Enzyme-linked immunosorbent assays using recombinant TgSAG2 and NcSAG1 to detect *Toxoplasma gondii* and *Neospora caninum*-specific antibodies in domestic animals in Turkey

Mo ZHOU¹,²#, Shinuo CAO¹#*, Ferda SEVINC³, Mutlu SEVINC³, Onur CEYLAN³, Mingming LIU¹, Guanbo WANG¹, Paul Franck Adjou MOUMOUNI¹, Charoonluk JIRAPATTHARASATE¹, Hiroshi SUZUKI¹, Yoshifumi NISHIKAWA¹ and Xuenan XUAN¹#*

¹National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080–8555, Japan
²State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin 150001, China
³Selcuk University, Faculty of Veterinary Medicine, Konya, Turkey

(Received 8 May 2016/Accepted 4 August 2016/Published online in J-STAGE 15 August 2016)

**ABSTRACT.** Considering the scarce information on occurrences of *Toxoplasma gondii* and *Neospora caninum* in domestic animals from Turkey, the aim of this study was to investigate the seroprevalence of these parasite infections in cattle, horses, sheep, goats and dogs in Turkey. The specific antibodies against *T. gondii* and *N. caninum* were detected by iELISAs based on the recombinant TgSAG2 or NcSAG1 in a total of 2,039 serum samples from eleven provinces. The seroprevalence of *T. gondii* infections was 46.3%, 4.0%, 20.0%, 12.9% and 19.8%, that of *N. caninum* infections was 0.3%, 7.4%, 2.1%, 3.2% and 16.6% in the horses, cattle, sheep, goats and dogs, respectively. These results indicated that *T. gondii* and *N. caninum* infections are prevalent in Turkish domestic animals.

**KEY WORDS:** ELISA, *Neospora caninum*, *Toxoplasma gondii*, Turkey

doi: 10.1292/jvms.16-0234; J. Vet. Med. Sci. 78(12): 1877–1881, 2016

*Correspondence to: XUAN, X., National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080–8555, Japan. e-mail: gen@obihiro.ac.jp
#*These authors contributed equally to this work.
©2016 The Japanese Society of Veterinary Science

**NOTE** Parasitology

*Toxoplasma gondii* (*T. gondii*) and *Neospora caninum* (*N. caninum*) are two closely related apicomplexan protozoa that infect many warm blood animals. *T. gondii* shares structural, genetic and immunological similarities to *N. caninum*, but they are antigenically different [10, 23]. *T. gondii* infections cause abortion or neonatal mortalities in human and warm-blooded animals [7, 28]. *N. caninum* is the causative agent of neosporosis, an infection that always causes reproductive failure in cattle, sheep, goats and horses, and neurological alterations in dogs [1, 10, 26]. Numerous epidemiologic studies of toxoplasmosis and neosporosis have been reported in many areas worldwide [2, 6, 20, 27]. However, the epidemiological information about the seroprevalence of *T. gondii* and *N. caninum* is limited in Turkey [19]. Therefore, the objective of this study was to determine the seroprevalence of *T. gondii* and *N. caninum* in a wide range of domestic animals in Turkey. Recombinant antigens are usually available in pure form, which provide better options in serological diagnosis [5, 16]. Surface antigen 2 of *T. gondii* (TgSAG2) and surface antigen 1 of *N. caninum* (NcSAG1) have been identified as important candidate of serological diagnosis for toxoplasmosis and neosporosis, respectively [5, 13, 15–17, 21]. In this study, we determined the seroprevalence of *T. gondii* and *N. caninum* in a wide range of domestic animals in Turkey using ELISA based on the recombinant TgSAG2 and NcSAG1, respectively.

The field samples analyzed in this study were collected from 11 provinces that located in the 6 regions of Turkey (Fig. 1). The serum samples were obtained from a total of 2,039 animals (616 horses, 377 cattle, 610 sheep, 249 goats and 187 dogs) within the study area. The horses were adult draft type belonging to the farmers in Adana, Bursa, Gaziantep, Istanbul, Izmir and Konya provinces. The cattle that raised primarily for milk production (dairy breed/cross breed) were selected from Adana, Afyon, Diyarbakir, Karaman, Kırklareli, Konya and Zonguldak provinces. Sheep that raised principally for the meat, wool and breeding were selected from Karaman, Konya and Zonguldak provinces. Most of the selected sheep were females and over one year old. The goats selected from the herds in Karaman and Konya provinces were reared for family milk and meat consumption. The dogs in the present study were house and stray dogs across the urban-rural areas of Konya province. All animal experiments were approved by the Scientific and Technological Research Council of Turkey. Care of animals and animal experimentation were performed in accordance with Animal Welfare Approved Standards for Turkeys (http://animalwelfareapproved.org/).

The recombinant TgSAG2-GST and NcSAG1-GST proteins used in this study were generated according to...
the method described previously [5, 16]. In brief, the PCR products of truncated TgSAG2 and NcSAG1 were inserted into the pGEX-4T vector (Amersham Pharmacia Biotech, San Francisco, CA, U.S.A.) and expressed in an *E. coli* BL-21 strain. A fresh 10 ml overnight culture of transformed *E. coli* was grown in 1 L of LB base broth containing 50 µg/ml of ampicillin at 37°C with shaking at 250 rpm until the optical density (OD) at 600 nm reached to 0.5. The expression of these proteins was induced by 5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) followed by incubation at 27°C overnight. The *E. coli* culture was centrifuged at 8,000 g for 15 min, and the cell pellet was then suspended in TNE buffer (50 mM Tris-HCl, pH 8.0, 100 mM NaCl, 2 mM EDTA and 1% Triton X-100) containing 50 mg/ml of lysozyme, 1% (w/v) N-Lauroylsarcosine sodium and protease inhibitors. These recombinant proteins were purified from the soluble fractions using Glutathione-Sepharose 4B beads, according to the manufacturer’s instructions (Amersham Pharmacia Biotech). Protein expressions were verified by SDS-PAGE stained with Coomassie blue. The concentration of the expressed protein was measured using the BCA assay.

Indirect ELISAs were carried out to detect specific antibodies to *T. gondii* (rTgSAG2-ELISA) and *N. caninum* (rNcSAG1-ELISA) according to the previous reports, with modifications [5, 16]. Briefly, rTgSAG2-GST, rNcSAG1-GST and GST were diluted to a final concentration of 4 µg/ml, 4 µg/ml and 4 µg/ml, in coating buffer (0.05 M carbonate-bicarbonate buffer, pH 9.6), respectively. The wells of the ELISA plate were coated with 100 µl of these antigens and incubated overnight at 4°C. Typically, after removing the coating solution, the plates were then blocked with PBS containing 3% (w/v) skim milk for 1 hr at 37°C. After washing, the plates were incubated with serum samples (diluted 1:100). The bound antibody was detected by treating with horseradish peroxidase (HRP)-conjugated (Bethyl, Montgomery, AL, U.S.A.) to anti-horse IgG, anti-bovine IgG, anti-sheep/goat IgG or anti-dog IgG (1:4,000) and ABTS [2,2′-azinobis (3-ethylbenzthiazolinesulfonic acid)] (Sigma, St. Louis, MO, U.S.A.). The color was allowed to develop at room temperature. And, 50 µl stop solution was added (2 M sulfuric acid) to each well to stop the action of horseradish peroxidase in the substrate. Optical density (OD) was measured by an MTP-500 microplate reader (Corona Electric, Hitachinaka, Japan) at 415 nm. The ELISA results were determined for each sample by subtracting OD415 value of GST protein from OD415 value of recombinant TgSAG2 or NcSAG1 proteins. The cut-off values were determined using the *T. gondii* or *N. caninum*-negative sera from different animals and calculated as the mean of OD415 value of negative sera plus three standard deviations. The cut off values were determined as 0.098, 0.103, 0.107, 0.102 and 0.118 for TgSAG2-ELISA; and 0.118, 0.093, 0.102, 0.141 and 0.124 for NcSAG1-ELISA in horses (n=30), cattle (n=30), sheep (n=50), goats (n=50) and dogs (n=20), respectively (Fig. 2). A sample was considered positive when the absorbance value of sample was higher than the cut-off value.

The prevalence of anti-*Toxoplasma* antibodies was estimated from the ratio of positive results to the total number of different animal examined with the exact binomial confidence interval of 95% (95% CI). Odds ratio (OR) with 95% confidence intervals based on likelihood ratio statistics is reported. Difference in the prevalence of these pathogens among different provinces was calculated using the binary logistic regression in SPSS (Release 18.0 standard version, SPSS Inc., Chicago, IL, U.S.A.).

The prevalence of *T. gondii* and *N. caninum* in horses, cattle, sheep, goats and dogs from 11 provinces of Turkey is summarized in Table 1. The overall seroprevalence of *T. gondii* (24.1%, 95% CI: 22.3–26.0%) is significantly higher than *N. caninum* (4.0%, 95% CI: 3.3–5.0%) in the surveyed regions (*P*<0.0001; OR: 7.49; 95% CI: 5.88–9.56). Furthermore, the anti-*Toxoplasma* antibodies were detected in all of the 11 provinces (100%). The overall seroprevalence of *T. gondii* in horses (46.3%, 95% CI: 42.4–50.2%) was...
been reported. The infection of N. caninum has been reported in sheep and goats have been reported. The Neospora was reported at a prevalence of 3.3% and 6% in goats from Brazil and Czech Republic, respectively. Furthermore, 8.1% of sheep from Uberlandia, and 16.8% of sheep and 6.9% of goats from Greece showed seropositive for anti-N. caninum antibodies in a previous study [2, 12, 24]. The seroprevalence of N. caninum in sheep and goats obtained by us is similar with other reports from different countries. Antibodies to Neospora species in equine populations were reported in many parts of the world, such as Brazil [7, 9], U.S.A. [11] and Iran [14]. However, little is known on the prevalence of antibodies to Neospora in subclinically infected horses in Turkey. Several studies have detected the antibodies against N. caninum even more than 30% of horses [11]. The current study found that the proportion of horses with antibodies against N. caninum was quite low (0.3%), which was lower than previously reports for other regions. The finding of this study indicates that the horses in Turkey are at risk of neosporosis.

The highest prevalence of toxoplasma in cattle was found in Konya province (9.2%; 95% CI: 5.4–15.4%), and followed by Kirkilareli (2%; 95% CI: 0.7–15.4%) and Afyon (1.3%; 95% CI: 0.2–6.8%). The specific antibody against T. gondii could not be detected in other four provinces. For neosporosis, Zonguldak showed the highest prevalence (39.1%; 95% CI: 22.2–59.2%) in cattle. The seroprevalence of N. caninum in cattle was 7.7% (95% CI: 2.7–20.3%), 3.8% (95% CI: 1.3–10.5%), 5.9% (95% CI: 1.1–27.0%), 7.7% (95% CI: 3.6–15.8%) and 4.6 (95% CI: 2.1–9.7%) in Adana, Afyon, Duyarbakir, Kirkilareli and Konya provinces, respectively. Mixed infection was found in one cow. The sheep from Konya showed the highest prevalence (24.0%; 95% CI: 20.3–28.2%) of toxoplasma, and followed by Karaman (9.2%; 95% CI: 5.2–15.7%) and Zonguldak (7.5%; 95% CI: 2.6–19.9%) provinces. The seropositivity of Neospora in sheep from Karaman and Konya was 0.8% (95% CI: 0.2–4.6%) and 2.7% (95% CI: 1.5–4.6%). Mixed infection was found in 3 sheep. In goat samples, the antibodies against T. gondii were found to be 8.3% (95% CI: 3.6–18.1%) in Karaman and 14.3% (95% CI: 10.0–20.0%) in Konya. The specific antibodies against N. caninum were detected in the goats only from Konya province showing low level of infection rate with 4.2% (95% CI: 2.2–8.1%). Mixed infection was found in 2 goats. The seroprevalence of T. gondii (19.8%; 95% CI: 14.7–26.1) and N. caninum (16.6%; 95%
This work was also supported by the Scientific and Technology, Culture, Sports, Science, and Technology, Japan. Grant-in-Aid for Scientific Research from the Ministry of

ACKNOWLEDGMENTS. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology, Japan. This work was also supported by the Scientific and Technological Research Council of Turkey (TUBITAK 1001-1130336).

REFERENCES

1. Barber, J. S. and Trees, A. J. 1996. Clinical aspects of 27 cases of neosporosis in dogs. Vet. Rec. 139: 439–443. [Medline] [CrossRef]

2. Bártová, E. and Sedlák, K. 2012. Toxoplasma gondii and Neospora caninum antibodies in goats in the Czech Republic. Vet. Med. 57: 111–114.

3. Bártová, E., Sedlák, K. and Literák, I. 2009. Toxoplasma gondii and Neospora caninum antibodies in sheep in the Czech Republic. Vet. Parasitol. 161: 131–132. [Medline] [CrossRef]

4. Cedillo-Peláez, C., Díaz-Figueroa, I. D., Jiménez-Seres, M. I., Sánchez-Hernández, G. and Correa, D. 2012. Frequency of antibodies to Toxoplasma gondii in stray dogs of Oaxaca, México. J. Parasitol. 98: 871–872. [Medline] [CrossRef]

5. Chahan, B., Gaturaga, I., Huang, X., Liao, M., Fukumoto, S., Hirata, H., Nishikawa, Y., Suzuki, H., Sugimoto, C., Nagasawa, H., Fujisaki, K., Igarashi, I., Mikami, T. and Xuan, X. 2003. Serodiagnosis of Neospora caninum infection in cattle by enzyme-linked immunosorbent assay with recombinant truncated NcSAG1. Vet. Parasitol. 118: 177–185. [Medline] [CrossRef]

6. Diakoua, A., Papadopoulos, E., Panousis, N., Karatzias, C. and Giadinis, N. 2013. Toxoplasma gondii and Neospora caninum seroprevalence in dairy sheep and goats mixed stock farming. Vet. Parasitol. 198: 387–390. [Medline] [CrossRef]

7. Dubey, J. P. and Beattie, C. P. 1988. General biology. p.220. In:

| Animal species | Regions    | No. of animals tested | T. gondii-seropositive | N. caninum-seropositive | Both seropositive |
|----------------|------------|-----------------------|------------------------|-------------------------|------------------|
|                |            |                       | Frequency              | Prevalence (95% CI)     | Frequency        | Prevalence (95% CI) |
| Horses         | Adana      | 34                     | 17                     | 31.5 (20.7–44.7)        | 0                | 0 (0–6.6)          |
|                | Bursa      | 209                    | 56.5 (51.4–61.5)       | 1                       | 0.3 (0.1–1.5)    | 1 (0.3–0.1–1.5)   |
|                | Gaziantep  | 5                      | 26.3 (11.8–49.0)       | 0                       | 0 (0–16.8)      | 0 (0–16.8)        |
|                | Istanbul   | 19                     | 48.7 (33.9–63.8)       | 0                       | 0 (0–9.0)       | 0 (0–9.0)         |
|                | Izmir      | 2                      | 3.7 (1.0–12.5)         | 0                       | 0 (0–6.6)       | 0 (0–6.6)         |
|                | Konya      | 33                     | 41.3 (31.1–52.2)       | 1                       | 1.3 (0.2–6.8)   | 0 (0–4.6)         |
|                | Total      | 285                    | 46.3 (42.4–50.2)       | 2                       | 0.3 (0.1–1.2)   | 1 (0.2–0.9)       |
| Cattle         | Adana      | 30                     | 0 (0–9.0)              | 3                       | 7.7 (2.7–20.3)  | 0 (0–9.0)         |
|                | Afyon      | 1                      | 1.3 (0.2–6.8)          | 3                       | 3.8 (1.3–10.5)  | 0 (0–4.6)         |
|                | Diyarbakir | 17                     | 0 (0–18.4)             | 1                       | 5.9 (1.1–27.0)  | 0 (0–18.4)        |
|                | Karaman    | 10                     | 0 (0–27.8)             | 0                       | 0 (0–27.8)      | 0 (0–27.8)        |
|                | Kirkclee   | 2                      | 2.6 (0.7–8.9)          | 6                       | 7.7 (3.6–15.8)  | 0 (0–4.7)         |
|                | Konya      | 132                    | 9.2 (5.4–15.4)         | 6                       | 4.6 (2.1–9.7)   | 1 (0.8–0.4–2)    |
|                | Zonguldak  | 23                     | 0 (0–14.3)             | 9                       | 39.1 (22.2–59.2)| 0 (0–14.3)        |
|                | Total      | 377                    | 4.0 (2.4–6.5)          | 28                      | 7.4 (5.2–10.5)  | 1 (0.3–0.1–1.5)   |
| Sheep          | Karaman    | 120                    | 9.2 (5.2–15.7)         | 1                       | 0.8 (0.2–4.6)   | 0 (0–3.1)         |
|                | Konya      | 450                    | 24.0 (20.3–28.2)       | 12                      | 2.7 (1.5–4.6)   | 3 (0.2–2.0)       |
|                | Zonguldak  | 40                     | 7.5 (2.6–19.9)         | 3                       | 0 (0–8.8)       | 0 (0–8.8)         |
|                | Total      | 610                    | 20.0 (17.0–23.4)       | 13                      | 2.1 (1.3–3.6)   | 3 (0.5–2.1–4)    |
| Goats          | Karaman    | 60                     | 8.3 (3.6–18.1)         | 0                       | 0 (0–6.0)       | 0 (0–6.0)         |
|                | Konya      | 189                    | 14.3 (10.0–20.0)       | 8                       | 4.2 (2.2–8.1)   | 2 (1.0–3.3–8)    |
|                | Total      | 249                    | 12.9 (9.3–17.6)        | 8                       | 3.2 (1.6–6.2)   | 2 (0.8–2.2–9)    |
| Dogs           | Konya      | 187                    | 19.8 (14.7–26.1)       | 31                      | 16.6 (11.9–22.6) | 8 (4.3–2.8–2)    |
|                | Total      | 2,039                  | 24.1 (22.3–26.0)       | 82                      | 4.0 (3.3–5.0)   | 15 (0.5–1.2)     |
Toxoplasmosis of animals and man. CRC Press, Boca Raton.

8. Dubey, J. P. and Thruliez, P. 1993. Persistence of tissue cysts in edible tissues of cattle fed *Toxoplasma gondii* oocysts. *Am. J. Vet. Res.* 54: 270–273. [Medline]

9. Dubey, J. P., Kerber, C. E. and Granstrom, D. E. 1999. Serologic prevalence of *Sarcocystis neurona*, *Toxoplasma gondii*, and *Neospora caninum* in horses in Brazil. *J. Am. Vet. Med. Assoc.* 215: 970–972. [Medline]

10. Dubey, J. P. 2003. Review of *Neospora caninum* and neosporosis in animals. *Korean J. Parasitol.* 41: 1–16. [Medline] [CrossRef]

11. Dubey, J. P., Mitchell, S. M., Morrow, J. K., Ryhan, J. C., Stewart, L. M., Granstrom, D. E., Romand, S., Thruliez, P., Saville, W. J. and Lindsay, D. S. 2003. Prevalence of antibodies to *Neospora caninum*, *Sarcocystis neurona*, and *Toxoplasma gondii* in wild horses from central Wyoming. *J. Parasitol.* 89: 716–720. [Medline] [CrossRef]

12. Faria, E. B., Gennari, S. M., Pena, H. F., Atayde, A. C., Silva, M. L. and Azvedo, S. S. 2007. Prevalence of anti-*Toxoplasma gondii* and anti-*Neospora caninum* antibodies in goats slaughtered in the public slaughterhouse of Patos city, Paraiba State, Northeast region of Brazil. *Vet. Parasitol.* 149: 126–129. [Medline] [CrossRef]

13. Hemphill, A., Fuchs, N., Sonda, S., Gottstein, B. and Henrich, B. 1997. Identification and partial characterization of a 36 kDa surface protein on *Neospora caninum* tachyzoites. *Parasitology* 115: 371–380. [Medline] [CrossRef]

14. Hosseini, M., Moraveji, M., Tahamian, Y., Rahimian, A., Mohammadi, G. and Namavari, M. 2011. Seroprevalence of *Neosporasp*. in Horses in North East of Iran. *Iran. J. Parasitol.* 6: 64–68. [Medline]

15. Howe, D. K., Crawford, A. C., Lindsay, D. and Sibley, L. D. 1998. The p29 and p35 immunodominant antigens of *Neospora caninum* tachyzoites are homologous to the family of surface antigens of *Toxoplasma gondii*. *Infect. Immun.* 66: 5322–5328. [Medline] [CrossRef]

16. Huang, X., Xuan, X., Kimbila, E. N., Battur, B., Miyazawa, T., Fukimoto, S., Mishima, M., Makaka, L. H., Suzuki, H., Sugimoto, C., Nagasawa, H., Fujisaki, K., Mikami, T. and Igarashi, I. 2002. Development and evaluation of an enzyme-linked immunosorbent assay with recombinant SAG2 for diagnosis of *Toxoplasma gondii* infection in cats. *J. Parasitol.* 88: 804–807. [Medline] [CrossRef]

17. Ibrahim, H. M., Huang, P., Salem, T. A., Talaat, R. M., Nasr, M. I., Xuan, X. and Nishikawa, Y. 2009. Short report: prevalence of *Toxoplasma caninum* and *Toxoplasma gondii* antibodies in northern Egypt. *Am. J. Trop. Med. Hyg.* 80: 263–267. [Medline]

18. Kamani, J., Mani, A. U., Kumshe, H. A., Dogo, G. I., Yidawi, J. P., Pauline, D. K., Nnabuife, H. E., Joan, P. and Egwu, G. O. 2009. Serosurvey for *Toxoplasma gondii* in dogs in Maiduguri, Borno State, Nigeria. *J. Infect. Dev. Ctries.* 4: 15–18. [Medline]

19. Oncel, T. and Yural, G. 2006. Occurrence of *Toxoplasma gondii* antibodies in sheep in Istanbul, Turkey. *Vet. Arch.* 76: 547–553.

20. Pita Gondim, L. F., Barbosa, H. V. Jr., Ribeiro Filho, C. H. and Saeki, H. 1999. Serological survey of antibodies to *Toxoplasma gondii* in goats, sheep, cattle and water buffaloes in Bahia State, Brazil. *Vet. Parasitol.* 82: 273–276. [Medline] [CrossRef]

21. Prince, J. B., Auer, K. L., Huskinson, J., Parmley, S. F., Araujo, F. G. and Remington, J. S. 1990. Cloning, expression, and cDNA sequence of surface antigen P22 from *Toxoplasma gondii*. *Mol. Biochem. Parasitol.* 43: 97–106. [Medline] [CrossRef]

22. Raeghi, S., Akaberi, A. and Sedeghi, S. 2011. Seroprevalence of *Toxoplasma gondii* in Sheep, Cattle and Horses in Urmia North-West of Iran. *Iran. J. Parasitol.* 6: 90–94. [Medline]

23. Reid, A. J., Vermont, S. J., Cotton, J. A., Harris, D., Hill-Cawthorne, G. A., Könén-Waisman, S., Latham, S. M., Mourier, T., Norton, R., Quail, M. A., Sanders, M., Shannugam, D., Sohal, A., Wasmuth, J. D., Brunk, B., Grigg, M. E., Howard, J. C., Parkinson, J., Roos, D. S., Trees, A. J., Berriman, M., Pain, A. and Wastling, J. M. 2012. Comparative genomics of the apicomplexan parasites *Toxoplasma gondii* and *Neospora caninum*: Coccidia differing in host range and transmission strategy. *PLoS Pathog.* 8: e1002567. [Medline] [CrossRef]

24. Salaberry, S. R., Okada, L. H., Nassar, A. F., de Castro, J. R. and Lima-Ribeiro, A. M. 2010. Prevalence of *Neospora caninum* antibodies in sheep flocks of Uberlândia county, MG. *Rev. Bras. Parasitol. Vet.* 19: 148–151. [Medline] [CrossRef]

25. Tassi, P. 2007. *Toxoplasma gondii* infection in horses. A review. *Parasitologia* 49: 7–15. [Medline]

26. Trees, A. J., Davison, H. C., Innes, E. A. and Wastling, J. M. 1999. Towards evaluating the economic impact of bovine neosporosis. *Int. J. Parasitol.* 29: 1195–1200. [Medline] [CrossRef]

27. Wanha, K., Edelhofer, R., Gabler-Eduardo, C. and Prosl, H. 2005. Prevalence of antibodies against *Neospora caninum* and *Toxoplasma gondii* in dogs and foxes in Austria. *Vet. Parasitol.* 128: 189–193. [Medline] [CrossRef]

28. Webster Joanne, P. 2010. Review of “Toxoplasmosis of Animals and Humans (Second Edition)” by J. P. Dubey. *Parasites & Vectors* 3: 112.

29. Yan, C., Fu, L. L., Yue, C. L., Tang, R. X., Liu, Y. S., Lv, L., Shi, N., Zeng, P., Zhang, P., Wang, D. H., Zhou, D. H., Zhu, X. Q. and Zheng, K. Y. 2012. Stray dogs as indicators of *Toxoplasma gondii* distributed in the environment: the first report across an urban-rural gradient in China. *Parasit. Vectors* 5: 5. [Medline] [CrossRef]