Veterinary Medicine: Research and Reports

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Toxoplasma gondii And *Neospora caninum* In Brain Tissue Of Rodents In North-West Iran

This article was published in the following Dove Press journal:
Veterinary Medicine: Research and Reports

Naser Nazari[1]
Saeedeh Shojaee[2]
Mehdi Mohebali[2]
Aref Teimouri[2,3]
Keyghobad Ghadiri[4]
Saber Raeghi[5]
Mohammad Reza Shiee[2,3]
Yousef Azarakhsh[6]
Arezoo Bozorgomid[4]

[1]Department of Medical Parasitology and Mycology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran; [2]Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran; [3]Students Scientific Research Center, Tehran University of Medical Sciences, Tehran, Iran; [4]Infectious Diseases Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran; [5]Department of Laboratory Sciences, Maragheh University of Medical Sciences, Maragheh, Iran; [6]Department of Biology, Razi University, Kermanshah, Iran

Purpose: Rodents live in proximity to humans and domestic animals. These creatures can serve as reservoir hosts for many zoonotic parasites; therefore, they increase the risk of human infections. The aim of this study was to investigate *Toxoplasma gondii* and *Neospora caninum* in rodents caught in Meshgin-Shahr District, Iran.

Patients and methods: In a cross-sectional study, brain samples were collected from 70 rodents caught in Meshgin-Shahr District during March and December 2015. The specimens were examined for exposure to *T. gondii* and *N. caninum* with molecular methods.

Results: Seventy rodents were caught, including 50 *Meriones persicus*, 11 *Mus musculus* and 9 *Cricetulus migratorius*. Thirty rodents were female and 40 were males. Using PCR (B1 gene), *T. gondii* was detected in 7.1% (5/70) of the rodents while *N. caninum* was not detected. The prevalence of *Toxoplasma* infection was higher in female rodents (4.28%) compared to male rodents (2.86%), but the difference was not significant.

Conclusion: The results showed a low risk of *T. gondii* and *N. caninum* among rodents. Finally, further research is needed to understand the role of these rodent species in the transmission of the above protozoan pathogens to humans and livestock in this area.

Keywords: rodents, *Neospora*, *Toxoplasma*, prevalence, PCR, Iran

Introduction

Rodents are the largest group of mammals with around 2700 species worldwide.[1] They are important intermediate hosts for several parasites affecting cats, dogs and wild carnivores such as *Toxoplasma, Neospora, Hammondia*, Sarcocystis, *Echinococcus* and *Toxocara*.[2] Rodents are also important zoonotic carriers of agents to humans, because they rapidly adapt to human habitats and ecologic and environmental changes.[3] *Toxoplasma gondii* and *Neospora caninum* are intracellular protozoan parasites belonging to the phylum Apicomplexa. Cats and dogs are the definitive hosts of *T. gondii* and *N. caninum*, respectively. Both parasites can use a wide range of domestic and wild animals as intermediate hosts.[4,5] Infection can be acquired vertically by transplacental transmission of tachyzoites or horizontally following ingestion of tissue cysts from uncooked or undercooked meat (bradyzoites), or ingestion of oocysts excreted in definitive hosts feces.[4,5]

Previous investigations have reported *N. caninum* infection in different species of rodents.[6,7] Infection in rodents has been associated with the spread of *N. caninum* infection and its effects on reproduction in dairy cattle.[8] *Toxoplasma* is one of the most common zoonotic parasites infecting one-third of the world's population.[9,10] *Neospora*, however, unlike *Toxoplasma*, is not considered a zoonotic protozoan.[11,12] *N. caninum*

Correspondence: Arezoo Bozorgomid
Infectious Diseases Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
Tel +98 918 872 8269
Email arezoobozorgomid@yahoo.com

submit your manuscript | www.dovepress.com
DovePress
http://doi.org/10.2147/VMRR.S224664

has been reported in different hosts such as hooded crows (*Corvus cornix*),[13] chickens (*Gallus domesticus*)[14] and stray dogs[15] in Iran, but there is no reported survey in rodents. There are scant reports of the prevalence of *T. gondii* infection in rodents in Iran. Serological surveys carried out in rodents have shown a prevalence of 0–31%.[16] A recent epidemiological study carried out by Seifollahi et al[17] revealed a prevalence of 5.7% in rodents. Therefore, considering the fact that both *T. gondii* and *N. caninum* have a global distribution and our knowledge of these species in Iran is limited, the aim of this study was to evaluate the molecular prevalence of *N. caninum* and *T. gondii* infections in rodents living in Meshgin-Shahr District, north-western Iran.

Patients And Methods

Study Site And Sampling

The location of sampling was Meshgin-Shahr District in north-western Iran (38°23′56″N and 47°40′55″E) (Figure 1). It is located near the Sabalan Mountains and has a cold mountainous climate. During the period of this cross-sectional study (March to December 2015), 70 rodents were trapped with live traps baited with fresh cucumber and walnut. Identification of rodents was based on their skull and tooth structures as presented by Etemad.[18]

Brain samples were collected, preserved in ethanol 40%, and transferred to the protozoology laboratory of the Public Health School of Tehran University of Medical Sciences for molecular evaluation. To avoid cross-contamination, a sterile scalpel was used to collect each sample. All procedures were approved by the Animal Ethics Committee of Kermanshah University of Medical Sciences, Iran (No. code: 94065) and performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

DNA Extraction And PCR Amplification

Genomic DNA from brain tissues of the rodents was extracted using the Qiagen DNA Minikit (Qiagen, Venlo, The Netherlands) according to the manufacturer's recommendations and stored at −20°C until further use. Polymerase chain reaction (PCR) was performed to detect the *T. gondii* and *N. caninum* DNA using the specific primers targeted B1 and Nc5, respectively.[19,20] DNA extracted from RH strain tachyzoites, NC-1 strain tachyzoites and bidistilled water was used as the positive control for *T. gondii* and *N. caninum* and the negative control for PCR reactions,

Figure 1 Map of Iran showing the geographical location of Ardabil Province and the study area, Meshgin-Shahr.

respectively. PCR reaction was performed in 15 µL volume including 1.5 mM $MgCl_2$, 200 µM of each dNTP, 0.5 µM of each primer, 1X PCR buffer, 1 U Taq DNA polymerase and 100 ng of *T. gondii* or *N. caninum* DNA. Amplification was conducted in a thermal cycler (Eppendorf, Germany) with an initial 5 mins denaturation at 95°C, followed by 35 amplification cycles, denaturation at 94°C for 45 s, 45 s annealing at specific annealing temperature for each primer and elongation at 72°C for 1 min, followed by a final extension step at 72°C for 5 mins. Annealing temperature was 61°C for gene B1 and 67°C for Nc5. PCR products were electrophoresed in a 1.5% agarose gel stained with Simply Safe (Eurx, Cat. No. E4600-01).

For data analysis, SPSS software (ver. 16.0) was used. The chi-square test was used to examine the relationships between rodent sex and the presence of *T. gondii* DNA. The $P < 0.05$ was considered as statistically significant.

Results

Seventy small rodents, including the three species of *Meriones persicus, Mus musculus* and *Cricetulus migratorius*, were collected. The most abundant species in the present study was *Meriones persicus* (71.43%). Thirty rodents were female and 40 were male. Totally, 5/70 (7.14%) brain samples of rodents were found positive for the *T. gondii* B1 gene (Figure 2). The prevalence of *Toxoplasma* infection was higher in female rodents (4.28%) compared to male rodents (2.86%). There was no significant difference between rodent sex and the presence of *T. gondii* DNA. One of the positive *T. gondii* amplicons was sequenced and had 100% nucleotide similarity with *T. gondii* isolates. The nucleotide sequence was deposited in GenBank under the accession number: MK184480. None of the rodents tested positive for *N. caninum* by PCR. Frequency of infection of *N. caninum* and *T. gondii* in the brain tissue of rodents is summarized in Table 1

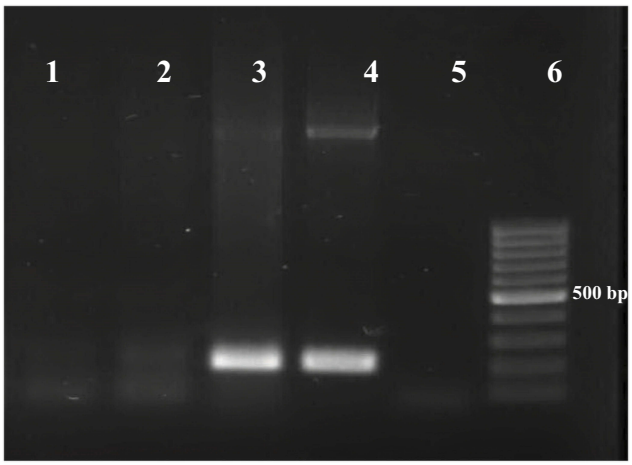

Figure 2 PCR amplification of the B1 gene.
Notes: Lanes 1 and 2: negative sample; lane 3: positive sample *Toxoplasma* isolate; lane 4: positive control; lane 5: negative control; lane 6: 100 bp DNA ladder.

Table 1 Frequency Of Infection Of *Neospora caninum* And *Toxoplasma gondii* In The Brain Tissue Of Rodents In Meshgin-Shahr District, Iran

Variables	Number Of Rodents Tested [n (%)]	Number Of Positive Rodents [n (%)]	
		Toxoplasma gondii	*Neospora caninum*
Gender			
Male	40 (57.14)	2 (2.86)	0
Female	30 (42.86)	3 (4.28)	0
Species			
Meriones persicus	50 (71.43)	1 (1.43)	0
Cricetulus migratorius	9 (12.86)	1 (1.43)	0
Mus musculus	11 (15.71)	3 (4.28)	0

Discussion

Our increased knowledge of the parasitological diseases of rodents in different parts of the world has a fundamental role in the evaluation of environmental health conditions and the assessment of health plans aimed at preventing certain prevalent infectious diseases in human and animal populations.

In the current investigation, *T. gondii* infection was detected in 7.1% of the rodents by molecular analysis, which is comparable to the results of Saki and Khademvatan[21] in rodents of Ahvaz District in south-western Iran. Thomasson et al[22] reported a prevalence of up to 40.78% for *T. gondii* in *Apodemus sylvaticus* in England. In a study by Fuehrer et al,[23] the DNA of *T. gondii* was detected in 0.7% of common voles and 4.7% of water voles in the western part of Australia.

Molecular surveys conducted in *Mus musculus* on the Czech-German border showed *N. caninum* in 13 out of 360 animals (3.6%).[6] A study in Mexico showed a *N. caninum* prevalence of 77% in *Mus musculus*, 71% in *Spermophilus variegatus*, and 50% in *Rattus norvegicus*.[7] Dellarupe et al[24] did not detect the DNA of *N. caninum* in any of the sampled rodents.

In the current study, none of 70 mouse brains was positive for *N. caninum* DNA in molecular surveys.

Several factors may have contributed to the failure to identify the *N. caninum* DNA in rodents in this study. For example, Barratt et al[25] suggested that screening wild rodents for the presence of cysts or *N. caninum* DNA in the brain alone would result in the under-reporting of the infection. According to Ferroglio et al,[26] the brain tissue of only 4 of 25 rodents was positive for DNA of *N. caninum*, whereas 19 muscle samples and 6 kidney samples showed evidence of exposure to infection. However in this study, it was not possible to detect *N. caninum* DNA from the tissue samples due to our financial limitations.

The district of Meshgin-Shahr is considered the coldest province in Iran with a very cold climate in the winter and a mild climate in the summer; therefore, decreased oocyst survival and sporulation under cool conditions could explain the low infection rates seen in the current study. Some studies suggest that cohabitation of rodent with the definitive hosts of *N. caninum* is a putative risk factor for seropositivity due to high level of exposure to oocysts.[7,27] Therefore, low prevalence rates in rodents can be associated with different distribution patterns of host species such as cats, dogs and livestock, which were found to be largely infected by *N. caninum*.

In this study, only one of the positive *T. gondii* amplicons was sequenced and had 100% nucleotide similarity with *T. gondii* isolates. Sequencing of the other four PCR *T. gondii*-positive samples was not successful, which could be explained by the small amount of *T. gondii* DNA in the samples. It can be concluded that, although the results of the current study indicated a low risk of *T. gondii* and *N. caninum* infection among rodents of this district, the infection could be much more widespread in animals; the prevalence might be underestimated due to low DNA concentrations in samples. In addition, only a few cases of *Toxoplasma* infection might develop into clinical lesions. Moreover, we must be cautious about the interpretation of our results, as different studies have used different genetic markers to detection *T. gondii* and *N. caninum* infection and these markers have different power to detection parasite.

Rodent plays an important role in the life cycle of *T. gondii* and *N. caninum* because they are generally preyed by domestic and feral cats and dogs hence can spread the parasite to humans and other animals. Besides, hunting and eating of rodents by man as an ancient practice in some nations increased the risk of acquiring zoonotic diseases such as toxoplasmosis and neosporiasis.[28] However, to the best of the authors' knowledge, this research is the first study of *Toxoplasma* and *Neospora* prevalence in rodents in the Meshgin-Shahr District. Further research is needed to understand the role of these rodent species in the epidemiology of such protozoan pathogens to humans and livestock in this area. In addition, we recommend evaluating other molecular markers for the detection of *T. gondii* and *N. caninum* infection in rodent.

Acknowledgment

This study received financial support from Kermanshah University of Medical Sciences, Kermanshah, Iran (Grant No. 94065).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Wilson DE, Reeder DM. *Mammal Species of the World: A Taxonomic and Geographic Reference*. 3nd ed. Baltimore, Maryland: Johns Hopkins University Press; 2005.
2. Krücken J, Blümke J, Maaz D, et al. Small rodents as paratenic or intermediate hosts of carnivore parasites in Berlin, Germany. *PLoS One*. 2017;12(3):e0172829. doi:10.1371/journal.pone.0172829
3. Chaisiri K, Chaeychomsri W, Siruntawineti J, Ribas A, Herbreteau V, Morand S. Diversity of gastrointestinal helminths among murid rodents from northern and northeastern Thailand. *Southeast Asian J Trop Med Public Health*. 2012;43(1):21–28.
4. de Barros LD, Miura AC, Minutti AF, Vidotto O, Garcia JL. *Neospora caninum* in birds: a review. *Parasitol Int*. 2018;67:397–402. doi:10.1016/j.parint.2018.03.009
5. Torrey EF, Yolken RH. *Toxoplasma* oocysts as a public health problem. *Trends Parasitol*. 2013;29(8):380–384. doi:10.1016/j.pt.2013.06.001
6. Hůrková-Hofmannová L, Qablan M, Jurankova J, Modrý D, Pialek J. A survey of *Toxoplasma gondii* and *Neospora caninum* infecting house mice from a hybrid zone. *J Parasitol*. 2014;100(1):139–141. doi:10.1645/13-255.1
7. Medina-Esparza L, Macías L, Ramos-Parra M, Morales-Salinas E, Quezada T, Cruz-Vázquez C. Frequency of infection by *Neospora caninum* in wild rodents associated with dairy farms in Aguascalientes, Mexico. *Vet Parasitol*. 2013;191(1–2):11–14. doi:10.1016/j.vetpar.2012.08.007
8. Dubey J, Schares G, Ortega-Mora L. Epidemiology and control of neosporosis and *Neospora caninum*. *Clin Microbiol Rev*. 2007;20(2):323–367. doi:10.1128/CMR.00031-06
9. Flegr J, Prandota J, Sovičková M, Israili ZH. Toxoplasmosis – a global threat. Correlation of latent toxoplasmosis with specific disease burden in a set of 88 countries. *PLoS One*. 2014;9(3):e90203.
10. Nazari N, Bozorgomid A, Janbakhsh A, Bashiri F. *Toxoplasma gondii* and human immunodeficiency virus co-infection in western Iran: a cross sectional study. *Asian Pac J Trop Med*. 2017;10(10):58–62.
11. Oshiro LM, Motta-Castro ARC, Freitas SZ, et al. *Neospora caninum* and *Toxoplasma gondii* serodiagnosis in human immunodeficiency virus carriers. *Rev Soc Bras Med Trop*. 2015;48(5):568–572. doi:10.1590/0037-8682-0151-2015
12. Calero-Bernal R, Horcajo P, Hernández M, Ortega-Mora LM, Fuentes I. Absence of *Neospora caninum* DNA in Human Clinical Samples. *Spain Emerg Infect Dis*. 2019;25(6):1226–1227. doi:10.3201/eid2506.181431

13. Abdoli A, Arbabi M, Pirestani M, et al. Molecular assessment of *Neospora caninum* and *Toxoplasma gondii* in hooded crows (*Corvus cornix*) in Tehran, Iran. *Comp Immunol Microbiol Infect Dis*. 2018;57:69–73. doi:10.1016/j.cimid.2018.06.008
14. Sayari M, Namavari M, Mojaver S. Seroprevalence of *Neospora caninum* infection in free ranging chickens (*Gallus domesticus*). *J Parasit Dis*. 2016;40(3):845–847. doi:10.1007/s12639-014-0590-8
15. Yakhchali M, Bahrami M, Asri-Rezaei S, Bokaie S. The enzymes and electrolytes profiles in sera of Iranian stray dogs naturally infected with *Neospora caninum*. *Annals Ann Parasitol*. 2017;63(1):63–68.
16. Khademvatan S, Foroutan M, Hazrati-Tappeh K, et al. Toxoplasmosis in rodents: a systematic review and meta-analysis in Iran. *J Infect Public Health*. 2017;10(5):487–493. doi:10.1016/j.jiph.2017.01.021
17. Seifollahi Z, Sarkari B, Motazedian MH, Asgari Q, Ranjbar MJ, Abdolahi Khabisi S. Protozoan parasites of rodents and their zoonotic significance in Boyer-Ahmad District, Southwestern Iran. *Vet Med Int*. 2016;2016:5.
18. Etemad E. *Rodents and Identification Key of Them*. Vol. I. Tehran: National Association of Natural Source Protection and Human Environment; 1979.
19. Burg JL, Grover CM, Pouletty P, Boothroyd J. Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii*, by polymerase chain reaction. *J Clin Microbiol*. 1989;27(8):1787–1792.
20. 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):2850–2852.
21. Saki J, Khademvatan S. Detection of *Toxoplasma gondii* by PCR and mouse bioassay in rodents of Ahvaz District, Southwestern Iran. *Biomed Res Int*. 2014;2014:1–5. doi:10.1155/2014/383859
22. Thomasson D, Wright E, Hughes J, et al. Prevalence and co-infection of *Toxoplasma gondii* and *Neospora caninum* in *Apodemus sylvaticus* in an area relatively free of cats. *Parasitology*. 2011;138(9):1117–1123. doi:10.1017/S0031182011000904
23. Fuehrer H-P, Blöschl I, Siehs C, Hassl A. Detection of *Toxoplasma gondii, Neospora caninum*, and *Encephalitozoon cuniculi* in the brains of common voles (*Microtus arvalis*) and water voles (*Arvicola terrestris*) by gene amplification techniques in western Austria (Vorarlberg). *Parasitol Res*. 2010;107(2):469–473. doi:10.1007/s00436-010-1905-z
24. Dellarupe A, Fitte B, Pardini L, et al. *Toxoplasma gondii* and *Neospora caninum* infections in synanthropic rodents from Argentina. *Rev Bras Parasitol Vet*. 2019;28(1):113–118. doi:10.1590/S1984
25. Barratt J, Al Qassab S, Reichel MP, Ellis JT. The development and evaluation of a nested PCR assay for detection of *Neospora caninum* and *Hammondia heydorni* in feral mouse tissues. *Mol Cell Probes*. 2008;22(4):228–233. doi:10.1016/j.mcp.2008.03.001
26. Ferroglio E, Pasino M, Romano A, Grande D, Pregel P, Trisciuoglio A. Evidence of *Neospora caninum* DNA in wild rodents. *Vet Parasitol*. 2007;148(3–4):346–349. doi:10.1016/j.vetpar.2007.06.031
27. Zanet S, Sposimo P, Trisciuoglio A, Giannini F, Strumia F, Ferroglio E. Epidemiology of *Leishmania infantum, Toxoplasma gondii*, and *Neospora caninum* in *Rattus rattus* in absence of domestic reservoir and definitive hosts. *Vet Parasitol*. 2014;199(3–4):247–249. doi:10.1016/j.vetpar.2013.10.023
28. Suwannarong K, Chapman RS, Lantican C, Michaelides T, Zimicki S. Hunting, food preparation, and consumption of rodents in Lao PDR. *PLoS One*. 2015;10(7):e0133150. doi:10.1371/journal.pone.0133150