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

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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

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 Karaman, Konya and Zonguldak provinces. Sheep that raised mainly 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 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 rGST 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-ethylbenzthiazinesulfonic 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-T. gondii and anti-N. caninum 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 prevalence of Neospora caninum has been evaluated in various species, including horses, sheep, and goats, in Turkey. Many studies indicate that the infection of this organism is not limited to dogs, as it is also common in horses, sheep, and goats in Turkey. The seroprevalence of Neospora caninum in horses, sheep, and goats varies from 0.2% to 6.9%, with 95% confidence intervals ranging from 0.2% to 6.8% (95% CI: 0.2–6.8%).

In this study, the seroprevalence of Toxoplasma gondii was determined in different animal species, including cattle, sheep, goats, and horses. The highest seroprevalence of Toxoplasma gondii in cattle was found in Konya province (9.2%; 95% CI: 5.4–15.4%), followed by Kirklareli (2%; 95% CI: 0.7–15.4%) and Afyon (1.3%; 95% CI: 0.2–6.8%). The specific antibody against Toxoplasma 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 Neospora 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, Diyarbakir, Kirklareli, 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 Neospora 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 was 19.8% (95% CI: 14.7–26.1) and N. caninum (16.6%; 95% CI: 11.9–22.6%) was found in 2 goats. The seroprevalence of T. gondii in horses was lower (4.0%, 95% CI: 2.4–6.5%) compared to cattle (7.4%, 95% CI: 2.4–6.5%).

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