www.czasopisma.pan.pl PAN POLSKA AKADEMIA NAUK www.journals.pan.pl

Polish Journal of Veterinary Sciences Vol. 26, No. 3 (2023), 349–357

DOI 10.24425/pjvs.2023.145039

Original article

Prevalence of *Neospora caninum* as an etiologic agent of animal abortion in Kurdistan Region of Iraq

R.R. Mohammed[1], M. Tavassoli[1], K.R. Sidiq[2], B. Esmaeilnejad[1]

[1] Department of Pathobiology, Faculty of Veterinary Medicine,
Urmia University, Nazloo Campus, PO Box 1177, Urmia, West Azerbaijan, Iran
[2] Department of Medical Laboratory Science, College of Medical and Applied Sciences,
Charmo University, 46023 Chamchamal, Sulaimani, Kurdistan Region, Iraq

Abstract

Neospora caninum (N. caninum) is the etiologic agent of neosporosis, a potential cause of severe reproductive disorders in cattle, small ruminants, equines, wild animals and canids across the world. The current study is performed to estimate molecular prevalence of *N. caninum* in small ruminants and equines that had abortion in Kurdistan region of Iraq. A total of 64 tissue samples (brain, placenta, heart, lung and liver) were taken from aborted foetuses, with a total of 122 dam blood samples taken from 63 sheep, 39 goats, 12 mares and 8 jennies in local breed fields. Besides, a risk factor analysis for *N. caninum* positive animals was performed. The observed prevalence of *N. caninum* DNA in the blood of sheep, goats, horses and donkeys were 20.6%, 17.9%, 21.4% and 25.0%, respectively, and 19.3%, 17.6%, 18.1 and 20.0% in the aborted foetuses of the animals, respectively. Moreover, occurrence of *N. caninum* was 20.3% in the blood of aborted dams, while it was 18.7% in their aborted foetuses. Confirmatory analysis was also done through constructing a phylogenetic tree to compare the partial sequences of the Nc-5 gene in our isolates (OP771519, OP771520, OP771521 and OP771522) with the GenBank sequences. This showed 98-100% sequence identity with other *N. caninum* strains in the GenBank database. Older small ruminants and equines had a higher risk of being positive for *N. caninum* and exposure to dogs were considered as significant risk factors for *N. caninum* infection in the studied animals ($p<0.05$). Thus, the results of this study suggest that *N. caninum* is one of the microbial abortive agents in small ruminants and equines in Kurdistan region of Iraq. It is hoped that the results of this study will help to control animal abortion in livestock and reduce the economic losses.

Keywords: abortion, equine, Kurdistan region-Iraq, molecular prevalence, *Neospora caninum*, small ruminants

---

Correspondence to: M. Tavassoli, e-mail: m.tavassoli@urmia.ac.ir, tel.: +98-4432770508, fax: +98- 4432770508

Introduction

Neosporosis is a cosmopolitan parasitic disease in various species of animals. It is induced by *Neospora caninum*, an obligate intracellular protozoan belonging to Toxoplasmatidae (Dubey et al. 2007, Novoa et al. 2023). It is known that *N. caninum* causes reproductive failure including miscarriage, stillbirth, and weak births in pregnant animals and neurological dysfunction of newborns (Marsh et al. 1998, Zhao et al. 2022). *N. caninum* has been reported worldwide with various prevalence. The disease is considered to be globally distributed abortifacient protozoan of cattle (Gondim and McAllister 2022) and can be a potential pathogens associated with congenital disorders, abortions and stillbirth in several animal species including small ruminants (sheep and goats), equines and camels worldwide (Reichel et al. 2013, Dubey et al. 2017, Amdouni et al. 2022, Nayeri et al. 2022).

N. caninum has advances life cycle in which sexual cycle occurs exclusively in canids that are identified as definitive hosts of the protozoa including domestic and wild dogs (Canis lupus familiaris) (Basso et al. 2001, Yang et al. 2022), coyotes (Dubey et al. 2014, Gharekhani et al. 2022), gray wolves and Dingoes (Gondim 2006, King et al. 2011). However, a wide range of warm-blooded animals such as cattle (Dubey et al. 2014, Manca et al. 2022), sheep (Al-Shaeli et al. 2020), buffaloes (Rodrigues et al. 2022), horses (Mazuz et al. 2020), goats (Costa et al. 2018), donkeys (Cong et al. 2018, Rahmani et al. 2022), camels (Ahmed et al. 2017, Gharekhani et al. 2020), rodents (Japa et al. 2018), cats (Sedlák et al. 2014) and birds (Tayyub et al. 2022) are considered as the intermediate hosts of *N. caninum*. Various stages of the parasite such as tachyzoite, tissue cyst, and oocyst can persist in ruminants in farms and herds for years (Dubey 2003).

N. caninum can be transmitted to definitive hosts by ingestion of raw tissues infected with the parasite or vertical transmission by the transplacental transmission of the parasite from the dam to the foetus, and can be transmitted to intermediate hosts through ingestion of sporulated oocysts with food and water or via vertical transmission (Dubey et al. 2006). The main route of infection in cattle is considered the vertical transmission (Dubey 2003) that leading to economic losses estimated at U$S 1.300 billion annually in the livestock industry worldwide (Reichel et al. 2013).

Moreover, *N. caninum* can be a potential factor causing a significant rate of miscarriages in ovine and caprine with a different pervasiveness (Dubey and Schares 2011, Nayeri et al. 2022). The worldwide prevalence of *N. caninum* has recently been estimated at around 12% molecularly and 17% serologically in sheep (Langoni et al. 2011, Mendoza-Morales et al. 2022, Nayeri et al. 2022) and highest in Colombia; 79% (Mendoza-Morales et al. 2022). The rate of *N. caninum* infection 7-16% in goats (Moore et al. 2007, Špilovská and Reiterová 2008), 14-45% in horses and 12-34% in donkeys (Gharekhani et al. 2020, Mazuz et al. 2020, Rahmani et al. 2022).

Traditionally, the knowledge about the relation of *N. caninum* to abortion in ovine, caprine and equine was limited in Iraq (Al-Farwachi and Albadrani 2018, Faraj and Ghattof 2018, Al-Shaeli et al. 2020). In recent years, the increased number of livestock abortions was reported by the local Veterinary Departments. However, the causes of the abortion in such animals remained unknown, even with no sufficient available data about the prevalence of the disease in equids. Thus, this study aimed at finding out the relation of *N. caninum* with abortion in ovine, caprine and equine in Kurdistan region of Iraq, using molecular diagnostic analyses.

Materials and Methods

Area of study

The study was done in the Sulaymaniyah province, Kurdistan Region, Iraq. It is situated between 35°04′ - 36°30′ latitude and 44°50′ - 46°16′ longitude and has a mountainous and mild climate with an average annual rainfall of 650 mm and an average annual temperature of 16.7°C. Sulaymaniyah is considered as the most important area for agriculture and animal husbandry.

Animals and sampling

The study was carried out between October 2020 and February 2022, when abortion outbreak occurred specially in small ruminants across the province. A total of 64 tissue samples (brain, placenta, heart, lung and liver) were taken from aborted foetuses, including 19 sheep, 11 goat, 5 mare and 3 jenny foetuses. Also, a total of 122 dam blood samples were taken from 63 sheep, 39 goats, 12 mares and 8 jennies in local breed fields. All samples were kept at -20°C until DNA purification at the Charmo Research Center based in Charmo University. In addition, some possible risk factor data, including the age of the dams, reproduction history, trimesters of abortion and accompanying dogs in the farm were recorded. This protocol was approved by Veterinary Faculty and was carried out according to standard animal experimentation protocol of the Veterinary Ethic Committee of Urmia University. The Animal Ethics Committee approval number was IR-UU-AEC 2022M14.

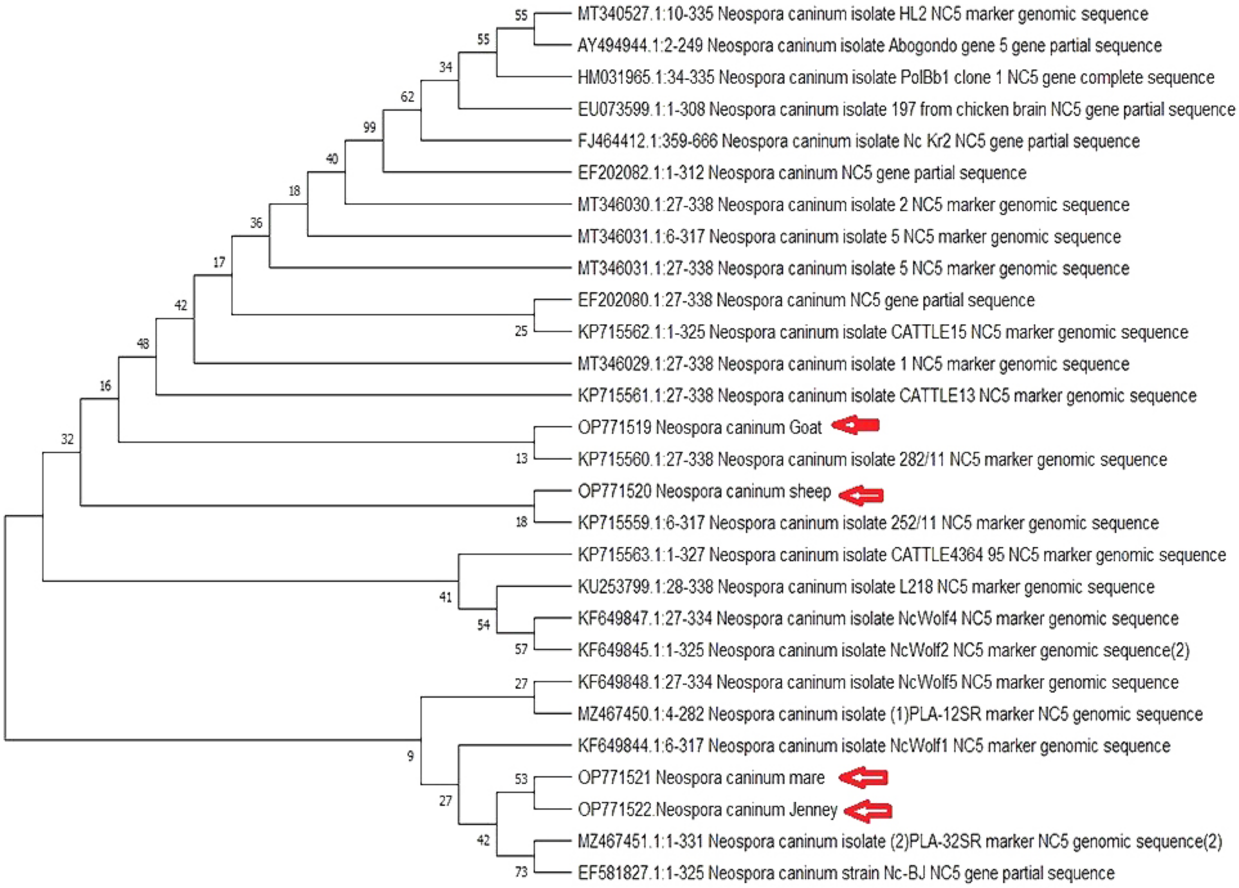

Fig. 1. Nc-5 gene-based phylogenetic analysis of four *Neospora caninum* isolates from tissue samples of foetuses using the neighbor-joining method with 1000 bootstrap replicates. The accession numbers of OP771519, OP771520, OP771521 and OP771522 are *N. caninum* isolates from goat, sheep, mare and jenny foetuses respectively.

DNA extraction and PCR amplification

Genomic DNA was extracted from the homogenized tissues of brain, liver, placenta, heart, and lung of the aborted foetuses, and blood of their dams using a commercial kit (Addprep® Genomic DNA Extraction Kit, South Korea), according to the manufacturer's instructions. The PCR was performed for all the samples to detect *N. caninum*, using a protocol that was previously described by (Müller et al. 1996). Based on the protocol a forward NEO1 primer (5'-CCCAGTGC GTCCAATCCTGTAAC-3') and a reverse primer NEO2 (5'-CTCGCCAGTCAACCTACGTCTTCT-3') were used for the amplification of a 338 bp fragment of the Nc-5 gene. The reaction mixture consisted of 12.5 µL of 2x master mix (Cinaclone Co., Iran), 5.5 µl of nuclease-free water, 1 µL of each primer and 5 µL of genomic DNA template in a total volume of 25 µL. The PCR amplification was conducted with initial-denaturation for 5 min at 94°C, 40 cycles at 94°C for 40 s (denaturation), annealing at 62°C for 40 s, extension at 72°C for 40 s, and final extension at 72°C for 10 min (Müller et al. 1996). Each PCR product was examined in (1%) agarose gel electrophoresis and visualized with ultraviolet transilluminator to verify the present of the expected bands (338 bp) of the target genes.

DNA sequencing and phylogenetic analysis

The amplified Nc-5 sequences were purified and sequenced by Macrogen Inc., Applied Biosystems 3500 in Seoul, South Korea. The phylogenetic analysis was performed using MEGA software (Version: 11) through the neighbor-joining method with 1000 bootstraps to accomplish several sequence alignments. A phylogenetic tree, depending on *N. caninum* Nc-5 locus, was constructed and compared with Nc-5 partial sequences that were already available in GenBank database. The sequences with high similarity were sorted out (Fig. 1).

Statistical analysis

The analysis of the potential risk factors in *N. caninum* related abortion was conducted using Fisher's exact test for categorical variables within the SPSS-software (version 26.0). The differences were considered statistically significant when $p<0.05$ and evaluated

Table 1. Prevalence of *N. caninum* in blood samples of the aborted dams.

Species	No. of blood sample	No. of positive	% Positive	95% Cl
Sheep	63	13	20.6	12.4-32.1
Goat	39	7	17.9	8.9-32.6
Horse	14	3	21.4	7.5-47.5
Donkey	12	3	25.0	10.7-60.3
Total	128	26	20.3	

95% Cl – confidence interval

Table 2. *Neospora caninum* isolates in the tissue samples of the aborted foetuses.

		Collected positive tissues					*NP
Species	No. of foetus	Placenta	Brain	Heart	Liver	Lung	
Sheep	31	+ ++	++	+	-	-	6
Goat	17	++	+	-	-	-	3
Horse	11	+	+	-	-	-	2
Donkey	5	-	+	-	-	-	1
Total	64	6	5	1	0	0	12

* NP total number of positive tissues

by the Chi-square (χ2) test by software R-4.1.2 for Windows.

Results

The prevalence of *N. caninum* DNA based on Nc-5 locus, was 20.3% (26/128) in aborted dam blood samples and 18.7% (12/64) of the aborted foetuses. According to the animal species, the infection rates in blood samples were 20.6% in ewes, 17.9% in goats, 21.4% in mares and 25.0% in jennies (Table 1). Besides, examination of tissue samples from aborted foetuses demonstrated *N. caninum* in 6 sheep, 3 goat, 2 horse and 1 donkey (Table 2). The detection of *N. caninum* in both aborted dams and foetuses was found to be statistically significant. PCR reactions of tissues illustrated that majority of foetuses were positive in the placenta, followed by brain. In contrast liver and lungs showed no positive results (Table 2).

Regarding the possible risk factors (Table 3), significant differences were observed between varied ages, *N. caninum* was significantly more often obtained from older dams than younger ones ($p<0.05$). Moreover, the results showed that a great number of abortions, 82 (64%), occurred in the second and third trimesters of the pregnancy. Among the studied animals exposure to dogs was considered as significant risk factors for *N. caninum* infection ($p <0.05$) and only 16.6% dams had history of previous abortions.

In addition, the alignment of isolated sequences showed 98-100% identity with the sequences of other *N. caninum* strains in GenBank database. The sequences of the *N. caninum* Nc-5 locus isolated from the goat, sheep, mare and jenny fetal tissues were submitted to the GenBank under accession numbers OP771519, OP771520, OP771521 and OP771522 respectively (Fig. 1). The phylogenetic tree analysis revealed that *N. caninum* isolates in goat (OP771519) and sheep (OP771520) foetuses have the closest node and clade with KP715559 and KP715560, respectively. However, the *N. caninum* isolates in mare and jenny foetuses are located in another branch and have the nearest node and clade with MZ467451 and MF581827 (Fig. 1).

Discussion

N. caninum is well-known as a major abortifacient protozoan of cattle worldwide (Wouda et al. 1992, Dubey et al. 2017), and there is also an increasing concern about its role in small ruminants and equines reproductive dysfunctions (e.g. abortions). *N. caninum* has been reported sporadically as one of the agents of reproductive failures in ovine and caprine (Diakou et al. 2013, González-Warleta et al. 2014). Neosporosis of sheep and goat have been well investigated in different countries around the world. However, few studies also showed the prevalence of *Neospora* infection in sheep and goats in various regions of Iraq. Additionally,

Table 3. The relation of risk factors with *N. caninum* infection in the aborted dams.

Species	# No. of animals	No. of infected host	P
Sheep Parameter			
Age of dam			
≤3 years	41	12	X2=5.344, p=0.024
>3 years	22	1	
Presence of dog			
Yes	38	11	X2=6.193, p=0.015
No	25	2	
Previous abortions			
Yes	21	1	X2=4.864, p=0.045
No	42	12	
Trimester of abortion			
First	23	1	X2=6.257, p=0.040
Second	16	4	
Third	24	8	
Goats Parameter			
Age of dam			
≤3 years	20	6	X2=4.674, p=0.044
>3 years	19	1	
Presence of dog			
Yes	17	6	X2=6.157, p=0.030
No	22	1	
Previous abortions			
Yes	21	1	X2=5.372, p=0.035
No	18	6	
Trimester of abortion			
First	14	1	X2=3.318, p=0.213
Second	9	1	
Third	16	5	
Mares Parameter			
Age of dam			
≤5 years	9	3	X2=6.873, p=0.0.27
>5 years	5	0	
Presence of dog			
Yes	9	1	X2=1.593, p=0.505
No	5	2	
Previous abortions			
Yes	5	0	X2=6.873, p=0.027
No	9	3	
Trimester of abortion			
First	4	0	X2=9.545, p=0.011
Second	5	0	
Third	4	3	
Jennies Parameter			
Age of dam			
≤5 years	5	3	X2=5.600, p=0.045
>5 years	7	0	
Presence of dog			
Yes	6	2	X2=0.444, p=1
No	6	1	
Previous abortions			
Yes	8	0	X2=8.000, p=0.018
No	4	3	
Trimester of abortion			
First	4	0	X2=8.000, p=0.055
Second	4	0	
Third	4	3	

Neospora-associated abortion in equines has not been readily distinguished and the etiology of equine abortion has not been extensively studied (Duarte et al. 2004, Locatelli-dittrich et al. 2006). There are also no data about the role of *N. caninum* and *N. hughesi* in equine (horses and donkeys) abortion in Iraq. Thus, our study applied molecular technique to investigate the involvement *Neospora* parasite as an etiology of abortion in equine and small ruminant dams in Kurdistan region-Iraq. PCR, finding specific DNA in limited samples, is one of the most accurate, extensively used molecular approach to finding the global prevalence of *N. caninum* infection in animals that had an abortion and aborted foetuses (Anderson et al. 2019, Bartley et al. 2019, Basso et al. 2022, Gharekhani et al. 2022, Rahmani et al. 2022).

In this study *N. caninum* DNA was found in 12 out of 64 (18.7%) of foetal tissue samples and 26 out of 128 blood samples (20.3%) of their dams by conventional PCR. This could suggest the vertical transmission of the parasite and its involvement in small ruminants and equines abortion. Consistently, neosporosis was previously reported in range of 17.4% to 28.6% in aborted and healthy bovines according to the diagnostic approaches with ELISA and PCR assays in Iraq (AL-Badrani 2012, Al-Farwachi et al. 2012, Nooruldeen et al. 2021). In Wasit province (Iraq) it was showed that the prevalence rate of *N. caninum* was 13.7% in aborted ovine foetuses (Al-Shaeli et al. 2020) and 18.9% in aborted caprine foetuses (Faraj and Ghattof 2018). In addition, the infection rates of *N. caninum* in sheep and goats were 19.3% and 5.7% in north-eastern Italy (Gazzonis et al. 2016), and 16.8% and 6.9% in Greece (Diakou et al. 2013). Neosporosis in ovine was reported in African countries like Senegal and Tunisia at different prevalence rates of 41.9% and 10.6%, respectively (Amdouni et al. 2018, Dahourou et al. 2019). However, the ovine neosporosis is endemic with the highest infection rate of 67.4% in Israel (Tirosh-Levy et al. 2022).

Numerous global studies found the seroprevalence and molecular prevalence of *N. caninum* infection in equines in various countries. It is noteworthy that many studies showed a range of prevalence values of the infection. For example, the seroprevalences of *Neospora* spp. were 11.8% in donkeys in Southern Italy (Machačová et al. 2013), 8% in horses in Nigeria (Bártová et al. 2017), 2% in donkeys to 5.62% in horses in Brazil (Gennari et al. 2016, Moreira et al. 2019), 9.1% of horses in Portugal (Waap et al. 2020) and 69% in donkey in Israel (Tirosh-Levy et al. 2020). In this study, the neosporosis in equines was also investigated. The blood samples of 14 mares and 12 jennies were tested by PCR. The neosporosis infection rates were 21.4% in mares and 25.0% in jennies. The transplacental transmission of the parasite were found in 2 foetuses of horse and 1 foetus of donkey. Comparing to the above countries, our finding shows higher prevalence of neosporosis of mares than in Italy, Nigeria, Brazil and Portugal; however lower than that in Israel (Mazuz et al. 2020, Tirosh-Levy et al. 2020). Transplacental transmission was also detected in 3 out of 9 fetuses (33.3%) in seronegative mares in Brazil (Antonello et al. 2012). Furthermore, a recent study in Iran reported *N. caninum* in 10 out of 29 (34.5%) aborted donkeys by using PCR of Nc-5 locus; besides the evidence of transplacental transmission of the parasite was detected in 40% of aborting jennies (Rahmani et al. 2022).

Our findings show that the prevalence of *N. caninum* infections are more common in the Kurdistan region than in other countries (Machačová et al. 2013, Bártová et al. 2017). These differences may be associated with several aspects such as the number of dogs in the location, local climate (humidity induce sporulation and enhance the survival of *N. caninum* oocysts in the ecosystem according to Ghattof and Faraj (Ghattof and Faraj 2015), farm management system and regional ecology (Dubey et al. 2007). The sensitivity of the detection method such as PCR could be another factor causing varied infection rate detected (Beck et al. 2009, Mazuz et al. 2020). Moreover, nearly all abortions were noticed during the second and third trimester of the pregnancy in aborting dams. Our findings are consistent with other reports that relate the existence of *N. caninum* DNA in foetuses with late-term abortions in dam or in stillborn (Almería et al. 2016, Mazuz et al. 2020).

Statistical analysis revealed significant difference in infection prevalence associated with increased age in both ruminant and equine species due to cumulative infections that are not cleared (Amdouni et al. 2018). The prevalence of *N. caninum* infection was found to be lower in young animals than in adults; the highest prevalence of *N. caninum* infection was found in animals older than 3 years from small ruminants and over 5 years in horses and donkeys. Moreover, the increased prevalence are noted, when dogs are present in the herd. The contamination of the animal feeding areas with infected oocysts, released from infected dogs, lead to observing statistically significant evidence of horizontal transmission in herds. Finally, our findings suggest that *N. caninum* plays a role as one of the abortive agents in small ruminates and equine in Iraq, as well as in other countries (Moreno et al. 2012, Mazuz et al. 2020, Della Rosa et al. 2021, Rahmani et al. 2022, Irehan et al. 2022). Thus *N. caninum* could be also evaluated in the differential diagnosis of reproductive problems to the offspring in small ruminants and equines.

Conclusion

The present study is the first report on the molecular prevalence of *N. caninum* as one of the causes of abortion in equine and small ruminants in Kurdistan region of Iraq. The results suggested that *N. caninum* is vertically transmitted, whereas transplacental transmission has an essential role in the epidemiology and circulation of the parasite. Further studies are required to find out other microbial etiologies of animal abortion in Kurdistan region of Iraq.

Acknowledgements

The authors wish to thank all the technical staff of the Laboratory Research Center from Charmo University for providing molecular tools. We thank Drs. Avin Kawa Ahmed, Awat Samiei, Shorsh Hamaxurshid and Farhad Mohammed. The authors would like to sincerely thank the members of the Faculty of Veterinary Medicine and Urmia University Research Council for the approval of this research. We thank the study participants and animal owners for their dedication to this project.

References

Ahmed NE, Al-Akabway LM, Ramadan MY, El-Gawad SM, Moustafa MM (**2017**) Serological and PCR-sequencing assays for diagnosis of *Toxoplasma gondii* and *Neospora caninum* infecting camels in Egypt. Benha Vet Med J 33: 200-210.

AL-Badrani BA, AL-Farwachi MI, AL-Hankawai OK (**2012**) Detection of *Toxoplasma gondii* and *Neospora caninum* antibodies in cattle in Mosul city, Iraq. Al-Qadisiyah J Vet Med Sci 11: 46-50.

Al-Farwachi MI, Al-Badrani BA, Al-Khafaji WS (**2018**) Serodiagnosis of ovine neosporosis in Mosul city , Iraq. Eurasian J Vet Sci 28: 190-193.

Al-Farwachi MI, Al-Hankawe OK, Al-Khafaji WS (**2012**) Serodiagnosis of Bovine Neosporosis in Mosul City, Iraq. Assiut Vet Med J 58: 1-4.

Al-Shaeli SJ, Ethaeb AM, Gharban HA (**2020**) Molecular and histopathological identification of ovine neosporosis (*Neospora caninum*) in aborted ewes in Iraq. Vet World 13: 597-603.

Almería S, Serrano-Perez B, Darwich L, Domingo M, Mur-Novales R, Regidor-Cerrillo J, Cabezón O, Pérez--Maillo M, Lopez-Helguera I, Fernández-Aguilar X, Puig-Ribas M, Ortega-Mora LM, García-Ispierto I, Dubey JP, López-Gatius F (**2016**) Foetal death in naive heifers inoculated with *Neospora caninum* isolate Nc-Spain7 at 110 days of pregnancy. Exp Parasitol 168: 62-69.

Amdouni Y, Abedennebi I, Amairia S, Abdelkader A, Chandoul W, Gharbi M (**2022**) First molecular detection of *Neospora caninum* from naturally infected slaughtered camels in Tunisia. Vet Med Sci 8: 2241-2247.

Amdouni Y, Rjeibi MR, Awadi S, Rekik M, Gharbi M (**2018**) First detection and molecular identification of *Neospora caninum* from naturally infected cattle and sheep in North Africa. Transbound Emerg Dis 65: 976-982.

Anderson JA, Alves DA, Cerqueira-Cézar CK, da Silva AF, Murata FH, Norris JK, Howe DK, Dubey JP (**2019**) Histologically, immunohistochemically, ultrastructurally, and molecularly confirmed neosporosis abortion in an aborted equine fetus. Vet Parasitol 270: 20-24.

Antonello AM, Pivoto FL, Camillo G, Braunig P, Sangioni LA, Pompermayer E, Vogel FS (**2012**) The importance of vertical transmission of *Neospora* sp. in naturally infected horses. Vet Parasitol 187: 367-370.

Bartley PM, Guido S, Mason C, Stevenson H, Chianini F, Carty H, Innes EA, Katzer F (**2019**) Detection of *Neospora caninum* DNA in cases of bovine and ovine abortion in the South-West of Scotland. Parasitol 146: 979-982.

Bártová E, Sedlák K, Kobédová K, Budíková M, Joel Atuman Y, Kamani J (**2017**) Seroprevalence and risk factors of *Neospora* spp. and *Toxoplasma gondii* infections among horses and donkeys in Nigeria, West Africa. Acta Parasitol 62: 606-609.

Basso W, Holenweger F, Schares G, Müller N, Campero LM, Ardüser F, Moore-Jones G, Frey CF, Zanolari P (**2022**) *Toxoplasma gondii* and *Neospora caninum* infections in sheep and goats in Switzerland: Seroprevalence and occurrence in aborted foetuses. Food Waterborne Parasitol 28: e00176.

Basso W, Venturini L, Venturini MC, Hill DE, Kwok OCH, Shen SK, Dubey JP (**2001**) First isolation of *Neospora caninum* from the feces of a naturally infected dog. J Parasitol 87: 612-618.

Beck HP, Blake D, Dardé ML, Felger I, Pedraza-Díaz S, Regidor-Cerrillo J, Gómez-Bautista M, Ortega-Mora LM, Putignani L, Shiels B, Tait A, Weir W (**2009**) Molecular approaches to diversity of populations of apicomplexan parasites. Int J Parasitol 39: 175-189.

Cong W, Nie LB, Qin SY, Wang WL, Qian AD, Meng QF (**2018**) Prevalence of *Neospora* spp. in donkeys in China. Parasite 25, 16.

Costa RC, Mesquita LP, Nunes MV, Oliveira IM, Oliveira LF, Souza AR, Maiorka PC, Varaschin MS (**2018**) *Neospora caninum* bioassay in gerbils using placental tissues from naturally infected goats. Vet Parasitol 249: 70-73.

Dahourou LD, Gbati OB, Savadogo M, Yougbare B, Dicko A, Combari AH, Kamga-Waladjo AR (**2019**) Prevalence of *Toxoplasma gondii* and *Neospora caninum* infections in households sheep “Elevage en case” in Dakar, Senegal. Vet World 12: 1028-1032.

Diakou A, Papadopoulos E, Panousis N, Karatzias C, Giadinis N (**2013**) *Toxoplasma gondii* and *Neospora caninum* seroprevalence in dairy sheep and goats mixed stock farming. Vet Parasitol 198: 387-390.

Della Rosa P, Fiorentino MA, Morrell EL, Scioli MV, Paolicchi FA, Moore DP, Cantón GJ, Hecker YP (**2021**) *Neospora caninum* and *Toxoplasma gondii* as causes of reproductive losses in commercial sheep flocks from Argentina. Curr Res Parasitol Vector Borne Dis 1: 100057.

Duarte PC, Conrad PA, Barr BC, Wilson WD, Ferraro GL, Packham AE, Carpenter TE, Gardner IA (**2004**) Risk of transplacental transmission of *Sarcocystis neurona* and *Neospora hughesi* in California horses. J Parasitol 90: 1345-1351.

Dubey JP (**2003**) Review of *Neospora caninum* and neosporosis in animals. Korean J Parasitol 41: 1-16.

Dubey JP, Buxton D, Wouda W (**2006**) Pathogenesis of Bovine Neosporosis. J Comp Pathol 134: 267-289.

Dubey JP, Hemphill A, Calero-Bernal R, Schares G (**2017**) Neosporosis in animals, 1st ed., CRC Press, Taylor and Francis group.

Dubey JP, Jenkins MC, Ferreira LR, Choudhary S, Verma SK, Kwok OC, Fetterer R, Butler E, Carstensen M (**2014**) Isolation of viable *Neospora caninum* from brains of wild gray wolves (Canis lupus). Vet Parasitol 201: 150-153.

Dubey JP, Schares G (**2011**) Neosporosis in animals-the last five years. Vet Parasitol 180: 90-108.

Dubey JP, Schares G, Ortega-Mora LM (**2007**) Epidemiology and control of neosporosis and *Neospora caninum*. Clin Microbiol Rev 20: 323-367.

Faraj AA, Ghattof HH (**2018**) Diagnosis of *Neospora caninum* using ELIZA and study of histopathological changes in dairy goat in Wasit province: Iraq. J Entom Zool Stud 6: 1256-1259.

Gazzonis AL, Garcia GA, Zanzani SA, Ortega Mora LM, Invernizzi A, Manfredi MT (**2016**) *Neospora caninum* infection in sheep and goats from north-eastern Italy and associated risk factors. Small Rumin Res 140: 7-12.

Gennari SM, Pena HF, Lindsay DS, Lopes MG, Soares HS, Cabral AD, Vitaliano SN, Amaku M (**2016**) Prevalence of antibodies against *Neospora* spp. and *Sarcocystis neurona* in donkeys from northeastern Brazil. Rev Bras Parasitol Vet 25: 109-111.

Gharekhani J, Yakhchali M, Berahmat R (**2020**) *Neospora caninum* infection in Iran (2004-2020): A review. J Parasit Dis 44: 671-686.

Gharekhani J, Yakhchali M, Heidari R (**2022**) Molecular detection and phylogenetic analysis of *Neospora caninum* in various hosts from Iran. Comp Immunol Microbiol Infect Dis 80: 101737.

Ghattof HH, Faraj AA (**2015**) Seroprevalence of *Neospora caninum* in goats in Wasit province Iraq. Int J Curr Microbiol Appl Sci 4: 182-191.

Gondim LF (**2006**) *Neospora caninum* in wildlife. Trends Parasitol 22: 247-252.

Gondim LF, McAllister MM (**2022**) Experimental *Neospora caninum* Infection in Pregnant Cattle: Different Outcomes Between Inoculation With Tachyzoites and Oocysts. Front Vet Sci 9: 911015.

González-Warleta M, Castro-Hermida JA, Regidor-Cerrillo J, Benavides J, Álvarez-García G, Fuertes M, Ortega-Mora LM, Mezo M (**2014**) *Neospora caninum* infection as a cause of reproductive failure in a sheep flock. Vet Res 45: 88.

Irehan B, Sonmez A, Atalay MM, Ekinci AI, Celik F, Durmus N, Ciftci AT, Simsek S (**2022**) Investigation of *Toxoplasma gondii, Neospora caninum* and *Tritrichomonas foetus* in abortions of cattle, sheep and goats in Turkey: Analysis by real-time PCR, conventional PCR and histopathological methods. Comp Immunol Microbiol Infect Dis 89: 101867.

Japa O, Morand S, Karnchanabanthoeng A, Chaisiri K, Ribas A (**2018**) Detection of *Neospora caninum* (Toxoplasmatidae) in wild small mammals from Thailand. Folia Parasitologica 65.

King JS, Jenkins DJ, Ellis JT, Fleming P, Windsor PA, Šlapeta J (**2011**) Implications of wild dog ecology on the sylvatic and domestic life cycle of *Neospora caninum* in Australia. Vet J 188: 24-33.

Langoni H, Greca HJ, Guimarães FF, Ullmann LS, Gaio FC, Uehara RS, Rosa EP, Amorim RM, Da Silva RC (**2011**) Serological profile of *Toxoplasma gondii* and *Neospora caninum* infection in commercial sheep from São Paulo State, Brazil. Vet Parasitol 177: 50-54.

Locatelli-Dittrich R, Dittrich JR, Richartz RR, Gasino JM, Antunes J, Pinckney RD, Deconto I, Hoffmann DC, Thomaz-Soccol V (**2006**) Investigation of *Neospora* sp. and *Toxoplasma gondii* antibodies in mares and in precolostral foals from Parana State, Southern Brazil. Vet Parasitol 135: 215-221.

Machačová T, Bártová E, Di Loria A, Sedlák K, Guccione J, Fulgione D, Veneziano V (**2013**) Seroprevalence and risk factors of *Neospora* spp. in donkeys from Southern Italy. Vet Parasitol 198: 201-204.

Manca R, Ciccarese G, Scaltrito D, Chirizzi D (**2022**) Detection of Anti-*Neospora caninum* Antibodies on Dairy Cattle Farms in Southern Italy. Vet Sci 9: 87

Marsh AE, Barr BC, Packham AE, Conrad PA (**1998**) Description of a new neospora species (Protozoa: apicomplexa: sarcocystidae). J Parasitol 5: 84-91.

Mazuz ML, Mimoun L, Schvartz G, Tirosh-Levy S, Savitzki I, Edery N, Blum SE, Baneth G, Pusterla N, Steinman A (**2020**) Detection of *Neospora caninum* infection in aborted equine fetuses in Israel. Pathogenes 9: 1-11.

Mendoza-Morales LF, Lagorio V, Corigliano MG, Sánchez--López E, Ramos-Duarte VA, Clemente M, Sander VA (**2022**) Neosporosis in sheep: A systematic review and meta-analysis of global seroprevalence and related risk factors. Acta Trop 233: 106569.

Moore DP, de Yaniz MG, Odeón AC, Cano D, Leunda MR, Späth EA, Campero CM (**2007**) Serological evidence of *Neospora caninum* infections in goats from La Rioja Province, Argentina. Small Rumin Res 73: 256-258.

Moreira TR, Sarturi C, Stelmachtchuk FN, Andersson E, Norlander E, de Oliveira FL, Machado Portela J, Marcili A, Emanuelson U, Gennari SM, Minervino AH (**2019**) Prevalence of antibodies against *Toxoplasma gondii* and *Neospora* spp. in equids of Western Pará Brazil. Acta Trop 189: 39-45.

Moreno B, Collantes-Fernández E, Villa A, Navarro A, Regidor-Cerrillo J, Ortega-Mora LM (**2012**) Occurrence of *Neospora caninum* and *Toxoplasma gondii* infections in ovine and caprine abortions. Vet Parasitol 187: 312-318.

Müller N, Zimmermann V, Hentrich B, Gottstein B (**1996**) Diagnosis of *Neospora caninum* and *Toxoplasma gondii* infection by PCR and DNA hybridization immunoassay. J Clin Microbiol 34: 2850-2852.

Nayeri T, Sarvi S, Moosazadeh M, Daryani A (**2022**) The Global Prevalence of *Neospora caninum* Infection in Sheep and Goats That Had an Abortion and Aborted Fetuses: A Systematic Review and Meta-Analysis. Front Vet Sci 9: 870904

Nooruldeen MY, Jaafar SE, Salih AI (**2021**) Seroprevalence of *Neospora caninum* infections in cattle in Kirkuk province. Iraqi J Vet Sci 35: 331-334.

Novoa MB, Soler JP, Cirone KM, Hecker YP, Valentini BS, Primo ME, Moore DP (**2023**) Use and comparison of serologic assays to detect anti-*Neospora caninum* antibodies in farmed red deer (Cervus elaphus). Vet Parasitol 313: 109839.

Rahmani SS, Malekifard F, Tavassoli M (**2022**) *Neospora caninum*, a cause of abortion in donkeys (*Equus asinus*) in Iran. Parasitol Res 121: 367-372.

Reichel MP, Ayanegui-Alcérreca MA, Gondim LF, Ellis JT (**2013**) What is the global economic impact of *Neospora caninum* in cattle - the billion dollar question. Int J Parasitol 43: 133-142.

Rodrigues AA, Brito DR, Kono IS, Reis SS, de Souza Lima Nino B, Nascimento TV, Barros LD, Garcia JL, de Cunha IA (**2022**) Seroprevalence of *Neospora caninum* and risk factors associated with infection in water buffaloes (*Bubalus bubalis*) from Maranhão State, Brazil. Vet Parasitol Reg Stud Reports 27: 100661.

Sedlák K, Bartova E, Machacova T (**2014**) Seroprevalence of *Neospora caninum* in cats from the Czech Republic. Acta Parasitol 59: 359-361.

Špilovská S, Reiterová K (**2008**) Seroprevalence of *Neospora caninum* in aborting sheep and goats in the Eastern Slovakia. Folia Vet 52: 33-35.

Tayyub M, Ali S, Javid A, Imran M (**2022**) Molecular detection of Toxoplasma gondii and Neospora caninum in rock pigeons (*Columba livia*) in Punjab, Pakistan. Parasitol Res 121: 1499-1505

Tirosh-Levy S, Savitsky I, Blinder E, Mazuz ML (**2022**) The involvement of protozoan parasites in sheep abortions – a ten-year review of diagnostic results. Vet Parasitol 303: 109664.

Tirosh-Levy S, Steinman A, Minderigiu A, Arieli O, Savitski I, Fleiderovitz L, Edery N, Schvartz G, Mazuz ML (**2020**) High Exposure to *Toxoplasma gondii* and *Neospora* Spp. in Donkeys in Israel: Serological Survey and Case Reports. Animals 10: 1921

Waap H, de Oliveira UV, Nunes T, Gomes J, Gomes T, Bärwald A, Dias Munhoz A, Schares G (**2020**) Serological survey of *Neospora* spp. and Besnoitia spp. in horses in Portugal. Vet Parasitol Reg Stud Reports 20: 100391.

Wouda W, van den Ingh TS, van Knapen F, Sluyter FJ, Koeman JP, Dubey JP (**1992**) Neospora abortion in cattle in The Netherlands. Tijdschr Diergeneeskd 117: 599-602.

Yang J, Ai J, Qi T, Ni X, Xu Z, Guo L, Sun Y, Li Y, Kang M, Li J (**2022**) *Toxoplasma gondii* and *Neospora caninum* Infections in Stray Cats and Dogs in the Qinghai-Tibetan Plateau Area, China. Animals 12: 1390

Zhao SS, Tao DL, Chen JM, Chen X, Geng XL, Wang JW, Yang X, Song JK, Liu Q, Zhao GH (**2022**) *Neospora caninum* infection activated autophagy of caprine endometrial epithelial cells via mTOR signaling. Vet Parasitol 304: 109685.