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Occurrence of anti-*Toxoplasma gondii* and *Neospora caninum* antibodies in camels (*Camelus dromedarius*) in the center of Iran

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Abstract: During the period from December 2008 to September 2009, we examined 254 serum samples (164 in hot-dry weather and 90 in cold-dry weather) from dromedary camels distributed all over Yazd Province, in the center of Iran. IgG antibodies were assayed by the modified agglutination test (MAT) and *Neospora* agglutination test (NAT) using whole tachyzoites of *Toxoplasma gondii* and *Neospora caninum*, incorporating 2-mercaptoethanol, for *T. gondii* and *N. caninum*, respectively. Anti-*T. gondii* antibodies were found in 37 (14.56%) of 254 camels with titers of 1:20 in 7, 1:40 in 6, 1:80 in 8, 1:160 in 1, 1:200 in 4, 1:400 in 6, 1:800 in 4, and 1:1600 in 1 camel. Out of 254 (3.94%) camel sera, 10 had antibodies to *N. caninum* with titers of 1:20 in 6 and 1:40 in 4 camels. There was no difference between the presence of antibodies for both parasites in male and female camels or in different weather conditions, but occurrence of anti-*T. gondii* antibodies was greater in older camels.

Key words: *Toxoplasma gondii*, *Neospora caninum*, dromedary camel, Iran

1. Introduction

Toxoplasma gondii and *Neospora caninum* are 2 closely related cyst-forming apicomplexan protozoa that infect many warm-blooded animals (1,2). Morphologic and biologic characteristics of *N. caninum* are similar to those of *T. gondii*, and until 1988 it was misdiagnosed as *T. gondii*. Both parasites form intracellular tachyzoites and tissue cysts in intermediate hosts. The importance of felids in the epidemiology of *T. gondii* infection stems from the fact that they are the only hosts that can excrete environmentally resistant oocysts. Unlike *T. gondii*, oocysts of *N. caninum* have not been demonstrated or isolated from cats (3). Dogs, coyotes, and gray wolves have been identified as definitive hosts for *N. caninum* (1,4,5). Dogs also act as the intermediate host for *N. caninum* (6).

T. gondii and *N. caninum* can cause abortion in ruminants (7–10). *T. gondii* infection is prevalent in all warm-blooded vertebrates, and *N. caninum* is a major pathogen for cattle and dogs, which occasionally causes clinical infections in other animals including camels (1).

Both parasites may be transmitted to intermediate hosts following ingestion of oocysts by animals via contaminated feed or water. Vertical transmission from mother to fetus through the placenta is an alternative route (4). The 3 principal ways by which humans are infected with *T. gondii* are congenital infection, ingestion of raw or undercooked meat containing tissue cysts, and ingestion of water or poorly washed raw fruits and vegetables contaminated with sporulated oocysts present in the feces of infected cats (11).

T. gondii infection does not usually cause clinical signs in healthy humans and animals, but it is sometimes embryotoxic. Infection in pregnant women can cause severe fetal diseases, including abortion, encephalitis, mental retardation, and blindness (12).

Evidence of widespread *T. gondii* infection in meat-producing animals has been found by serological studies (13–16). *T. gondii* is widely prevalent in human beings and animals in Iran (17–23).

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Dromedary camels (*Camelus dromedarius*) are an important multipurpose animal of arid and semiarid parts of the world. In Yazd Province they are mainly kept for the purpose of meat production. They are slaughtered in registered industrial slaughterhouses in Iran, under strict hygienic controls and inspections. According to the last enumeration, there were about 153,000 camels in Iran, 21,690 of them counted in Yazd Province (24). The objective of the present study was to investigate the occurrence of anti-*T. gondii* and *N. caninum* antibodies in dromedary camels in Yazd Province located in the center of Iran.

2. Materials and methods

2.1. Collection of serum samples

During a period of 1 year, from the beginning of December 2008 to the end of September 2009, 5 different regions were selected from 11 camel-rearing areas in Yazd Province (Figure) for 2-stage cluster random sampling. Factors likely affecting the seropositivity rate in the analyses were age, sex, and weather conditions. From a total of 254 dromedary camels, 164 were from hot-dry seasons and 90 were from cold-dry seasons. Due to transport or slaughtering of the sample animals, the animals in different seasons were not the same, although they were from the same population. The tested animal population comprised 207 males and 44 females. This was due to the fact that the females were kept for breeding purposes and the owners often do not permit sampling from females. Blood samples were collected from camels ranging in age from 6 months to 30 years. Animals did not have any clear symptoms of diseases at the time of sampling. Blood samples were centrifuged at 1000 × *g* and sera were stored at −20 °C until analysis.

2.2. Serologic test for *T. gondii*

The sera were tested for the presence of *T. gondii* antibodies using the modified agglutination test (MAT) based on direct agglutination of fixed parasites with sera pretreated with 2-mercaptoethanol to prevent nonspecific IgM agglutination, as described by Desmonts and Remington (25) and Dubey and Desmonts (26). The *T. gondii* antigen used in the MAT was prepared from parasites that were harvested from cells grown in mice in the Pasteur Institute of Iran. The sera were started at 1:10 serum dilution to prevent false negatives. We continued the test until a negative dilution was observed.

The MAT was conducted in 96-well U-bottomed microtiter plates. The sera were diluted with 0.01 M phosphate-buffered saline at pH 7.2. The antigen suspension was composed of 2.5 mL of borate buffer, pH 8.9, containing 0.4% bovine serum albumin, 35 µL of 2-mercaptoethanol, 50 µL of 2 mg/mL Evans blue, and 140 µL of the suspension of whole tachyzoites. In each well, 50 µL of the antigen solution and 50 µL of the serum to be tested were mixed, and the microtiter plate was incubated at 37 °C for 12 h (25,26).

2.3. Serologic test for *N. caninum*

The sera were also tested by the *Neospora* agglutination test (NAT), as described by Romand et al. (27). This test is identical to the MAT for *T. gondii*, but *N. caninum* tachyzoites are used instead of *T. gondii*. The sera were run at 1:10 dilution.

For the above 2 tests, a complete carpet of agglutinated parasites was considered a positive result, and a clear-cut button-shaped deposit of parasite suspension at the bottom of the well was interpreted as a negative reaction.

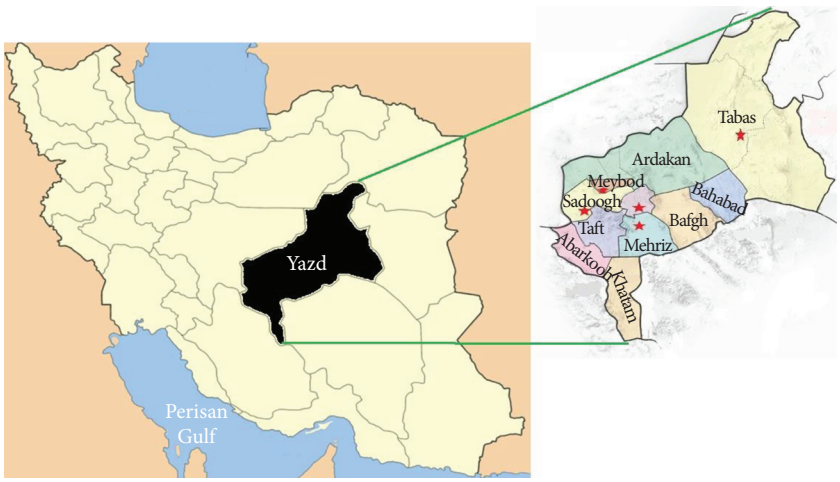

Figure. Location of Yazd Province in Iran. The samples were taken from 5 subregions, which are marked by stars.

2.4. Statistical analysis

The results obtained from the serum evaluation were analyzed statistically by logistic regression using SPSS 16. The chi-square test was also performed for *T. gondii*, and Fisher's exact test was used for analyzing *N. caninum* results. Alpha was 0.05 for all tests for the association between the presence of *Toxoplasma* and *Neospora* antibodies in camels regarding age, sex, and weather conditions.

3. Results

Anti-*Toxoplasma gondii* antibodies were found in 37 out of 254 camels (14.57%) with titers of 1:20 in 7, 1:40 in 6, 1:80 in 8, 1:160 in 1, 1:200 in 4, 1:400 in 6, 1:800 in 4, and 1:1600 in 1 camel. *N. caninum* antibodies were detected in 10 out of 254 tested camels, giving a 3.94% seropositivity rate with titers of 1:20 in 6 and 1:40 in 4.

From 208 male camels, 31 (14.90%) and 8 (3.85%) were positive for *T. gondii* and *N. caninum* antibodies, respectively. From 46 females, 6 (13.04%) were positive for *T. gondii* and 2 (4.35%) for *N. caninum*.

Analysis with logistic regression revealed that camels of higher ages were significantly associated with greater *T. gondii* infection. The odds ratio (OR) was 1.216 with a 95% confidence interval (CI) ($P < 0.001$). However, no significant association was seen between age and occurrence of *N. caninum* (OR = 1.092, CI = 95%; $P > 0.05$).

The frequency of infection among both sexes and both weather conditions was similar for both parasites, and there was no relation between those factors and the occurrence of *T. gondii* or *N. caninum* ($P > 0.05$). The results of this study are shown in the Table.

4. Discussion

To the best of our knowledge, this is the first preliminary study that investigated the occurrence of *T. gondii* and *N. caninum* antibodies among dromedary camels in Yazd Province, central Iran.

Previous studies in different countries were often designed without considering *Neospora caninum* protozoa, which are highly related to *T. gondii*. This prompted us to conduct a survey on camels in the central region of Iran to determine the seroprevalence of both *T. gondii* and *N. caninum* infections in this animal, which is mostly used for meat and milk consumption in this area.

Our findings showed 14.57% seropositivity among examined camels for *T. gondii* and 3.94% for *N. caninum*. The *T. gondii* infection was increased statistically with the age of the camels, but this was not true for *N. caninum*, which may be due to an insufficient number of positive animals. Statistical analyses revealed no difference between seroprevalence of *T. gondii* and *N. caninum* in sex groups and different weather conditions. The 14.57% prevalence of *T. gondii* antibodies in the present study is similar to the 17.4% prevalence of *T. gondii* found in Egypt (28) and the 16% prevalence in Saudi Arabia (29). Hilali et al. (28) and Hosseininejad et al. (30) also showed similar results for prevalence of *N. caninum* (3.72% and 3.22%, respectively) in Egypt and Iran. However, in a study by Wernery et al. (31) in the United Arab Emirates, 13.7% of examined camels were found to be seropositive to *N. caninum* by enzyme-linked immunosorbent assay.

Using an immunofluorescence antibody test, Sadrebazzaz et al. (32) showed that antibodies against *N. caninum* and *T. gondii* were present in 5.83% and 4.16%, respectively, of camels from the northeastern region of Iran. This difference in results may be due to different procedures, the initial serum dilution, or different climate and weather conditions.

The data suggest that the occurrence of toxoplasmosis in one-humped camels in the center of Iran is relatively considerable, and consumption of camel meat may pose a risk to humans in the area of study.

In contrast to the occurrence of clinical toxoplasmosis, clinical neosporosis in camels has not yet been reported.

Table. *T. gondii*- and *N. caninum*-specific antibodies in dromedary camel in Yazd, Iran, according to sex and weather conditions.

	n	*T. gondii* seropositivity rate (n) %	*N. caninum* seropositivity rate (n) %
Sex			
Male	208	(31) 14.90	(8) 3.85
Female	46	(6) 13.04	(2) 4.35
Weather conditions			
Hot-dry	164	(24) 14.63	(6) 3.66
Cold-dry	90	(13) 14.44	(4) 4.44
Total	254	(37) 14.57	(10) 3.94

Acute toxoplasmosis was observed in a 6-year-old camel. Dyspnea was the main clinical sign, and many tachyzoites were found in the lungs and plural exudates (33).

Toxoplasmosis is a globally distributed zoonosis with serious impact on unborn fetuses and also immunosuppressed individuals (34,35). The prevalence and impact of *T. gondii* on human health are highly variable geographically, and sources of infection vary greatly in different human populations with different culture and eating habits (35,36). In a cross-sectional study conducted on adult humans, the group avoiding meat consumption had a significantly lower prevalence rate of infection (18%) as compared with another group that consumed meat (40%) (37). *T. gondii* is responsible for approximately 21% of all deaths attributed to foodborne pathogens in the US, and the Centers for Disease Control estimates that 50% of all human exposure to *T. gondii* is foodborne (38). In Europe, up to 63% of human *Toxoplasma* infections are attributable to the consumption of undercooked or cured meat products (39).

In conclusion, although our results cannot provide an estimate of the percentage of infected camel meats, the consumption of camel meat may be one of the sources of infection for humans in the center of Iran. Therefore, meat and other edible parts of animals should be cooked thoroughly before consumption.

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