# *Neospora caninum* Infection in Rodents: A Molecular Study in Dairy Cattle Farms in Arak, Iran

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**Abstract**

*Neospora caninum* is an apicomplexan protozoa which is an important cause of abortion and economic loss in dairy and beef industries. This parasite has an indirect prey-predator lifecycle, which provides an opportunity for domestic and wild species to play a role in the lifecycle of *N. caninum*. Ongoing research is being conducted to ascertain the involvement of other vertebrates in the epidemiology and transmission of this parasite. Rodents are abundant in many habitats, including livestock farms, and their role in the maintenance and spread of *N. caninum* remains unresolved. In this study the plausible role of feral rodents in the transmission of *N. caninum*, was investigated in wild rodents captured from several dairy farms with a history of abortion and neosporosis in Arak city, Iran. During the study, rodent samples were collected from 14 farms with high abortion rate. All the trapped rodents were identified as *Mus musculus*. The rodents were necropsied and the brain samples were tested by Nested-PCR. No evidence for *N. caninum* infection was detected in any of the rodents' samples.

**Keywords**: *Neospora caninum*, Dairy cattle, Abortion, Molecular biology, *Mus musculus*

## 1. Introduction

*Neospora* sp. is a protozoan parasite causing abortion and reduced fertility in animals specially in cattle. The wide range of intermediate hosts makes the parasite widely distributed around the world (2, 3). Neosporosis causes sporadic abortions and abortion storms on farms causing severe reproductive and economic losses in the cattle. Besides the parasite is responsible for neuromuscular disease in dogs around the world (2,4,5). The vertical transmission of *Neospora*, in addition to the horizontal transmission through oocyst ingestion, plays an important role in the maintenance and spread of the infection within a cattle herd (5,6). The established role of dogs and certain wild canids as the definitive hosts in the lifecycle and prevalence of N. caninum is widely recognized. Different serological tests including enzyme-linked immunosorbent assays (ELISAs), indirect fluorescent antibody testing (IFAT), and agglutination tests in addition to the PCR-based (polymerase chain reaction) methods can be used to diagnose the infection. Small animals were incriminated to the sylvatic cycle of the infection. Rodents play an important role in the transmission of various microorganisms and they are criticized to have a role in the complex lifecycle of *N. caninum* in cattle farms. Studies on the role of rodents in the epidemiology of neosporosis has revealed the infection in different rodent species with varying relative frequency from Zero to 40% (3,7-12). The prevalence rate of cattle neosporosis is reported 23.6% and 20% in Iran and other countries, respectively (13). Globally, a prevalence rate of 5% among rodents has been reported, while this rate is 16% in Iran.

The data on the presence and prevalence of *Neospora* infection is sparse and ongoing. Various bird and rodent species were reported to harbor the parasite reservoir (3,7,9). These infested animals may play an important role in the epidemiology of the disease as their infected tissues may be the source of the infection for other hosts in the parasite's lifecycle. This study was performed to further investigate the plausible role of feral rodents on the distribution and infection of neosporosis. For this aim, wild rodents captured from several dairy farms with a history of neosporosis and abortion in Arak city, Iran were investigated molecularly for the presence of *N. caninum*

## 2. Methods

This study took place on the dairy farms in Arak (34°05'30.26"N 49°41'20.98"E), a county in Markazi province, Iran. Sampling from dairy cattle farms with a history of abortion due to neosporosis (14) was done from around the fodder barn, the manger, the watershed, milking parlor and outdoor area. Regarding 95% confidence level, 5% margin of errors and 4% population proportion, the least sample size was determined to be 60. Wooden traps and mouse glue trap were used for sampling. The trapped mice were euthanized with ether, identified morphologically and necropsied for obtaining fresh brain samples (12). The ethical approval for this study was obtained from the ethics committee of the Faculty of Veterinary Medicine, University of Tehran (28864/6/2). Upon necropsy any visible clinical lesions were recorded and brain was excised, homogenized aseptically with PBS (pH=7.4).

The obtained samples were centrifuged at 21500xg for 5 minutes and DNA extraction steps were followed on the sediment by DNA extraction Kit (Cinnaclone, Iran) (15). The samples were tested for the presence of *Neospora* using Nested-PCR. Primers for NC-5 gene were applied using NC-6, NC-21, NC-7 and NC-10 as nested-PCR (16-18). The PCR reaction contained 0.2 µM of each primer, 200 µM of each dNTP, and 1.5 mM of MgCl2, 2.5 U of Taq DNA polymerase, and 2 µl of DNA template in a total volume of 25 µl. For each set of PCR amplification, *N. caninum* isolates as internal positive control, and reaction without DNA template as the negative control were included. The thermal cycler PCR program was as following: pre-denaturing 5 min at 94 °C; 94 °C for 30 s in 40 cycles of, 63 °C for 30 s and 72°C for 1 min and a final 5 min extension at 72 °C. The amplified PCR products were

visualized on 1.5% Agarose gel pre-stained with Nancy-520 DNA Gel Stain (Sigma-Aldrich, Dorset, UK) under UV light and gel images were recorded by Gel document.

## 3. Results and Discussion

Totally, 68 rodents were captured from 14 farms with high abortion rate. All the captured rodents were identified as *Mus musculus*. None of the CNS samples was positive neither via PCR nor in the nested PCR tests.

Several investigations have explored the involvement of rodents in the epidemiology of *Neospora caninum*. In the West Indies (16) PCR revealed an infection rate of 8.6% while serology showed an infection rate of 5.1% in *Mus musculus*. 13.8% infection rate was reported by PCR in Italy in *Mus musculus* (19). In Argentina, there have been reports of infection in *Mus musculus* by IFAT (0.8%) (20). The Netherlands has reported 15.4% infection rate in house mice (15). In Mexico, PCR indicates a high infection rate of 77%, while immunohistochemistry shows a rate of 15% (12). The Czech Republic–German hybrid zone reports a 3.6% infection rate (21). Studies in Iran used agglutination, IFAT, PCR and nested-PCR tests and reported various rodent species to be infected with an infection rate of zero to 31.9% (10,13). In the present study no infection was detected in the samples.

There are several reports where the parasite was not identified in rodents' samples. Fernández-Escobar et al. (11) conducted a study and found no presence of *N. caninum* in house mice, although they did report a prevalence of 1.3% in other micromammals such as rats, shrews, and other species of mice. Similarly, Nazari et al. (23) used molecular methods to examine urban rodents and were unable to detect *N. caninum*, but 39% of the samples tested positive using IFAT (10). Machačová et al. (24) reported a serologic prevalence of 0.4% in 621 captured wild mammals, but no positive results were found in the captured house mice. These disparities could potentially arise from the examined organs. While some studies reported liver as the best target organ for *Neospora* detection in rodents, others have implied that brain or heart samples suits better (11, 25).

Prevalence data analysis requires special attention due to the constraints encountered due to the applied detection techniques. According to Jenkins (16), when focusing on the ITS loci a higher number of positive outcomes could be obtained in samples obtained from dairy farm environments, than Nc5 PCR. Bedsides there are reports that failed to confirm the molecularly detected infection through immunohistochemistry (2, 11, 22).

Regarding the sampling habitat, rodents residing in dry-land habitats were found to have a higher likelihood of being infected with *N. caninum* compared to those trapped in different habitats such as forests, rain-fed lands. Rodents inhabiting cattle farms with *N. caninum* abortion were more frequently infected than in peri-urban areas (20, 25, 26).

Diverse detection techniques, different sampling locations in relation to proximity to cattle farms, various rodent species, and examination of various organs may all contribute to the result diversification. It is worth mentioning that PCR or serology are commonly used detection methods to identify *N. caninum* infections in various animal species. These methods detect parasite DNA or specific antibodies in the host, but they do not necessarily indicate a viable or successful infection (27). However, the involvement of other animal species including rodents and birds in the maintenance of the parasite is still under study. It has been proven that pigeons and gerbils are the most susceptible hosts (2).

Although the presence of antibodies or the parasite's DNA in animals other than bovids and canids may make these animals a plausible host, it has not been proven in experimental studies. Despite the susceptibility of *Mus musculus* to infection, the role played by in the urban cycle of *N. caninum* infection appears to be negligible. It may be noted that the present study surveyed brain tissue from the farm captured *Mus musculus*. In order to surpass the limitations on experiment parameters, it is recommended to incorporate multiple diagnostic and confirmatory

techniques on different organs, alongside a more extensive sampling approach that encompasses a wider range of rodent species.

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**Author Contributions**

Fatemeh Arabkhazaeli: Conceptualization, Data curation, Investigation, Methodology, Software, Supervision, Writing – original draft, Writing – review & editing. Seyed Davood Hosseini: Supervision, Validation, Writing – review & editing. Mobina Farrokhnia: Writing – original draft. Mohammad Khani: Investigation, Writing – original draft.

**Conflict of Interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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