Pakistan J. Zool., vol. 50(4), pp 1199-1203, 2018. **DOI**: http://dx.doi.org/10.17582/journal.pjz/2018.50.4.1199.1203

Serological Prevalence of *Neospora caninum* in Indigenous Dromedary Camels (*Camelus dromedarius*) in Saudi Arabia

Riyadh S. Aljumaah*, Mohammed A. Alshaikh, Abdulrahman Jarelnabi, Mutassim M. Abdelrahman and Mansour F. Hussein

Department of Animal Production, College of Food and Agriculture Sciences, King Saudi University, P.O. Box 2460, Riyadh, 11451 Saudi Arabia

ABSTRACT

The serological prevalence of *Neospora caninum* in Saudi Arabian camels was determined using an indirect enzyme linked immunosorbent assay (ELISA). All camels tested were clinically normal. Out of 253 camels of either sex, 117 (21.99%) were found to be serologically positive to specific anti-*N.caninum* antibodies. Logistic regression analysis and estimation of odds ratio revealed significant association of the sex and location of the camels with *N. caninum* seropositivity. On the other hand, neither the breed nor age of the camels was significantly associated with seropositivity.Studies are needed to evaluate other potential risk factors and to assess the pathogenic effects and economic consequences of neosporosis in camels.

Article Information
Received 19 October 2017
Revised 22 November 2017
Accepted 30 November 2017
Available online 10 May 2018

Authors' Contribution
RSJ and MMA designed the study.
MAA and AJ analyzed the samples.
MFH edited the paper.

Key words
***Neospora caninum*, dromedary camels, ELISA, Saudi Arabia.**

INTRODUCTION

Neospora caninum is an heteroxenous protozoan parasite of worldwide distribution that uses dogs, coyotes and grey wolves as definitive hosts, in addition to a wide range of other mammals including cattle, sheep, goats, buffaloes, camelids, wild ruminants, felids and even marine mammals, as intermediate hosts (Fujii *et al*., 2007; Dubey and Schares, 2011). Natural infection with *N. caninum* has also been reported in several species of birds, rodents and lagomorphs (Dubey and Schares, 2011). Dogs serve both as definitive and intermediate hosts (Dubey, 2003). *N. caninum*is a major cause of bovine abortion and neonatal mortality (Haddad *et al*., 2005; Dubey *et al*., 2007; Andreotti *et al*., 2010; Dubey and Schares, 2011) leading to heavy economic losses in many parts of the world (Dubey *et al*., 2007). The parasite may also cause abortion and other disease conditions in sheep and goats (Dubey *et al*., 1990, 1993, 1996; Moreno *et al*., 2012). *N. caninum*is is also highly pathogenic to dogs in which it may cause various clinical conditions, the commonest of which is paralysis of the hind limbs (Barber and Trees, 1996; Georgieva *et al*., 2006).

No information about *N. caninum* infection in animals in Saudi Arabia has been published, apart from a preliminary report in which low *N. caninum* antibody titers were recorded in 17(4.1%) out of 412 camels tested by indirect immunofluoresence antibody assay (Al-Anazi, 2011). The report was limited to Riyadh Province in the central Saudi Arabia and did not cover other parts of the Kingdom. Besides, it was limited to adult camels in which neither sex nor breed were known. Reports of the prevalence of *N. caninum* in dromedary camels in other countries are also scarce. Most of the work published in other countries in camel is also very limited in nature (Hilali *et al*., 1998; Sadrebazzaz *et al*., 2006; Wernery *et al*., 2008; Hosseininejad *et al*., 2009; Hamidinejat *et al*., 2013). Neosporosis has also been reported in South American camelids (Serrano-Martínez *et al*., 2007; Moré *et al*., 2008).

The aim of the present communication was to determine the serological prevalence of *N. caninum* in indigenous Saudi Arabian dromedary camels of either sexes, representing different age groups, breeds and geographical locations.

MATERIALS AND METHODS

Selection of animals

During January-December 2013, a total of 532 camels of either sex were investigated for serological prevalence of antibodies against *N. caninum*. The animals were selected randomly at camel enclosures, markets, slaughter-houses and free ranged herds in different regions of Saudi Arabia. They were divided into four age groups: pre-weaned (<2 yrs), young (2-4 yrs), adult (4-10 yrs) and old camels

* Corresponding author: rjumaah@ksu.edu.sa
0030-9923/2018/0004-1199 $ 9.00/0

(<10 yrs). The animals belonged to *Maghatir*, *Majahim*, *Sufr* and *Hummer* breeds. All camels were apparently healthy when sampled. However, some camels harbored *Hyalomma dromedarii* ticks. None of the adult females was pregnant while some females were lactating. Many of the sampled camels belonged to herds with previous history of reproductive disorders of unknown aetiology.

Serological test

Ten ml blood samples were collected from the jugular vein of each camel into plain vacutainer tubes (Becton, Dickinson and Co., Franklin Lakes, N.J., USA) and allowed to clot at room temperature for 3 h. Sera were separated by centrifugation at 1,500 g for 15 min and stored at -20°C. Tests for antibodies against *N. caninum*were performed using CHEKIT-*N. caninum* enzyme immunoassay (IDEXX laboratories, Bommeli Diagnostics, AG, Bern, Switzerland).

The test reportedly has a sensitivity and specificity of 97.56% and 98.51%, respectively (Wu *et al*., 2002) and is often used for serological screening of neosporosis in cattle and small ruminants, wild animals and dogs (Dubey and Schares, 2011). The test was performed according to manufacturer's protocol except that peroxidase-conjugated goat anti-camel IgG (Triple J. Farms, 777 Jorgensen Place, Bellingham, WA 98226, USA) was used to detect positive camel sera. The test was performed in microtiter plates pre-coated with *N. caninum*antigen. Known positive and negative control sera were run in each test plate. The optical density (OD), corresponding to color intensity and hence antibody concentration in the sample, was determined at 450 nm in a microtiter plate reader. The OD percentage (OD%) of the samples was calculated as follows:

$$OD\% \text{ of the test sample} = 100\ (S - N)/(P - N)$$

Where, S, N and P are the OD values of the test, negative control and positive control sera, respectively. Samples with OD percentage value ≥40 were considered positive.

Statistical analysis

Data were analyzed with the prevalence of *N. caninum* coded as a binary dependant variable (0 for sero-negative and 1 for seropositive animals). Frequencies and means of *N. caninum* prevalence were computed using Statistical Analysis System V. 9.1 software for Windows. A probability value of $p \leq 0.05$ was considered statistically significant. Logistic regression analysis was utilized to examine the associations of independent variables, namely breed, age, sex and location, with seropositivity to *N. caninum*. Odds ratios were calculated from the logistic model to evaluate the risk probability for being positive to *N. caninum* test.

Table I.- Seroprevalence of *N. caninum* in Saudi Arabian camels.

Factors	Animals		Prevalence of *Neospora*			
			+ve		-ve	
	n	(%)	n	(%)	n	(%)
Breed						
Majahim	367	(68.98)	79	(21.53)	288	(78.47)
Maghatir	49	(9.21)	5	(10.20)	44	(89.80)
Hummer	54	(10.15)	17	(31.48)	37	(68..52)
Sufer	62	(11.65)	16	(25.81)	46	(74.19)
Age						
Pre weaning	15	(2.82)	1	(6.67)	14	(93.33)
Young	97	(18.23)	27	(27.84)	70	(72.16)
Adult	298	(56.02)	64	(21.48)	234	(78.52)
Old	122	(22.93)	25	(20.49)	97	(79.51)
Sex						
Female	503	(94.55)	109	(21.67)	394	(78.33)
Male	29	(5.45)	8	(27.59)	21	(72.41)
Region						
Central	62	(11.65)	14	(22.58)	48	(77.42)
Eastern	64	(12.03)	2	(3.13)	62	(96.88)
Northern	35	(6.58)	1	(2.86)	34	(97.14)
Southern	178	(33.46)	35	(19.66)	143	(80.34)
Western	193	(36.28)	65	(33.68)	128	(66.32)
Overall	532	(100.00)	117	(21.99)	415	(78.01)

RESULTS AND DISCUSSION

Out of 532 camels tested, 117 were serologically positive to *N. caninum*-specific antibodies, giving an overall prevalence of 21.99% (Table I). This relatively high prevalence suggests that camels might serve as potential intermediate hosts for *N. caninum* in Saudi Arabia. Logistic regression analysis and odds ratio estimation (Tables II, III) revealed a significant association with *N. caninum* positivity for the sex as well as location of the camels. By contrast, only weak association with positivity was found for the breed and age of the camels.

The present study shows that the serological prevalence of anti- *N. caninum* antibodies in Saudi Arabian camels (22%) is nearly four folds that reported by Al-Anazi (2011). It is also the highest serological prevalence reported in dromedary camels in other countries, namely 3.72% in Egypt (Hilali *et al*., 1998), 3.22%,4.16% and 3.9% in Mashad, Isfahan and Yazd regions, respectively, in Iran (Sadrebazzaz *et al*., 2006; Hosseininejad *et al*., 2009; Hamidinejat *et al*., 2013) and 13.7% in the United Arab Emirates (Wernery *et al*., 2008). With the exception of the report from the Emirates, these surveys were based

either on indirect immunofluorescence test (IFAT) or modified agglutination test (MAT), suggesting that both of these tests might be less sensitive fordetecting *N. caninum* antibodies than the ELISA test used in the present study. ELISA tests have also shown to have higher sensitivity and specificity for the serodiagnosis of *N. caninum*in cattle and sheep than IFAT (Bjorkman *et al*., 1997; Romero and Frankena, 2004; Andereotti *et al*., 2009).

Table II.- Logistic regression model of studied risk factors for prevalence of *N. caninum*in camels in Riyadh province (n=532).

Factors	β	SE	Wald χ^2	P-Value
Intercept	-1.6999	0.3877	19.2260	<.0001
Breed				
Hummer	0.5187	0.2970	3.0501	0.0807
Maghater	-0.7496	0.4148	3.2651	0.0708
Majahim	-0.1486	0.2184	0.4632	0.4961
Sufer	Reference			
Sex				
Female	-0.5695	0.2818	4.0852	0.0433
Male	Reference			
Age				
Pre-weaned	-0.6409	0.9452	0.4597	0.4977
Young	0.3359	0.3857	0.7584	0.3838
Adult	0.1997	0.3577	0.3116	0.5767
Old Adult	Reference			
Location				
Central	0.4128	0.5011	0.6784	0.4101
Eastern	-1.3875	0.6840	4.1143	0.0425
Northern	-1.1657	0.8421	1.9163	0.1663
Southern	0.6956	0.3256	4.5648	0.0326
Western	Reference			

Significant differences in the seroprevalence of *N. caninum* were found in different sampling locations in the Kingdom, with the highest prevalence being recorded in the Western region and lowest in Northern and Eastern regions. These findings agree with previous studies on Iranian camels (Sadrebazzaz *et al*., 2006; Hosseininejad *et al*., 2009; Hamidinejat *et al*., 2013) and in other species of animals, indicating that the prevalence of *N. caninum* differed not only between countries but also between different geographical locations within the same country (Dubey *et al*., 2007; Dubey and Schares, 2011). A significant effect of sex on *N. caninum* seropositivity was also observed in the present camels.

Table III.- Odds ratio (OR) estimates of the risk probability to being positive to *N. caninum* test.

Effect / Comparisons	OR	95% confidence limits	
Breed			
Majahim *vs* Maghater	1.814	0.633	5.197
Majahim *vs* Hummer	0.558	0.282	1.105
Majahim *vs* Sufer	0.527	0.186	1.497
Maghater *vs* Hummer	0.308	0.093	1.021
Maghater *vs* Sufer	0.291	0.069	1.230
Hummer *vs* Sufer	0.945	0.282	3.171
Sex			
Female *vs* Male	0.27	0.09	0.80
Age			
Pre-weaned *vs* Young	0.496	0.040	6.133
Pre-weaned *vs* Adult	0.346	0.028	4.249
Pre-weaned *vs* Old Adult	0.403	0.032	5.036
Young *vs* Adult	0.698	0.354	1.378
Young *vs* Old Adult	0.813	0.368	1.795
Adult *vs* Old Adult	1.164	0.675	2.010
Location[1]			
Central *vs* Northern	6.607	1.016	42.982
Central *vs* Southern	0.807	0.255	2.549
Central *vs* Western	0.376	0.126	1.121
Northern *vs* Southern	0.122	0.024	0.624
Northern *vs* Western	0.057	0.011	0.287
Southern *vs* Western	0.466	0.286	0.757

[1]Eastern location was excluded from the comparisons due to low number of incidences.

Regarding the effect of age, a lower seroprevalence was observed in pre-weaned camels compared to other age groups. However, regression analysis and OR estimation indicated only weak association between age and *N. caninum* positivity. Similar observations were recorded in Iranian camels (Hamidinejat *et al*., 2013) and cattle (Woodbine *et al*., 2008). Some studies referred to increased prevalence with age in cattle, sheep and goats (Al-Majali *et al*., 2008; Eiras *et al*., 2011; Al-Jomaili *et al*., 2013). In these species, the transmission of *N. caninum* occurs both transplacentally and horizontally (Dubey and Schares, 2011) but the rate of vertical versus horizontal transmission varies among herds (Bartels *et al*., 2007). A non-significant association between camel breeds and *N. caninum* positivity was also recorded in the present camels. In a large-scale study in cattle by Eiras *et al*.

(2011), the seroprevalence of *N. caninum* in beef cattle breeds was similar to dairy cattle breeds. Others reported that the prevalence of *N. caninum* varied between different cattle breeds (Armengol *et al*., 2007; Santolaria *et al*., 2011). However, Dubey and Schares (2011) cautioned that some of these results could have been due to differences in the production system used for different breeds rather than to breed-related difference. Further studies are needed to evaluate the association of these and other risk factors, such as environmental conditions, husbandry methods, feed, presence of dogs, occurrence of concurrent infections etc., with the prevalence of *N. caninum* in camels.

Finally, the absence of clinical manifestations in serologically positive camels agrees with observations in other species of farm animals in which the infection, other than causing abortion, is often asymptomatic (Dubey *et al*., 2007; Elsheikha *et al*., 2013). However, abortion is aserious consequence of neosporosis in cattle and occasionally sheep and goats (Moreno *et al*., 2012). To our knowledge, no studies have been made to determine the association, if any, between *N. caninum* infection and abortion indromedary camels. On the other hand, the parasite was incriminated as an important cause of abortion in South American camelids (Serrano-Martinez *et al*., 2007). Using immunohistochemical technique or polymerase chain reaction, these authors recorded *N. caninum* in 16 (28%) out of 50 aborted fetuses of the llama (*Lama glama*) and alpaca (*Vicogna pacos*) in Peru. These results underscore the need to evaluate the potential economic impact of neosporosis in camels.

CONCLUSION

The serological prevalence of Neospora caninum of Saudi Arabian camels is very high (22%) compared with other countries. A significantly positive association of sex and location with *N-caninum* was detected, but not applicable for camel's age and breed. Further research is needed to assess the pathogenic effects and economic consequences of neosporosis in camels raised in Saudi Arabia.

ACKNOWLEDGMENT

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for funding this work through the research group project No. RGP-042.

Statement of conflict of interest

Authors have declared no conflict of interest.

REFERENCES

Al-Anazi, A.A.D., 2011. Prevalence of *Neospora caninum* and *Toxoplasma gondii* in sera from camels (*Camelus dromedarius*) in Riyadh Province, Saudi Arabia. *J. Egypt. Soc. Parasitol*., **41**: 245-250.

Al-Jomaily, A.I.A. and Al-Rubaie, H.M.A.S., 2013. A study the prevalence of Neospora caninum in serum and milk of sheep in Al-Fallujah city. *Al-Anbar J. Vet. Sci*., **6**: 114-118.

Al-Majali, A.M., Jawasreh, K.I., Talafha, H.A. and Talafha, A.Q., 2008. Neosporosis in sheep and different breeds of goats from Southern Jordan: Prevalence and risk factors analysis. *Am. J. Anim. Vet. Sci*., **3**: 47-52. https://doi.org/10.3844/ajavsp.2008.47.52

Andereotti, R., Matos, M.F.C., Gonçalves, K.N., Oshiro, I.M., Lima-Junior, M.S.C., Paiva, F. and Leite, L.F., 2009. *Neospora caninum* antibodies in sheep. *Rev. Bras. Parasitol. Vet*., **18**: 20-24.

Armengol, R., Pabón, M., Santolaria, P., Cabezón, O., Adelantado, C., Yániz, J., López-Gatius, F. and Almería, S., 2007. Low seroprevalence of *Neospora caninum* infection associated with the limousin breed in cow-calf herds in Andorra. *Europe. J. Parasitol.,* **93**:1029-1032.

Barber, J.S. and Trees, A.J., 1996. Clinical aspects of 27 cases of neosporosis in dogs. *Vet. Rec*., **139**: 439-443. https://doi.org/10.1136/vr.139.18.439

Bartels, C.J.M., Huinink, I., Beiboer, M.L., van Schaik, G., Wouda, W., Dijkstra, T. and Stegeman, A., 2007. Quantification of horizontal transmission of *Neospora caninum* infection in Dutch dairy herds. *Vet. Parasitol*., **148**: 83-92. https://doi.org/10.1016/j.vetpar.2007.06.004

Bjorkman, C., Holmdahl, O.G.M. and Ugla, A., 1997. An indirect enzyme-linked immunoassay (ELISA) for demonstration of antibodies to *Neospora caninum* in serum and milk of cattle. *Vet. Parasitol*., **68**: 251-260. https://doi.org/10.1016/S0304-4017(96)01076-X

Dubey, J.P., Hartley, W.S., Lindsey, D.S. and Topper, M.J., 1990. Fatal congenital Neospora infection in a lamb. *Parasitology*, **76**: 127-130. https://doi.org/10.2307/3282640

Dubey, J.P. and Lindsey, D.S., 1993. Neosporosis. *Parasitol. Today*, **9**: 452-458. https://doi.org/10.1016/0169-4758(93)90099-2

Dubey J.P., Morales, J.A., Villaobos, P., Lindsey, D.S., Blagburn, B.L. and Topper, M.J., 1996. Neosporosis-associated abortion in dairy goats. *J. Am. Vet. Med. Assoc*., **15**: 263-265.

Dubey, J.P., 2003. Review of *Neospora caninum* in animals. *Korean J. Parasitol*., **41**: 1-16. https://doi.org/10.3347/kjp.2003.41.1.1

Dubey, J.P., Schares, G. and Ortiga-Mora, L.M., 2007. Epidemiology and control of neosporosis and *Neospora caninum*. *Clin. Microbiol. Rev*., **20**: 323-367. https://doi.org/10.1128/CMR.00031-06

Dubey, J.P. and Shares, G., 2011. Neosporosis in animals - the last five years. *Vet. Parasitol*., **180**: 90-108. https://doi.org/10.1016/j.vetpar.2011.05.031

Eiras, C., Arnaiz, I., Álvares-García, I.M., Ortega-Mora, I.M., Sanjuánl, M.L., Yus, E. and Dieguez, F.J., 2011. *Neospora caninum* seroprevalence in dairy and beef cattle from the northwest region of Spain, Glaicia. *Prev. Vet. Med*., **98**: 128-132. https://doi.org/10.1016/j.prevetmed.2010.10.014

Elsheikha, H.M., McKinlay, C.L., Elsaied, N.A. and Smith, P.A., 2013. Effects of *Neospora caninum* infection on brain microvascular endothelial cells bioenergetics. *Parasites and Vectors,* **6**: 24. https://doi.org/10.1186/1756-3305-6-24

Fujii, K., Kakumuto, C., Kobayashi, M., Saito, S., Kariya, T., Watanabe, Y., Zuan, X.Igrashji, I. and Suzuki, M., 2007. Seroepidemiology of *Toxoplasma gondii* and *Neospora caninum* in seals aroun Hokkaido, Japan. *J. Vet. Med. Sci*., **69**: 393-398. https://doi.org/10.1292/jvms.69.393

Georgieva, D.A., Prelezov, P.N. and Koinarski, V.T., 2006. *Neospora caninum* and neosporosis in animals: A review. *Bulg. J. Vet. Med*., **9**: 1-26.

Haddad, J.P.A., Dohoo, I.R. and VanLeewen, J.A., 2005. A review of *Neospora caninum* in dairy and beef cattle-a Canadian perspective. *Can. Vet. J.,* **46**: 230-243.

Hamidinejat, H., Ghorbanpour, M., Rasooli, A., Nouri, M., Hekmatimoghaddam, S., Namavari, M.M., Pourmehdi-Borojeni, M. and Sazmand, A., 2013. Occurrence of anti-*Toxoplasma gondii* and *Neospora caninum* antibodies in camels (*Camelus dromedarius*) in the center of Iran. *Turk. J. Vet. Anim. Sci*., **37**: 277-281.

Hilali, M., Romand, S., Thulliez, P., Kwok, O.C.H. and Dubey, L.P., 1998. Prevalence of *Neospora caninum* and *Toxoplasma gondii* antibodies in sera from camels from Egypt. *Vet. Parasitol*., **28**: 175-178.

Hosseininejad, M., Pirali-Kheirabadi, K. and Hosseini, F., 2009. Seroprevalence of *Neospora caninum* in camels (*Camelus dromedarius*) in Isfahan province, center of Iran. *Iranian J. Parasitol*., **4**: 61-64.

Moré, G., Pardini, L., Basso, W., Marin, R. Bacigalupe, D., Auad, G., Venturini, L. and Vernturini, M.C., 2008. Seroprevalence of *Neospora caninum*, *Toxoplasma gondii* and *Sarcocystis* sp. in llamas (*Lama glama*) from Jujuy, Argentina. *Vet. Parasitol*., **155**: 158-160. https://doi.org/10.1016/j.vetpar.2008.04.003

Moreno, B., Collàntes-Fernandez, E., Villa, A., Navarro, A., Regidor-Cerillo, J. and Ortega-Mora, L.M., 2012. Occurrence of *neospora caninum* and *Toxoplasma gondii* infections in ovine and caprine abortions. *Vet. Parasitol*., **187**: 312-318. https://doi.org/10.1016/j.vetpar.2011.12.034

Romero, J.J. and Frankena, K., 2004. Bovine neosporosis: A review. *J. Anim. Vet. Adv*., **3**: 901-913.

Sadrebazzaz, A., Haddadzadeh, H. and Shayan, P., 2006. Seroprevalence of *Neospora caninum* and *Toxoplasma gondii* in camels (*Camelus dromedarius*) in Mashad, Iran. *Parasitol. Res*., **98**: 600-601. https://doi.org/10.1007/s00436-005-0118-3

Santolaria, P., Almería, S., Martínez-Bello, D., Nogareda, C., Mezo, M., Gonzalez-Warleta, M., Castro-Hermida, J.A., Pabón, M., Yániz, J.L. and López-Gatius, F., 2010. Different humoral mechanisms against Neospora caninum infection in purebreed and crossbreed beef/dairy cattle pregnancies. *Vet. Parasitol.,* **178**: 70-76.

Serrano-Matinez, E., Collantes-Fernàndez, E., Chàvez-Verlànsquez, A., Rodriguez-Bertos, A., Casas-Astos, E., Risco-Castillo, V., Rosadio-Alcantara, R. and Ortega-Mora, L.M., 2007. Evaluation of *Neospora caninum* and Toxoplasma gondii infection in alpaca (*Vicugna pacos*) and llama (*Lama glama*) aolbrted fetuses from Peru. *Vet. Parasitol*., **150**: 39-45. https://doi.org/10.1016/j.vetpar.2007.08.048

Wernery, U., Thomas, R., Raghavan, R., Syriac, G., Joseph, S. and Georgy, N., 2008. Seroepidemiological studies for the detection of antibodies against 8 infectious diseases in dairy dromedaries of the United Arab Emirates using modern laboratory techniques – Part II. *J. Camel Pract. Res*., **15**: 139-145.

Woodbine, K.A., Medley, G.F., Moore, S.J., Ana Ramirez-Villaescusa, A., Mason, S. and Green, L.E., 2008. A four year longitudinal sero-epidemiology study of *Neospora caninum* in adult cattle from 114 cattle herds in south west England: Associations with age, herd and dam-offspring pairs. *BMC Vet. Res.,* **4**: e35. https://doi.org/10.1186/1746-6148-4-35

Wu, J.T.Y., Dreget, S., Chow, E.W.Y. and Bowlby, E.E., 2002. Validation of 2 commercial *Neospora caninum* antibody enzyme linked immunosorbent assays. *Can. J. Vet. Res*., **66**: 264-271.