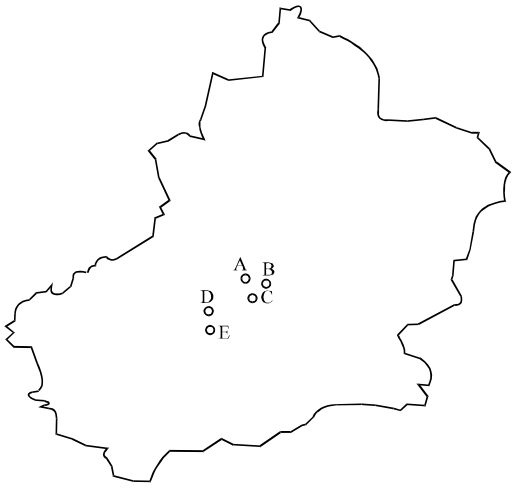

Fig. 1: Geographical localization of the five sampling sites in Xinjiang province, Northwest China from which blood samples of Tarim red deer were collected (A: Hejing; B: Hesuo; C: Bohu; D: Kuche and E: Shaya county)

The competitive Enzyme-Linked Immunosorbent Assay (c-ELISA) was used for the assay of antibodies to *N. caninum* in this study (cELISA VMRD® Inc., WA, USA). Briefly, 50 μL of serum samples were transfer into antigen-coated ELISA plate for each sample including negative and positive control. The ELISA plate was incubated at room temperature for 1 h following by washing with washing buffer solution for 3 times. About 50 μL of antibody-peroxidate conjugate was then added into each well and incubated for 20 min at room temperature. After incubation, the plate was washed with washing buffer solution for 3 times. About 50 μL of substrate were added into the well and incubated for 20 min, then added stop solution for termination reaction. Finally, the plate was measured through the optical density at 630 nm immediately. The results were expressed as the percentage of inhibition. Sera were considered negative if the sample caused <30% inhibition (cut-off) and positive if the sample caused ≥30% inhibition as indicated by the manufacturer.

Statistical analysis: Differences in seroprevalence were analyzed using the χ^2-test at $p<0.05$ of significance level. The calculations were performed using the STATISTICA Software (Series 1203b, Version 6.1 for Windows, Statsoft inc.).

RESULTS AND DISCUSSION

For *T. gondii*, out of 218 tested samples, 28 were positive, resulting in an average seroprevalence of 12.84% (28/218). For *N. caninum*, from 218 tested samples, 17 were positive resulting in a seroprevalence of 8.00% (17/218) as shown in Table 1. Significant

Table 1: Prevalence of anti-*T. gondii* and anti-*N. caninum* antibodies in 218 sera samples from Xinjiang province, Northwest China

		Prevalence of anti-*T. gondii* antibodies		Prevalence of anti-*N. caninum* antibodies	
Sampling sites	No. of sera samples	No. of positive	Percentage of positive	No. of positive	Percentage of positive
Hejing (A)	56	3	5.38 (3/56)[a]	9	16.07 (9/56)[b]
Hesuo (B)	47	5	10.64 (5/47)[b]	1	2.13 (1/47)[a]
Bohu (C)	52	9	17.31 (9/52)[b]	5	9.62 (5/52)[b]
Kuche (D)	32	4	12.50 (4/32)[b]	2	6.25 (2/32)[a]
Shaya (E)	31	7	22.58 (7/31)[b]	3	9.68 (3/31)[b]
Total	218	28	12.84 (28/218)	17	8.00 (17/218)

Different lowercase letters in the same column indicate significant differences at $p<0.05$

Table 2: Prevalence of anti-*T. gondii* and anti-*N. caninum* antibodies in 218 sera samples from Tarim red deer with abortion experience in Northwest China

		Prevalence of anti-*T. gondii* antibodies		Prevalence of anti-*N. caninum* antibodies	
Individuals	No. of sera samples	No. of positive	Percentage of positive	No. of positive	Percentage of positive
No abortion experience	186	19	10.22 (19/186)[a]	5	2.69 (3/186)[a]
With abortion experience	32	9	28.13 (9/32)[b]	12	37.50 (14/32)[b]
Total	218	28	12.84 (28/218)	17	8.00 (17/186)

Different lowercase letters in the same column indicate significant differences at $p<0.05$

differences ($p<0.05$) in prevalence of anti-*T. gondii* and anti-*N. caninum* antibodies were seen among five sampling sites. The highest prevalence (22.58%) of anti-*T. gondii* antibodies was examined in Shaya country while the highest seroprevalence (16.07%) of anti-*N. caninum* antibodies was observed in Hejing country (Table 1). Moreover, significant differences ($p<0.05$) in prevalence of anti-*T. gondii* and anti-*N. caninum* were seen between individuals with and without abortion experience (Table 2). The co-prevalence (2.75%, 6/218) of antibodies against *T. gondii* and *N. caninum* in samples was also examined (Table 3).

In this study, seroprevalence of anti-*T. gondii* and anti-*N. caninum* antibodies in Tarim red deer were 12.84 and 8.00%, respectively. Significant differences in prevalence of anti-*T. gondii* and anti-*N. caninum* antibodies in 218 sera samples were seen among five sampling sites. Differences in prevalence rates observed between individuals with and without abortion experience were statistically significant; the prevalence of antibodies was higher in individuals with abortion experience than that of individuals without abortion experience. The results showed that the infections of *T. gondii* and *N. caninum* were closely related to the abortion in Tarim red deer.

To date, many serologic diagnostic tests such as Indirect Fluorescent Antibody Tests (IFAT), Enzyme-Linked Immunosorbent Assays (ELISA), Modified Agglutination Test (MAT), Direct Agglutination Tests (DAT) and Western blot analysis have been developed for the diagnosis of *T. gondii* and *N. caninum* infection. MAT is a test of proven accuracy to detect *anti-T. gondii* antibodies in animal and human sera. IFAT and ELISA are two main tests used for serologic examination for *N. caninum* infection. Considering that IFAT is time-consuming and expensive compared with ELISA therefore, it is not used routinely for screening populations for *N. caninum* infection. c-ELISA (VMRD Laboratories, USA) has been shown to be unreactive to antigens of 2 closely related apicomplexan protozoa, *Toxoplasma gondii* and *N. caninum*. Hence, MAT and c-ELISA were adopted for the detection of *anti-T. gondii* and anti-*N. caninum* antibodies in this survey, respectively.

Toxoplasmosis and neosporosis are associated with many risk factors such as the presence on the farm of dogs and cats. Cats and dogs were considered as the definitive hosts of Toxoplasmosis and neosporosis,

Table 3: The co-prevalence of antibodies against *T. gondii* and *N. caninum* in positive samples

		Antibody against *T. gondii*	
Antibodies	Observation	No. of positive	No. of negative
Antibody against *N. caninum*	No. of positive	6	11
	No. of negative	22	0

respectively which played important roles in these protozoan diseases (Dubey and Lindsay, 1996; Barber and Trees, 1998; Bartels *et al*., 1999; Wanha *et al*., 2005; Thomasson *et al*., 2011; Lopes *et al*., 2011). The presence of cats and dogs on farms has been a potential risk to provide the increasing chance of horizontal transmission through the ingestion of oocysts, shed by infected cats or dogs. In this survey, five sampling farms are located in remote rural areas where there had cats and dogs in farms and nearby environment. In addition, wild rodents were also found in deer farms. Tarim red deer probably become infected by ingesting food or water contaminated by oocysts excreted by cats, dogs and wild rodents in the area. In addition, frequent exchange of bucks among deer farms may be contributed to the prevalence of these diseases.

Tarim red deer (*Cervus elaphus yarkandensis*) is one of subspecies of red deer which is mainly farmed in Tarim basin, Xinjiang province, Northwest China. In these areas, the meat from Tarim red deer is used as a food source. The high incidence of Toxoplasmosis and neosporosis in Tarim red deer may lead to the problem of public health. This survey highlights the importance of preventive measures that must be put into practice over the intermediate hosts in order to reduce infection in Tarim red deer and farm workers.

CONCLUSION

The present study is the first serological survey of *T. gondii* and *N. caninum* infection in farmed Tarim red deer living in Xinjiang province which provides useful epidemiological data for the control strategies of toxoplasmosis and neosporosis in Tarim red deer.

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