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Short Communication

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 follows an indirect prey-predator lifecycle, allowing both domestic and wild species to participate in its transmission. Ongoing research aims to ascertain the involvement of other vertebrates in the epidemiology and transmission of this parasite. Rodents,which are abundant in many habitats including livestock farms, although their role in the maintenance and spread of *N. caninum* remains unresolved. In this study, the potential 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 trapped rodents were identified as *Mus musculus*. Following necropsy, brain samples were collected and tested using Nested-PCR. No evidence for *N. caninum* infection was detected in any of the rodents' samples.

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1. Introduction

Neospora sp. is a protozoan parasite that cause abortion and reduced fertility in animals, especially in cattle. Due to its wide range of intermediate hosts, *N. caninum* is distributed globally (2, 3). Neosporosis causes sporadic abortions and abortion storms on farms, causing severe reproductive and economic losses in cattle herds. Additionally,the parasite is responsible for neuromuscular disease in dogs worldwide (2,4,5). Both vertical transmission and horizontal transmission through oocyst ingestionplay 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 definitive hosts in the lifecycle and prevalence of N. caninum is widely recognized. Diagnosis can be achieved through various 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. Small animals were incriminated to the sylvatic cycle of the infection. Rodents play an important role in the transmission of various microorganisms and are criticized to have a role in the complex lifecycle of *N. caninum* in cattle farms. Studies investigatingthe role of rodents in the epidemiology of neosporosis has revealed the infection in different rodent species with varying relative frequency ranging from Zero to 40% (3,7-12). The prevalence of neosporosis in cattle is reported to be 23.6% and 20% in Iran and other countries, respectively (13). Among rodents, global prevalence is estimated at 5%, while in Iran it reaches up to 16%.

Despite ongoing research, data on the presence and prevalence of *Neospora* infection in rodents and birds remain limited. 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. To this end, wild rodents captured from several dairy farms in Arak city, Iran, with a history of neosporosis and abortion, were molecularly investigated for the presence of *N. caninum*

2. Materials and Methods

This study was conducted on the dairy farms in Arak (34°05'30.26"N 49°41'20.98"E), located in in Markazi province, Iran. Sampling was carried out in areas with a documented history of abortion due to neosporosis ,including the fodder barn, the manger, the watershed, milking parlor and outdoor area. Based on a 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. Trapped mice were euthanized with ether, morphologically identified and necropsied to collect fresh brain samples (12). Ethical approval for this study was obtained from the Ethics Committee of the Faculty of Veterinary Medicine, University of Tehran (28864/6/2). During necropsy, any visible clinical lesions were recorded, and brain was aseptically excised and homogenized in PBS (pH=7.4).

The obtained samples were centrifuged at 21500xg for 5 minutes and DNA was extracted from the sediment using DNA extraction Kit (Cinnaclone, Iran) (15). 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 mixture contained 0.2 µM of each primer, 200 µM of each dNTP, 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 served as negative controls . Thermal cycler PCR program was as following: pre-denaturing 5 minutes at 94 °C; 40 cycles of 94 °C for 30 seconds of, 63 °C for 30 seconds and 72°C for 1 minute ,and a final final extension at 72°C for 5 minutes . 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

A total of 68 rodents were captured from 14 farms with high abortion rate. All captured rodents were identified as *Mus musculus*. None of the CNS samples tested positive via either PCR or the nested PCR tests.

4. Discussion

Several studies have investigated 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*. Italy reported a 13.8% infection rate by PCR in *Mus musculus* (19) and 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.

Several investigations have similarly failed to detect *N. caninum* 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 a1.3% prevalence 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*, although 39% of the samples tested positive using IFAT (10). Machačová et al. (24) reported a0.4% serologic prevalence 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 favored brain or heart samples (11, 25).

Prevalence data analysis requires special attention due to the constraints encountered, which is 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 farms than Nc5 PCR. Additionally, 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 forestsor rain-fed lands. Rodents inhabiting in 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 inconsistent results. 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 currently considered the most susceptible hosts (2).

Although the presence of antibodies or the parasite's DNA in animals rather 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, its role 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 outdo the limitations on experiment parameters, it is recommended to incorporate multiple diagnostic and confirmatory techniques on different organs, alongside a more extensive sampling approach, encompassing a wider range of rodent species.

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Authors' Contribution

Conceptualization, Data curation, Investigation, Methodology, Software, Supervision, Writing – original draft, Writing – review & editing: F. A.

Supervision, Validation, Writing – review & editing: S D. H.

Writing – original draft: M. F.

Investigation, Writing – original draft: M. K.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

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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Data Availability

The data that support the findings of this study are available on request from the corresponding author.

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