Revista Brasileira de Parasitologia Veterinária
Brazilian Journal of Veterinary Parasitology
ISSN 1984-2961 (Electronic)
www.cbpv.org.br/rbpv

Original Article

Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in Dromedary camels (*Camelus dromedarius*) from Saudi Arabia

Soroprevalência de *Toxoplasma gondii* e *Neospora caninum* em camelos dromedário (*Camelus dromedarius*) da Arábia Saudita

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How to cite: Mohammed OB, Amor N, Omer SA, Alagaili AN. Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in Dromedary camels (*Camelus dromedarius*) from Saudi Arabia. *Braz J Vet Parasitol* 2020; 29(1): e019119. http://doi.org/10.1590/S1984-29612020008

Abstract

Serological screening of 199 serum samples from Dromedary camels—from different cities in Saudi Arabia—was performed using enzyme-linked immunosorbent assay for detecting antibodies against two cyst-forming coccidian parasites, namely *Toxoplasma gondii* and *Neospora caninum.* Antibodies against *T. gondii* were detected in 68 (34.2%) samples, while those against *N. caninum* were present in 33 (16.6%) samples. The highest seroprevalence of *T. gondii* antibodies was reported in samples from Taif (51.2%), while the lowest seroprevalence was reported in samples from Riyadh and Hofuf (15.1%). The highest seroprevalence of *N. caninum* antibodies was reported in samples from Jizan (35.9%) while the lowest was reported in samples from Taif (2.4%). A total of 47 male and 21 female camels exhibited antibodies against *T. gondii*, while 19 male and 14 female camels showed antibodies against *N. caninum*. Concurrent detection of both *T. gondii* and *N. caninum* antibodies was observed in 18 camels. It has been demonstrated that *T. gondii* and *N. caninum* antibodies are prevalent in camels from different cities of the Kingdom of Saudi Arabia.

Keywords: Dromedary camel, Saudi Arabia, Seroprevalence, *Neospora caninum, Toxoplasma gondii*.

Resumo

A triagem sorológica para a detecção de anticorpos para *Toxoplasma gondii* e *Neospora caninum* no camelo dromedário foi realizada investigando 199 amostras de soro coletadas em diferentes cidades da Arábia Saudita. As amostras foram testadas utilizando imunoensaios enzimáticos para a detecção de anticorpos de ambos os parasitas coccídeos formadores de cistos (Laboratórios IDEXX, Bommeli Diagnostics, AG, Berna, Suíça). Anticorpos contra *T. gondii* foram detectados em 68 (34,2%) amostras, enquanto 33 (16,6%) apresentaram anticorpos contra *N. caninum.* A maior soroprevalência de anticorpos contra *T. gondii* (51,2%) foi relatada em Taif, enquanto a menor soroprevalência (15,1%) foi relatada em Riyadh e Hofuf. A maior soroprevalência de anticorpos contra *N. caninum* foi relatada em Jizan (35,9%), enquanto a menor foi em Taif (2,4%). Um total de 47 machos e 21 fêmeas revelou anticorpos para *T. gondii*, enquanto 19 machos e 14 fêmeas revelaram anticorpos para *N. caninum*. A detecção de ambos os anticorpos contra *T. gondii* e *N. caninum* foi de 18 indivíduos. Foi demonstrado que os anticorpos contra *T. gondii* e *N. caninum* são predominantes em camelos de diferentes cidades do Reino da Arábia Saudita.

Palavras-chave: Camelo dromedário, Arábia Saudita, Soroprevalência, *Neospora caninum*, *Toxoplasma gondii*.

Received October 31, 2019. Accepted January 21, 2020.
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Introduction

Neospora caninum and *Toxoplasma gondii* are obligate intracellular protozoan parasites that infect a wide range of domestic and non-domestic animals, as well as humans (Dubey, 2010; Donahoe et al., 2015). The zoonotic nature of toxoplasmosis has been well established, whereas despite serological evidence in humans, the disease caused by *N. caninum* is not considered zoonotic (Tranas et al., 1999; Lobato et al., 2006). *T. gondii* (a cyst-forming coccidium) was first discovered in 1908 and named a year later; however, its life cycle was fully elucidated in the 1970s (Dubey & Frenkel, 1972). The other cyst-forming coccidium, *N. caninum*, was first identified in 1984 (Bjerkas et al., 1984) and its detailed life cycle was elucidated in 1998 (McAllister et al., 1998).

Both parasites are believed to be transmitted to intermediate hosts via consumption of food contaminated with the sporulated oocysts shed by the definitive hosts. Domestic cat and other members of the family Felidae are the definitive hosts for *T. gondii* (Dubey, 2010), while dogs and coyotes are the definitive hosts for *N. caninum* (McAllister et al., 1998; Gondim et al., 2004). Vertical transmission from mother to fetus through the placenta is an alternative route for transmission of both parasites (McAllister et al., 2000; Dubey, 2010).

N. caninum is a bovine pathogen and abortion is the principal clinical manifestation of infected pregnant animals (Dubey & Schares, 2011). Intrauterine fetal death may occur during late gestation; the dead fetuses are usually expelled and show moderate autolysis. Fetuses succumbing in less than five months or during early gestation may be mummified and retained in the uterus for several months. Repeat breeding may be a consequence of fetal death during early stage of gestation in infected cows (Moore et al., 2002). One of the clinical features of toxoplasmosis is abortion, along with neurological symptoms in its intermediate hosts (Dubey & Beattie, 1988; Dubey, 2010).

Various reports on *N. caninum* and *T. gondii* antibodies in camels (*Camelus dromedarius*) from Saudi Arabia and the neighboring countries have been published (Hussein et al., 1988; Hilali et al., 1998; Sadrebazzaz et al., 2006; Wernery et al., 2008; Hosseininejad et al., 2010; Alanazi, 2011; Hamidinejat et al., 2013; Aljumaah et al., 2018). Most of these reports detected a low prevalence of *N. caninum* (3.2% to 5.8%) whereas the report by Aljumaah et al. (2018) reported a considerably higher prevalence of 22%. *T. gondii* antibodies in these studies were reported in the range between 4.2% and 17.4%.

Only two studies dealing with the seroprevalence of *N. caninum* from camels (*C. dromedarius*) were conducted in Saudi Arabia; the first one was limited to Riyadh Province (Alanazi, 2011). The second study was by Aljumaah et al. (2018) from different regions of Saudi Arabia who reported high prevalence of *N. caninum* antibodies. There was a contrast between the findings of the two studies where seroprevalence was found 5.6% in the first study while it was 21.99% in the second study. Furthermore, both studies dealt only with *N. caninum* and did not show the cities from where the samples were collected. Cities are important for the availability of camels slaughtered for human consumption. Studies on *T. gondii* in camels were only few and restricted to Riyadh and Hofuf, the study from Hofuf was not even on seroprevalence and it was on investigating the role of camels as an intermediate host for the parasite, no study has covered different cities in Saudi Arabia (Hussein et al., 1988; Hilali et al., 1995; Alanazi, 2011, 2013).

In the present study, we reported the antibody prevalence of both *T. gondii* and *N. caninum* in the Dromedary camel (*Camelus dromedarius*) from blood samples collected from different cities of Saudi Arabia in order to understand the potential role of this animal species in the epidemiology of these parasites and the possible risk of zoonosis.

Materials and Methods:

Blood samples were obtained from apparently healthy camels by drawing 5 ml of jugular blood into plain vacutainer tubes (Becton, Dickinson and Company 1 Becton Drive, Franklin Lakes, NJ, USA) and then left to clot. Serum was collected after subjecting the

clotted blood to centrifugation at 900 *g* for 10 minutes and stored at -20 °C until further use. A total of 199 blood samples were collected from camels from different cities in Saudi Arabia. Hofuf (n=38) represented the eastern region, Riyadh (n=38) represented the central region, Tabuk (n=43) represented the northern region, Jizan (n=39) represented the southern region and Taif (n=41) represented the western region. None of the sampled camels showed apparent signs of any disease and none of the females appeared to be pregnant.

An Enzyme-Linked Immuno Sorbent Assay (ELISA)-utilizing a kit available from Idexx (HerdCheck® Anti-*Neospora*; and Idexx Toxo-test IDEXX Laboratories; Bommeli Diagnostics, AG, Bern, Switzerland)-was used to detect anti-*Neospora* and anti-*Toxoplasma* IgG-antibodies in the camel serum samples. The kits were used as per the manufacturers' instructions. In the laboratory, the detection of the primary antibodies to *T. gondii* and *N. caninum* in the camel serum samples was brought about by using the secondary antibody anti-llama IgG-horseradish peroxidase conjugate (Bethyl Laboratories, Montgomery, TX) at a dilution of 1:15,000. This conjugate was previously used at the laboratory for the detection of antibodies to the Middle East Respiratory Syndrome corona virus (MERS CoV) in camel serum samples (Alagaili et al., 2014). The presence or absence of antibodies was determined via reading the reaction result on the ELISA reader at 450 nm. The presence or absence of specific antibodies for each test sample was determined by comparing the optical density percentage of the test samples with those of the control samples provided with the kit. Samples showing values of ≥ 40% were considered positive using the following formula:

$$\text{OD\% of the test sample} = \frac{(\text{OD of sample} - \text{OD of negative control})}{(\text{OD of positive control} - \text{OD of negative control})} \times 100$$

Statistical analysis of data obtained for the seroprevalence of *N. caninum* and *T. gondii* were computed using the Chi square test in the GraphPad statistical software (Prism 6.0). Chi square test values were considered significant if $p \leq 0.05$. The relative risk (RR) and 95% confidence interval were calculated according to Altman (1991).

Results

The overall antibody prevalence of *T. gondii* was found to be 34.2%, with the highest prevalence being reported in serum samples from Taif (51.2%), while the lowest was recorded from camels from Riyadh and Hofuf (15.8%), as shown in Table 1 and Figure 1. Antibodies against *T. gondii* were detected in 47 (35.1%) females and 21 (32.1%) males. *T. gondii* was more prevalent in males than in females in Taif (56.8%) and Jizan (54.2%), while the highest female seroprevalence of *T. gondii* was reported in samples from Tabuk (58.8%) (Table 2). The risk ratio of *T. gondii* prevalence is significantly higher in Taif, Jizan, and Tabuk than in Riyadh and Hofuf (Table 1).

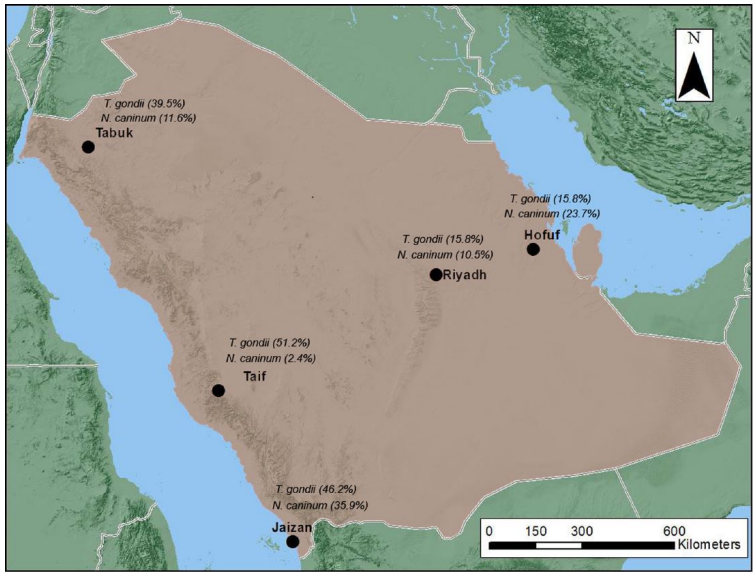

Figure 1. A map of Saudi Arabia showing cities where camels were sampled together with the results of the seroprevalence of *T. gondii* and *N. caninum* from camels sampled.

Table 1. Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in camels from different cities and comparison of risk ratio (RR) in different cities of Saudi Arabia.

City	Number examined	*Toxoplasma gondii*			*Neospora caninum*		
		Number positive (%)	RR (95%CI)	*P*	Number positive (%)	RR (95%CI)	*P*
Hofuf	38	6 (15.8)	1		9 (23.7)	9.7 (1.3-73.1)	**0.03**
Riyadh	38	6 (15.8)	1 (0.4-2.8)	1.00	4 (10.5)	4.3 (0.5-36.9)	0.18
Tabuk	43	17 (39.5)	2.5 (1.1-5.7)	**0.02**	5 (11.6)	4.8 (0.6-39.1)	0.15
Jizan	39	18 (46.2)	2.9 (1.3-6.6)	**0.009**	14 (35.9)	14.7 (2.0-106.7)	**0.008**
Taif	41	21 (51.2)	3.2 (1.5-7.2)	**0.003**	1 (2.4)	1	
Total	**199**	**68 (34.2)**			**33 (16.6)**		

RR=risk ratio; CI= confidence interval.

Table 2. Results of seroprevalence of *T. gondii* and *N. caninum* in male and female camels from different regions in Saudi Arabia (M=Male; F=Female).

City	Number of animals			*Toxoplasma gondii*			*Neospora caninum*		
	M	F	Total	M (%)	F (%)	Total (%)	M (%)	F (%)	Total (%)
Hofuf	15	23	38	1 (6.7)	5 (21.7)	6 (15.8)	3 (20)	6 (26.1)	9 (23.7)
Riyadh	29	11	38	5 (17.2)	1 (9.1)	6 (15.8)	4 (13.8)	0 (0)	4 (10.5)
Tabuk	26	17	43	7 (26.9)	10 (58.8)	17 (39.5)	2 (7.7)	3 (17.6)	5 (11.6)
Jizan	24	15	39	13 (54.2)	5 (33.3)	18 (46.2)	9 (37.5)	5 (33.3)	14 (35.9)
Taif	37	4	41	21 (56.8)	0 (0)	21 (51.2)	1 (2.7)	0 (0)	1 (2.4)
Total	**134**	**65**	**199**	**47** (35.1)	**21** (32.3)	**68** (34.2)	**19** (14.2)	**14** (21.5)	**33** (16.6)

The overall antibody prevalence of *N. caninum* was 16.6%, with the highest prevalence being reported in serum samples from Jizan (35.9%); the lowest prevalence was found in samples from Taif (2.4%) (Table 1; Figure 1). Antibodies against *N. caninum* were reported in 19 males and 14 females (Table 2). The risk ratio of *N. caninum* prevalence is significantly higher in Jizan and Hofuf than in Riyadh, Tabuk, and Taif (Table 1).

Males have significantly higher prevalence of *T. gondii* antibodies compared to those against *N. caninum* (p=0.0001), while the difference between the prevalence of antibodies against these two parasites in females was not significant (p=0.24). There was no significant difference between the prevalence of *T. gondii* and *N. caninum* antibodies among males and females (p=0.75 and p=0.22, respectively).

Concurrent detection of both *T. gondii* and *N. caninum* antibodies was also reported during the present study. *Toxoplasma gondii* antibodies were detected in 18 camels which tested positive for antibodies against *N. caninum* too. Of those 12 were from Jizan, 3 from Tabuk, 2 from Hofuf and 1 from Taif.

Discussion

In the present study, a seroprevalence analysis of two cyst-forming coccidia (*T. gondii* and *N. caninum*) in the Dromedary camel (*Camelus dromedarius*) in different cities covering different regions in Saudi Arabia was performed. The seroprevalence of *T. gondii* was found to be 34.2%, while that of *N. caninum* was found to be 16.6%.

The seroprevalence of *T. gondii* was comparable to that reported in previous studies on camels from Saudi Arabia and its neighboring countries (Hussein et al., 1988; Hilali et al., 1998; Wernery et al., 2008; Hosseininejad et al., 2010; Alanazi, 2011; 2013; Hamidinejat et al., 2013; Mentaberre et al., 2013). Extremely high prevalence—as high as 90.9% (from Turkey) (Utuk et al., 2012), 67% (from Butana plains, eastern Sudan) (Elamin et al., 1992) and 40.5% (from Fentale district, central Ethiopia) (Gebremedhin et al., 2014)—of *T. gondii* antibodies in camels have been reported. In our study, the difference in the prevalence of *T. gondii* antibodies between male and female camels was not significant and was in agreement with that reported in previous studies (Elamin et al., 1992; Wang et al., 2013; Gebremedhin et al., 2014). However, this is in contrast with what has been reported earlier by Hussein et al. (1988), who detected significantly higher prevalence of *T. gondii* antibodies in female camels than in male camels; they attributed this difference to the husbandry practices. In most studies carried out on the prevalence of *T. gondii* antibodies in camels, it was found that old camels generally had a higher prevalence compared to younger camels (Hussein et al., 1988; Gebremedhin et al., 2014). Calves are also affected by the infection, but it was not certain whether they acquired the infection as a result of innocuous consumption of cysts arising from Felidae family members or as a result of vertical transmission from mothers such as that in the case of *N. caninum* in camels from the Canary Islands (Mentaberre et al., 2013).

There are three reports on the prevalence of *N. caninum* in animals in Saudi Arabia and two of them are about camels (Alanazi, 2011; Alanazi et al., 2014; Aljumaah et al., 2018). In the present study, a prevalence of 16.6% was reported, while Alanazi (2011) and Aljumaah et al. (2018) reported a prevalence of 5.6% and 21.99% from camels, respectively. Different studies on the seroprevalence of *N. caninum* in camels from other countries reported different rates and the highest (86%) was reported in camels from the Canary Islands, Spain (Hilali et al., 1998; Sadrebazzaz et al., 2006; Wernery et al., 2008; Hosseininejad et al., 2010; Hamidinejat et al., 2013; Mentaberre et al., 2013). Mentaberre et al. (2013) attributed the high prevalence of antibodies against *N. caninum* in camels from the Canary Islands to the absence of the definitive host in the surveyed farms, resulting in the vertical transmission of this parasite in camels, as has been reported earlier in cattle (López-Gatius et al., 2004).

In the present study, camels from Taif city showed the highest prevalence of *T. gondii* antibodies and the lowest *N. caninum* antibodies. This could be attributed to the fact that

cats, which are the definitive host for *T. gondii*, are more abundant than dogs in Taif. It is quite possible that the transmission of infection occurs via contamination of animal feed or water. The definitive hosts of both *T. gondii* and *N. caninum* were common in the areas where the camels were sampled. Dogs are kept as sentinel animals by shepherds while cats may contaminate camel feed and the infective oocysts may stay viable until the feed is eaten by the camels. Particularly in the Tabuk area, it was noticed that several dogs were in the vicinity where the samples were collected and some of the camel owners complained that they have had some animals aborting for no obvious reason.

Detection of antibodies to *T. gondii* or *N. caninum* from camels is dependent of the level of infection and the availability of the definitive hosts and obviously on the test utilized in the investigation. Most of the laboratory tests employed in serological surveys dealing with *N. caninum* were based on serological tests such as indirect fluorescent antibody test (IFAT) or modified agglutination test (MAT). Only a few studies employed ELISA for the detection of antibodies, such as Wernery et al. (2008) and Aljumaah et al. (2018). All three investigations, including the present study, where ELISA was used, have detected a high percentage of seropositive animals; this is likely due to the efficiency of the secondary antibodies used in the test. Andreotti et al. (2009) had reported that the specificity of the ELISA was 98.3% compared to that of IFAT when testing sheep serum samples. In the present study, detection of antibodies for both parasites in the same individual was not surprising, as the concurrent detection of both *T. gondii* and *N. caninum* antibodies has previously been reported in goats from the Czech Republic (Bartova & Sedlak, 2012). It is possible that cross reactivity might have occurred in the samples positive for both parasites; however, the serological method used in the present study is highly specific and sensitive.

Consuming camel meat is common in Saudi Arabia and it is likely that undercooked meat might be a potential source of both, *T. gondii* and *N. caninum* infections in humans as a result of consuming camel meat, especially that of young camels. This inference was validated through the presence of viable cysts of *T. gondii* in non-infected cats, which were fed infected camel meat (Hilali et al., 1995). Dogs that were fed camel meat shed *Hammondia heydorni* and *Sarcocystis cameli*. However, the identification of *H. heydorni* based on the morphology of the oocysts was questionable and it could probably be mistaken for *N. caninum*, as the dimensions of the cysts of both parasites are quite similar (Schares et al., 2005).

Transmission of *T. gondii* by male goats through semen has been experimentally demonstrated (Dubey & Sharma, 1980; Santana et al., 2010). It is unknown whether a similar transmission can occur in camels. Artificial insemination is not practiced in camels and only the traditional method of mating is practiced; therefore, any infected camel could be a potential hazard to the females it mates with.

Antibodies against both *N. caninum* and *T. gondii* have been detected in camels from different cities in different regions of Saudi Arabia. There was no information regarding the disease situation of both *T. gondii* and *N. caninum* in cattle in Saudi Arabia. The role which may be played by camels and equine in the epidemiology of these diseases in cattle in Saudi Arabia cannot be underestimated (Alanazi et al., 2014).

Acknowledgements

This study was financially supported by the Deanship of Scientific Research at the King Saud University through Vice Deanship of Research Chairs. The authors thank the Deanship of Scientific Research and RSSU at King Saud University for their technical support.

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