

Detection of *Toxoplasma gondii* and *Neospora caninum* antibodies in Wild Boars (*Sus scrofa*) in Eastern Turkey

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ABSTRACT

The aim of this study was to examine for the presence of anti-*Toxoplasma gondii* and *Neospora caninum* antibodies in wild boars and to study the impact of infection between the sylvatic and domestic life cycles of these apicomplexan parasites. For this purpose, sera were collected from hunter-killed wild boars (*Sus scrofa*) during the winter period of 2011 from Erzurum province of Turkey. Collected sera were examined for antibodies against *T. gondii* and *N. caninum* by Sabin Feldman dye test (SFDT) and competitive-enzyme-linked immunosorbent assay (c-ELISA), respectively. Out of 12 collected samples, 4 (33.3%) of sera were found to be seropositive at the dilution of 1:16 for *T. gondii* however no seropositivity in any of the samples was detected against *N. caninum*. To the best knowledge of the authors, this is the first serologic study to detect anti-*T. gondii* antibodies in wild boars in Turkey.

Keywords: Wild Boar; *Sus scrofa*; Eastern Turkey; *Toxoplasma gondii*; *Neospora caninum*; Sabin-Feldman; c-ELISA.

INTRODUCTION

Toxoplasma gondii is one of the most important zoonotic protozoa that infect a variety of birds and mammals, including humans. Felids are the definitive hosts and many animal species are the intermediate hosts. Among these intermediate hosts, feral pigs are infected with *T. gondii* have been found to be infected at different rates between 4.4 to 45.9% (1). Antibodies to *T. gondii* have been reported in wild boars from Austria, Brazil, Czech Republic, France, Germany, Italy, Japan, Slovak Republic, Spain, United States (Georgia and South Carolina) (1, 2). The meat of wild boars with tissue cysts of *T. gondii* may be an important source for human toxoplasmosis when people consume these meats undercooked or without control (3).

Neospora caninum, an important parasitic disease of cattle, causes reproductive failure, especially abortion (4). Neosporosis is primarily a disease of cattle and dogs but antibodies have been detected a series of species, including domestic, wildlife and zoo animals (5). Dogs can acquire infection by ingestion of infected tissues; and intermediate hosts can be infected either by horizontal, postnatal or by vertical transmission (6). Due to the economic impact of neosporosis, existing studies are concentrated on farm animals and there is limited serological data about wild life (4-6). Anti-*N. caninum* antibodies have been reported in wild boars from Spain and the Czech Republic (7, 8).

For both *T. gondii* and *N. caninum* wild pigs may be important indicators for following environmental contamina-

tion since they are omnivorous animals obtaining infection from their local environment (9). Serologic tests (IFA, MAT, LAT, DT and c-ELISA) are used to monitor anti-*T. gondii* and *N. caninum* antibodies in wild pigs (1, 8, 9).

The aim of this study was to investigate the presence of anti-*T. gondii* and *N. caninum* antibodies in wild boars and to examine the impact between the sylvatic and domestic life of pigs in relation to these apicomplexan parasites.

MATERIAL AND METHODS

Sample collection

Blood samples were collected from 12 wild boars that were provided by certified hunters from Horasan and Pasinler regions of Erzurum province in Turkey in the year 2011. From information provided by hunters, the majority of these wild boars were adult in the age range 1-3 years, and all of them were female. The blood samples were collected for serological studies from the heart, following the death of animals after shooting. The blood samples were transferred into vacuum tubes, allowed to clot and then centrifuged at 4000 rpm for 10 minutes at room temperature. Subsequently they were placed into eppendorf tubes and stored at -20°C until use.

Serologic examination

All sera were examined for *T. gondii* antibodies using the SF dye test (SFDT) as described (10). The examinations were carried out at the National Reference Laboratory for Parasitology, Public Health Institution of Turkey. The procedure included two steps in preparation for performing the test. Healthy 3-4 week old white Swiss albino mice were injected with the virulent RH strain of *T. gondii*. *T. gondii* RH antigen was collected from the peritoneal fluid of mice after 48 hrs post injection. As an activator serum, human serum seronegative for *T. gondii* was used including factors such as magnesium, properdin, C₂, C₃, C₄. Alkaline methylene blue dye was prepared with 9.73 ml of 0.53 % Na₂CO₃ (Sigma, Seelze, Germany), 0.27 ml of 1.91 % Na₂B₄O₇·10H₂O (Merck, NJ, USA) and 25 mg of methylene blue (Difco, Detroit, MI, USA). Following inactivation of complement at 56°C for 30 minutes, 25 µl of test sera were prepared with normal saline in dilutions of 1:4, 1:16, 1:64, 1:256 and 1:1024. The antigen was added to the sera preparations of 25 µl activator serum at approximately 25 *T. gondii* tachyzo-

ites observed in a microscopic field of 40X magnification. The mixture was incubated in a water-bath at 37°C for 50 minutes. 25 µl of alkaline methylene blue was added to the mixture and kept in 4°C for 10 minutes. Examination was carried out using light microscopy with 40X objective to gauge whether *T. gondii* tachyzoite were stained. If more than 50 % of tachyzoites on one microscopic field were not stained, this dilution step was accepted as positive. Titers of 1:16 and greater were considered as positive (11). Positive and negative controls, which were confirmed by IFAT method, were included in the above procedure.

Serologic examination (c-ELISA)

Antibodies to *N. caninum* were detected by using a commercially available competitive enzyme-linked immunosorbent assay (c-ELISA) kit (VMRD, USA). The test was done by following the instructions of manufacturer. The mean optical density (OD) at 630 nm was determined for all wells using a microplate reader (ELx 800 UV, Universal Microplate Reader, Bio-Tek Instruments, Inc., Winooski, VT, USA). The percent inhibition for each test sample was determined using the below mentioned formula:

$$\text{Inhibition (\%)} = 100 - \left[\frac{\text{Sample O.D.} \times 100}{\text{Mean Negative Control O.D.}} \right]$$

The samples with values of ≥ 30% inhibition were regarded as positive and those with the values < 30% inhibition were regarded as negative (12).

RESULTS

Anti-*T. gondii* antibodies in SFDT test were detected in 4 of 12 (33.3%) wild boar sera to be seropositive at 1:16 dilution and, no seropositive cases (0%) were detected for *N. caninum* antibodies.

DISCUSSION

The wild boar is the only member of the suidae family present in Turkey and bioecological data about this animal are limited (13). Except in touristic regions, pork meat consumption is restricted in Turkey; however, wild boars are important game species and have the potential to cause damage to agricultural crops in Turkey (13). When the wild boar population increases, hunting organizations are arranged to cull wild boars and in so doing decrease the crop damage. Due to restricted

meat consumption, hunted animals are often left behind, close to the farm lands and consumed by domestic and wild carnivores. This scenario leads wild boars into importance in the sylvatic and domestic life cycles of *T. gondii* and *N. caninum*.

Studies concerning *N. caninum* infections in wild boars is limited (8, 14). Almería *et al.* studied 298 wild boars in Spain and found a prevalence of 0.3% (14). Bártová *et al.* studied on 565 wild boars and they detected the prevalence as 18.1% in Czech Republic (8). Researchers used c-ELISA for monitoring and IFAT for confirmation. In our study we could not detect any seropositivity with c-ELISA. In domestic pigs, experimental and natural infections have shown a low *N. caninum* incidence that suggests that environmental exposure to *N. caninum* is rare. This opinion may explain why we could not detect seropositivity for wild boars in our survey (14). Therefore, it appears that wild boar may not play an important role between the sylvatic and domestic life cycle of *N. caninum* infection.

Ranucci *et al.* studied 400 wild boars in Italy and found the prevalence of *T. gondii* as 14% using IFAT (2). Gauss *et al.* (15) studied on 507 wild boars and found the prevalence of *T. gondii* 38.4% with MAT in Spain. Richomme *et al.* studied 148 wild boars and found the prevalence 17.6% with MAT in France (16). Hejlíček *et al.* (17) studied on 124 wild boars and found a prevalence of 15% with SFDT in the Czech Republic. Furthermore the prevalence rates of *T. gondii* were found as 18.2% (n=170) and 34.4% (n=257) in Georgia and South Carolina provinces of United States respectively with MAT, 4.4% (n=90) and 5.6% (n=108) in Kukumoto and Iriomote Island of Japan respectively with LAT (1).

In our study we detected *T. gondii* antibodies in 4 out of 12 (33.3%) animals with SFDT. The differences between the results may be associated with different climatic conditions, sample sizes, serological tests used and different species of final hosts, their population density and their feeding behaviors. Although our sample size is small, 33.3% prevalence may be important for the transition between sylvatic to domestic life cycles by domestic and wild felids.

Limitations of this study include the small sample size and the fact that only female wild pigs were tested. In this regard this study only gives an indication of the distribution of these diseases among wild pigs may be considered a preliminary study to further larger and wider ranging studies in this area of Turkey. Differences in infection rates between the

sexes has not been extensively studied for either *Toxoplasma gondii* and *Neospora caninum* although a study in dogs has demonstrated a greater prevalence of *N. caninum* antibodies in female dogs than in males (18, 19).

In conclusion, it is hoped that the results of this preliminary study will contribute to the understanding of these apicomplexan parasites' epidemiology in the wild life. Further studies are required for developing effective control programs and a clear understanding of these diseases both in Turkey and worldwide.

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