



Prevalence and Risk Factors Associated to *Neospora caninum* (Apicomplexa: Toxoplasmatidae) in Pet Dogs From Hamadan, West of Iran, 2016

Jamal Gharekhani^{1,2*}, Mohammad Yakhchali², Reza Khaltabadi-Farahani^{3,4}

¹Department of Laboratory Sciences, Central Veterinary Laboratory, Iranian Veterinary Organization, Hamadan, Iran.

²Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

³Department of Molecular Biology, Pasteur Institute of Iran, Tehran, Iran.

⁴Department of Molecular Biology, National Veterinary Reference Laboratory, Iranian Veterinary Organization, Tehran, Iran.

***Corresponding author:**

Jamal Gharekhani,
Department of Laboratory
Sciences, Central Veterinary
Laboratory, Iranian Veterinary
Organization, Hamadan, Iran,
Postal Code: 6519611156,
Tel: + (98)81 32651801,
Fax: + (98)81 32644474,
Email: Gharekhani_76@
yahoo.com

Received: 6 Jan. 2020
Accepted: 10 Mar. 2020
ePublished: 30 Mar. 2020



Background

Neospora caninum as a parasite (Apicomplexa: Toxoplasmatidae) with global prevalence was first reported in puppies from Norway (1). Canine and a wide-range of herbivore animals are the definitive and intermediate hosts for *N. caninum* (2).

The infected dogs may represent neuromuscular neurologic disorders such as progressive flaccid paralysis of hind limbs and jaw, gradual atrophy of muscles, dysphagia, and heart failure (3). Although puppies are generally born without clinical symptoms, they ordinarily present progressing toward ascending paralysis of the rear limbs in 3 weeks after birth (3,4). Diagnosis is impossible by using only clinical signs. Accordingly, serological tools, namely, enzyme-linked immunosorbent assay (ELISA) are proposed to distinguish *N. caninum* in epidemiologic works. So far, molecular techniques have been used for affirming the serological assessment (2,5).

N. caninum infection was detected among 2.1% and 54.6% in various types of dogs, samples, and diagnostic methods in different regions of Iran (6-21). Considering

Abstract

Background: Neosporosis is considered as a ubiquitous disease in Iran and other countries. This research was expected to determine the prevalence and related risk factors of *Neospora caninum* in household dogs in Hamadan Municipality, Iran.

Methods: A total of 184 whole blood was evaluated for the presence of antibodies to *N. caninum* by the enzyme-linked immunosorbent assay (ELISA). All seropositive animals were affirmed by molecular techniques.

Results: Based on serology and molecular methods, *N. caninum* infection was detected in 4.9% (95% CI = 4.9 ± 3.1%) of animals. In addition, the highest infection rate was significantly recognized in female dogs (57.1%) with under 6 months old (54.4%). Additionally, the clinical signs of neosporosis were observed in 2 out of 4 positive dogs ($P < 0.0001$, odds ratio [OR] = 24.71). Finally, the infection had no significant connection ($P > 0.05$) with breeding, food regime, housing, and direct contact with infected animals.

Conclusions: In general, the serological and molecular outcomes were parallel together. It was concluded that this is a universal assessment of risk factors related to *N. caninum* in Iranian house dogs for the first time.

Keywords: *Neospora caninum*, Pets, Dogs, Iran, ELISA, PCR

the above-mentioned explanations (Table 1), the present study aimed to assess the infection rate and risk factors related to *N. caninum* in the Iranian household dogs of Hamadan municipality, located in the west of Iran. Furthermore, a historical mini-review on *N. caninum* infection in the dogs of Iran is presented in the Discussion Section.

Methods

Sampling Location, Animals, and Serology

Hamadan Municipality is situated in the west part of Iran (34.77° N and 48.58° E) which is surrounded by mountains and has normal yearly temperature of 11.3°C. During March-September 2016, a total of 184 whole blood was randomly sampled from household dogs that were referred to Veterinary Clinics in Hamedan. Data relating to animals was recorded and sera samples were evaluated after their preparation in order to identify antibodies to *N. caninum* using the ELISA (ID-Vet Company, France).

Table 1. Prevalence of *Neospora caninum* Infection in Dogs in Different Regions of Iran

Region		Type of Animals	Type of Samples	Infection Rate (%)	Technique	Reference	
Location	Municipalities						
Western Iran	Hamadan	Stray	Blood	52.8	ELISA*	(6)	
		Shepherd	Blood	18	ELISA	(7)	
		Stray	Blood	5	ELISA	(21)	
		Farm	Blood	8.6	ELISA	(8)	
Northwestern Iran	Lorestan	Farm	Stool	2.1	PCR	(9)	
		Tabriz	Stray	Blood	31	IFAT	(10)
		Urmia	Stray	Blood	27	IFAT	(11)
		Sarab, Tabriz	Shepherd	Blood	10.6	IFAT	(12)
Northeastern Iran	Meshkin-Shahr, Ardabil	Rural	Blood	30.4	ELISA	(13)	
		Mashhad	Farm	Stool	2.2	PCR	(14)
Central of Iran	Tehran	Farm	Blood	46	IFAT	(15)	
		Farm	Blood	28	IFAT	(14)	
		Urban	Blood	20	IFAT	(15)	
		Urban	Blood	11.3	IFAT	(16)	
		Stray	Blood	2.2	ELISA	(17)	
		Stray	Brain and other tissues	35.7	Nested-PCR	(18)	
Southwestern Iran	Isfahan	Stray	Blood	17.7	IFAT	(19)	
		Clinic animals, mix	Blood	10.3	IFAT	(20)	
		Shahrekord	Stray	Blood	22	IFAT	(18)
Southern Iran	Shahrekord and Ahvaz	Stray	Blood	43.3	ELISA	(19)	
		Farm	Blood	44.4	MAT	(20)	
Southern Iran	Shiraz	Farm	Blood	54.6	Dot-ELISA	(20)	

Note. ELISA: Enzyme-linked immunosorbent assay; PCR: Polymerase chain reaction; IFAT: Indirect fluorescent-antibody test; MAT: Modified agglutination test; *All of ELISA methods were used a commercially Kit, ID-Vet® Company, France.

Molecular Examination

The genomic DNA extraction of *N. caninum* in the whole blood samples of seropositive animals was performed by using the Dyna-Bio™ blood kit (Takapouzist Company, Iran). According to the protocol of Müller et al (22), a pair of Np21 plus and Np6 plus primers (Forward: 5'CCCAGTGCGTCCAATCCTGTAAC3' and Reverse: 5'CTCGCCAGTCAACCTACGTCTTCT3') was used to amplify a 330 bp-fragment-length of NC5 gene. Additionally, the nested polymerase chain reaction (PCR) was performed to amplify a 100 bp-fragment of the PCR product by using 1 µL of each PCR product and a pair of primers (Forward: 5'GTGTTGCTCTGCTGACGTGT-3' and Reverse: 5'-TACCAACTCCCTCGGTTCAC-3') (23).

Data Analysis

The chi-square test (χ^2) was used to evaluate the relation

among the infection rate and different variables (SPSS 16.0, Chicago, IL, USA) and the probability of ≤ 0.05 was considered significant.

Results

The antibodies to *N. caninum* were detected in 4 (4.9%, CI 95% = 1.8-8%) out of 184 examined dogs (Table 2). All seropositive animals were confirmed by nested PCR (Figure 1). The infection rate in female animals (7.6%) was 6.4-fold higher compared to male animals ($P=0.047$). The highest infection rate was observed in dogs with 1-2 years old (6.7%) but no significant association was found between infection rate and age groups ($P>0.05$). In addition, 5.4% and 3.7% infection rate were found in pure and mixed breed dogs, respectively ($P=0.630$). The clinical signs of neosporosis were recorded in 2 seropositive dogs (50%, $P<0.0001$, OR=24.71) while the infection had no connection with a food regime

Table 2. The Infection Rate of *Neospora caninum* in Household Dogs in Hamadan in Iran

Risk Factors		No. of Sample (%)	No. of Positive (%)	Statistical Analysis
Sex	Male	79 (42.9)	1 (1.3)	$\chi^2= 3.911, P= 0.047,$ OR= 6.43
	Female	105 (57.1)	8 (7.6)	
Breed	Pure	130 (70.7)	7 (5.4)	$\chi^2= 0.231, P= 0.630$
	Mixed	54 (29.3)	2 (3.7)	
Age (month)	< 6	119 (64.7)	7 (5.9)	$\chi^2= 0.317, P= 0.853$
	6-12	50 (27.2)	1 (2)	
	12-24	15 (8.1)	1 (6.7)	
Food regime	Home made	46 (25)	1 (2.2)	$\chi^2= 0.178, P= 0.914$
	Commercial food	21(11.4)	0 (0)	
	Mixed	117 (63.6)	8 (6.8)	
Housing	Indoors	51 (27.7)	1 (1.9)	$\chi^2= 2.009, P= 0.366$
	In- and outdoors	122 (66.3)	3 (2.5)	
	In outdoors	11 (6)	5 (45.5)	
Contact with other animals	Yes	152 (82.6)	8 (5.3)	$\chi^2= 0.259, P= 0.610$
	No	32 (17.4)	1 (3.1)	
Clinical signs	Yes	4 (2.2)	2 (50)	$\chi^2= 17.885, P< 0.0001,$ OR= 24.71
	No	180 (97.8)	7 (3.9)	
Total		184 (100)	9 (4.9)	CI 95%= 4.9%± 3.1

Note. OR: Odds ratio; CI: Confidence interval.

($P=0.914$), housing ($P=0.366$), and direct contact with another animal ($P=0.610$).

Discussion

The interest of keeping pet animals is progressively in spotlights, and therefore, their health status is crucial in the transmission of infection agents to humans. A concern to *N. caninum* infection in dogs might play a zoonotic role because of its close biologic relationship with *Toxoplasma gondii* and the occurrence of accidental oocyst ingestion and/or the consumption of raw meat containing tissue cysts. In addition, antibodies to *N. caninum* were reported in humans with a deficiency in the immune system (24). According to Yakhchali et al (10), assessing seroprevalence and hence the exposure of dogs to *N. caninum* are considered as the significant parts of investigating the possible transmission routes of the parasite and identifying dogs in which there is the possibility of neosporosis. Additionally, it should be noted that the negative results observed during the examinations of animals do not necessarily confirm the absence of infection (2).

On the global scale, the seroprevalence rate of *N. caninum* was estimated in 17.14% of the dog' population (25) while 4.9% of our examined animals were seropositive. This finding contradicts that of the other studies (5,26,27)

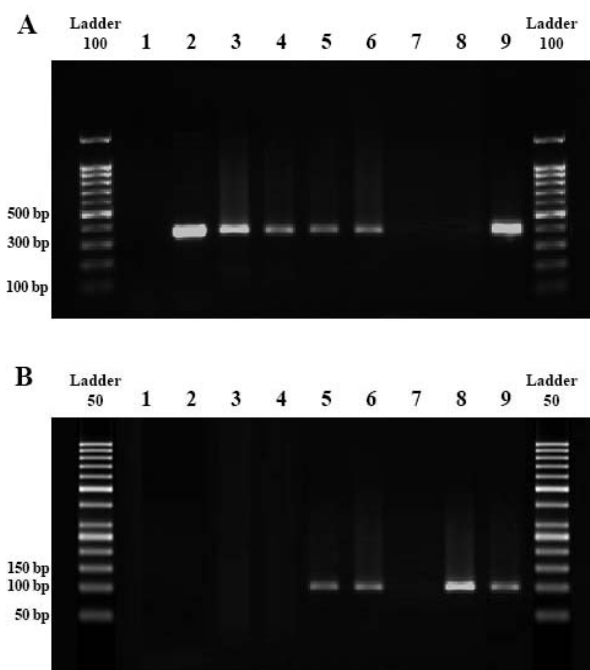


Figure 1. Gel Electrophoresis Results of *Neospora caninum* Detection
A (PCR gel): Negative control (L1); Positive control (L2,9); Positive samples (L3-6; bands of 330 bp); Negative samples (L7,8).
B (nested-PCR gel): Negative control (L1); Positive control (L8); Positive samples (L5,6,9; bands of 100 bp); Negative samples (L2-4,7).

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conducted in Turkey (10%), Switzerland (7.3%), Brazil (10-26.5%), the United Kingdom (5.8%-16.6%), and the United States (2-7%). These discrepancies might be ascribed to apply various laboratory methods, climatic variations, and dog population types (6). Low prevalence in the examined dogs may be due to their living conditions, limited direct exposure to potential sources of infection, feeding habits, and the owners' attention for eliminating raw meat from the dogs' diet, along with impossible climates such as low humidity and temperature for a pathogen, which is effective in sporulation time in the region (13,14).

The infection rate of *N. caninum* was significantly higher in female than male dogs (Table 2), which is in agreement with the findings of Goździk et al (28) while in contrast with those of other studies in Turkey (27) and Iran (6,10,12,14). Alexander and Stinson (29) noted that female dogs were progressively susceptible to *N. caninum*. The hormonal contrasts may have a significant role in host susceptibility to *N. caninum*. Since estrogen excretion enhances antibody production and androgen suppresses T and B cell immune responses, in females, the immunity to infection can be broken down as a result of various factors such as nutrition, age, pregnancy, and environmental changes (7).

In the current study, the highest infection rate was recognized in dogs of 12-24 months old (Table 2). In a similar investigation, Basso et al (30) revealed the highest antibodies to *N. caninum* in dogs of more than one year old (47.7%). Interestingly, the seroprevalence rate increased in dogs with postnatal exposure to *N. caninum* through horizontal transmission in prior reports in Iran (10,14,15). The high infection in older animals is due to their chance of exposure to infection over time (17).

N. caninum infection in the pure breed (5.4%) was higher than mixed breed (3.7%) with no significant difference. Nearly all reports confirmed that there is no correlation between breed susceptibility to *N. caninum* with seroprevalence (31). Further, Nazir et al (32) reported that there were no significant differences between seroprevalence and breeds belonging to Alsatian (12.6%), Bully (20.6%), Pug (20.4%), Bullterrier (18.9%), German Shepherd (31.4%), Labrador Retriever (19.6%), Crossbreeds (28.7%), and Mongrel breeds (35.4%). In contrast, Robbe et al (33) reported higher seropositivity to *N. caninum* in pure breed compared to the other type. Supplementary universal investigations are suggested subsequently because of less information about the impact of breeds in the epidemiology of canine neosporosis.

The clinical signs of neosporosis in dogs, usually neurological disorders, are not pathognomonic because they are very helpful in the exact diagnosis of diseases parallel to laboratory tools (4). In our study, musculoskeletal and neurological signs were recorded in

2 infected animals. Additionally, the infection rate had no association with food regime, housing, and direct contact with another animal (Table 2). The infection with *Neospora* is associated with animal food materials. Dogs with access to prey or raw food materials outside their home derived from intermediate hosts can increase the risk for seropositivity (5).

In conclusion, the outcomes affirmed the presence and exposure of examined dogs to *N. caninum* in Hamadan. This investigation, to the best of our knowledge, was the primary presentation of risk factors related to *N. caninum* infection in Iranian pet dogs. Further investigations on household animal populations are necessary for determining the prevalence and possible routes of infection in dogs from other parts of the country. Finally, using diagnostic screening tools and anti-*Neospora* compounds in pet dog clinics is recommended for controlling the disease.

Conflict of Interest

The authors declare that they have no competing interests.

Acknowledgments

The authors wish to thank all clinicians for helping in the sample collection process. This work was not upheld by any foundation.

Ethical Statement

The samples were taken from the dogs with official permission and under the supervision of the Institutional Animal Ethics and Research Committee of Iranian Veterinary Organization, Hamadan office, Iran (Certificate No. 32/1396.4.1).

Authors' Contribution

JG designed the study, sampling, laboratory techniques, data analysis and writing the paper. MY helped for designing the study and revised the final version of manuscript. RK helped in the design of molecular protocol. All authors reviewed the manuscript.

References

1. Bjerkås I, Mohn SF, Presthus J. Unidentified cyst-forming sporozoon causing encephalomyelitis and myositis in dogs. *Z Parasitenkd.* 1984;70(2):271-4. doi: 10.1007/bf00942230.
2. Dubey JP. Review of *Neospora caninum* and neosporosis in animals. *Korean J Parasitol.* 2003;41(1):1-16. doi: 10.3347/kjp.2003.41.1.1.
3. Dubey JP, Knickman E, Greene CE. Neonatal *N. caninum* infections in dogs. *Acta Parasitol.* 2005;50(2):176-9.
4. Barber JS, Trees AJ. Clinical aspects of 27 cases of neosporosis in dogs. *Vet Rec.* 1996;139(18):439-43. doi: 10.1136/vr.139.18.439.
5. Dubey JP, Schares G, Ortega-Mora LM. Epidemiology and control of neosporosis and *Neospora caninum*. *Clin Microbiol Rev.* 2007;20(2):323-67. doi: 10.1128/cmr.00031-06.
6. Gharekhani J, Tavoosidana GR, Akbarein H. Serological study of *Neospora caninum* infection in dogs and cattle from west of Iran. *Comp Clin Path.* 2014;23(5):1203-7. doi: 10.1007/s00580-013-1763-z.
7. Gharekhani J, Yakhchali M, Abbasi-Doulatshahi E, Barati E. Seroprevalence of *Neospora caninum* and *Toxoplasma gondii* infections in stray dogs of Hamadan suburb, west of Iran, 2018. *Avicenna J Clin Microbiol Infect.* 2019;6(2):57-60. doi:

8. Ghafarifar F, Sabevarinejad G, Dalimi A, Forouzandeh-Moghadam M. Molecular detection of *Neospora caninum* from naturally infected dogs in Lorestan province, west of Iran. *Arch Razi Inst.* 2014;69(2):185-90. doi: 10.7508/ari.2014.02.010.
9. Gharedaghi Y. Seroprevalence of *Neospora caninum* in stray dogs of Tabriz, Iran. *J Anim Vet Adv.* 2012;11(6):723-6. doi: 10.3923/javaa.2012.723.726.
10. Yakhchali M, Javadi S, Morshedi A. Prevalence of antibodies to *Neospora caninum* in stray dogs of Urmia, Iran. *Parasitol Res.* 2010;106(6):1455-8. doi: 10.1007/s00436-010-1824-z.
11. Khanmohammadi M, Fallah E. Prevalence of *Neospora caninum* antibodies in shepherd dogs in Sarab district, East Azerbaijan province, Iran. *Afr J Microbiol Res.* 2011;5(28):5062-6. doi: 10.5897/AJMR11.796.
12. Sharifdini M, Mohebbali M, Keshavarz H, Hosseinienejad M, Hajjaran H, Akhondi B, et al. *Neospora caninum* and *Leishmania infantum* co-infection in domestic dogs (*Canis familiaris*) in Meshkin-Shahr district, Northwestern Iran. *Iran J Arthropod Borne Dis.* 2011;5(2):60-8.
13. Razmi G. Fecal and molecular survey of *Neospora caninum* in farm and household dogs in Mashhad area, Khorasan province, Iran. *Korean J Parasitol.* 2009;47(4):417-20. doi: 10.3347/kjp.2009.47.4.417.
14. Malmasi A, Hosseinienejad M, Haddadzadeh H, Badii A, Bahonar A. Serologic study of anti-*Neospora caninum* antibodies in household dogs and dogs living in dairy and beef cattle farms in Tehran, Iran. *Parasitol Res.* 2007;100(5):1143-5. doi: 10.1007/s00436-006-0385-7.
15. Haddadzadeh HR, Sadrebazzaz A, Malmasi A, Talei Ardakani H, Khazraii Nia P, Sadreshirazi N. Seroprevalence of *Neospora caninum* infection in dogs from rural and urban environments in Tehran, Iran. *Parasitol Res.* 2007;101(6):1563-5. doi: 10.1007/s00436-007-0678-5.
16. Pouramini A, Jamshidi S, Shayan P, Ebrahimzadeh E, Namavari M, Shirian S. Molecular and serological detection of *Neospora caninum* in multiple tissues and CSF in asymptomatic infected stray dogs. *Iran J Vet Med.* 2017;11(2):105-12. doi: 10.22059/ijvm.2017.61602.
17. Hosseinienejad M, Mahzounieh M, Shams Esfandabadi N. *Neospora caninum* suspects as one of the most important causes of abortion in large dairy farms in Isfahan, Iran. *Iran J Parasitol.* 2017;12(3):408-12.
18. Hosseinienejad M, Hosseini F, Mahzounieh M, Raisi-Nafchi A, Moshrraf M. Seroprevalence of *Neospora caninum* infection in dogs in Chaharmahal-va-Bakhtiari province, Iran. *Comp Clin Path.* 2010;19(3):269-70. doi: 10.1007/s00580-009-0864-1.
19. Hosseinienejad M, Hosseini F. Seroprevalence of *Neospora caninum* and *Toxoplasma gondii* infection in dogs from west and central parts of Iran using two indirect ELISA tests and assessment of associate risk factors. *Iran J Vet Res.* 2011;12(1):46-51. doi: 10.22099/ijvr.2011.40.
20. Khordadmehr M, Hosseini S, Mohsenifar E, Namavari M, Khordadmehr S. Seroprevalence of *Neospora caninum* in farm and household dogs determined by ELISA. *Online J Vet Res.* 2012;16(4):172-81.
21. Gharekhani J, Yakhchali M. *Neospora caninum* infection in dairy farms with history of abortion in west of Iran. *Vet Anim Sci.* 2019;8:100071. doi: 10.1016/j.vas.2019.100071.
22. Müller N, Zimmermann V, Hentrich B, Gottstein B. Diagnosis of *Neospora caninum* and *Toxoplasma gondii* infection by PCR and DNA hybridization immunoassay. *J Clin Microbiol.* 1996;34(11):2850-2.
23. Macedo CA, Macedo MF, Cardim ST, Paiva MC, Taroda A, Barros LD, et al. *Neospora caninum*: evaluation of vertical transmission in slaughtered dairy cows (*Bos taurus*). *Rev Bras Parasitol Vet.* 2013;22(1):13-7. doi: 10.1590/s1984-29612013000100004.
24. McCann CM, Vyse AJ, Salmon RL, Thomas D, Williams DJ, McGarry JW, et al. Lack of serologic evidence of *Neospora caninum* in humans, England. *Emerg Infect Dis.* 2008;14(6):978-80. doi: 10.3201/eid1406.071128.
25. Anvari D, Saberi R, Sharif M, Sarvi S, Hosseini SA, Moosazadeh M, et al. Seroprevalence of *Neospora caninum* infection in dog population worldwide: a systematic review and meta-analysis. *Acta Parasitol.* 2020. doi: 10.2478/s11686-019-00163-4.
26. Sager H, Moret CS, Müller N, Staubli D, Esposito M, Schares G, et al. Incidence of *Neospora caninum* and other intestinal protozoan parasites in populations of Swiss dogs. *Vet Parasitol.* 2006;139(1-3):84-92. doi: 10.1016/j.vetpar.2006.02.021.
27. Coskun SZ, Aydın L, Bauer C. Seroprevalence of *Neospora caninum* infection in domestic dogs in Turkey. *Vet Rec.* 2000;146(22):649. doi: 10.1136/vr.146.22.649.
28. Goździk K, Wrzesień R, Wielgosz-Ostolska A, Bień J, Kozak-Ljunggren M, Cabaj W. Prevalence of antibodies against *Neospora caninum* in dogs from urban areas in Central Poland. *Parasitol Res.* 2011;108(4):991-6. doi: 10.1007/s00436-010-2143-0.
29. Alexander J, Stimson WH. Sex hormones and the course of parasitic infection. *Parasitol Today.* 1988;4(7):189-93. doi: 10.1016/0169-4758(88)90077-4.
30. Basso W, Venturini L, Venturini MC, Moore P, Rambeau M, Unzaga JM, et al. Prevalence of *Neospora caninum* infection in dogs from beef-cattle farms, dairy farms, and from urban areas of Argentina. *J Parasitol.* 2001;87(4):906-7. doi: 10.1645/0022-3395(2001)087[0906:poncii]2.0.co;2.
31. Fernandes BC, Gennari SM, Souza SL, Carvalho JM, Oliveira WG, Cury MC. Prevalence of anti-*Neospora caninum* antibodies in dogs from urban, periurban and rural areas of the city of Uberlândia, Minas Gerais--Brazil. *Vet Parasitol.* 2004;123(1-2):33-40. doi: 10.1016/j.vetpar.2004.05.016.
32. Nazir MM, Maqbool A, Akhtar M, Ayaz M, Ahmad AN, Ashraf K, et al. *Neospora caninum* prevalence in dogs raised under different living conditions. *Vet Parasitol.* 2014;204(3-4):364-8. doi: 10.1016/j.vetpar.2014.05.041.
33. Robbe D, Passarelli A, Gloria A, Di Cesare A, Capelli G, Iorio R, et al. *Neospora caninum* seropositivity and reproductive risk factors in dogs. *Exp Parasitol.* 2016;164:31-5. doi: 10.1016/j.exppara.2016.02.003.