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Original Article

Molecular Confirmation of *Neospora caninum* as the Etiological Agent of Abortion in a Dairy Cattle Herd in Qazvin Province, Iran

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Abstract

Background: *Neospora caninum*, a cyst-producing parasite is identified as one of the main causes of reproductive failure in cattle worldwide. Neosporosis results in increased rates of abortion and reduced milk production, leading to financial losses. This study aimed to identify *Neospora caninum* infection in aborted cattle and their fetuses, at a dairy cattle farm, focusing on the involvement of stray dogs in spreading the agent within the herd.

Methods: During a three-month investigation in 2023, twenty-one cattle in a farm in Qazvin Province, Iran, showed abortion at 2, 4, 5, and 7 months of pregnancy, while stray dogs had free access to various sections of the farm. Serum samples were collected from each of the aborted dams. Seven aborted fetuses, along with two placentas, were also transported to the laboratory in frozen ice packs for testing for *N. caninum*. The fetal and placenta samples underwent examination through molecular assessment.

Results: Anti-*Neospora* antibodies were detected in the sera of 7 out of 21 cattle, corresponding to a seroprevalence rate of 33.3%. Among these, three out of seven aborted fetuses tested positive for *Neospora* via real-time PCR, all of derived from seropositive cattle. *Neospora caninum* was not detected in any of the placental samples examined.

Conclusion: *N. caninum* significantly influences the occurrence of abortions in dairy cattle. Although transmission of infection from an infected dam to a fetus is the most prominent route of the disease transmission, the proximity of stray dogs to cattle herds plays a key role in increasing number of infected cases.

Introduction

Neospora caninum, an obligate intracellular, cyst-forming protozoan, is the causative agent of bovine neosporosis (1). Infection with *N. caninum* results in significant economic losses in cattle production, primarily due to increased abortion rates and

reduced milk yield (2). Epidemiological studies indicate that this parasite is a leading cause of reproductive failure in cattle (3). In a systemic review and meta-analysis accomplished by Nayeri et al. in 2022, the prevalence of *N. caninum* in aborted cattle was 47% and 45% using ELIZA and IFA diagnostic techniques respectively (4). The presence of *N. caninum* in dairy farms of Brazil, leads to a rise of 10.2% in the risk of abortion. Most of the cattle abortions due to neosporosis take place in mid to late gestation (5). As a result, development of weak newborns and neurological disorders may occur (1).

Seroprevalence studies have illustrated an extensive seropositivity of 3.8%–76.2% in cattle due to *Neospora* infection in Iran, in a meta-analysis study (6). Ansari-Lari estimated the seroprevalence of the infection in Iranian dairy cattle as 23.6% (7). Gharekhani and Heidari, reported that 26% of Iranian native cattle with history of abortion were seropositive for *N. caninum* (8).

The life cycle of *N. caninum* is heteroxenous (9). Canine can be both definitive and intermediate host while bovine, ovine and equine are regarded as intermediate host (10, 11). The principal route of the infection transmission is from pregnant cattle to fetus (12). In addition, consumption of water and feed infected with the sporulated oocysts which dogs shed is horizontal route of the disease transmission (13). The association between livestock neosporosis and the presence of infected dogs is considerable (14). The rate of horizontal transmission of *N. caninum* was 93.7% in Brazil. The study considered the presence of stray dogs near dairy cattle herds as a significant factor in the higher number of infected cases (15).

In Iran, most dairy cattle herds—particularly those raised in extensive production systems—have direct or indirect contact with stray dogs, recognized as a major risk factor for *Neospora caninum* transmission (16, 17). Furthermore, the movement of dogs between farms can facilitate the spread of infection (18). Consequently, controlling stray dog populations is considered a critical measure for preventing neosporosis in dairy cattle. Persistent exposure of cattle to dogs, especially stray animals, perpetuates the parasite's presence within herds, increasing the risk of abortion and vertical transmission to subsequent generations (15).

The present study aimed to detect *Neospora caninum* infection in aborted cattle and their fetuses in a dairy cattle farm and emphasizes on the role of stray dogs in the circulation of the agent in the herd.

Materials and Methods

The study design and sample collection

During a three-month investigation in 2023, in an industrial dairy farm in Qazvin Province, Iran, twenty-one cows experienced fetal loss at gestational ages of 2, 4, 5, and 7 months. Serum samples were collected from all the aborted dams. Additionally, seven aborted fetuses along with two placentas were transported to the laboratory on ice packs for *Neospora caninum* detection. The fetal and placental specimens were preserved in a sterile urine container for molecular analysis.

Enzyme-Linked Immunosorbent Assay (ELISA)

Blood samples were collected from the jugular veins of the aborted cattle into the tubes without EDTA. The presence of *Neospora caninum* antibodies was assessed using the IDEXX *Neospora* Ab Test ELISA kit (Westbrook, Maine, USA), following the manufacturer's protocol. Absorbance was measured at 450 nm.

DNA extraction and Real-time PCR

DNA was extracted from the homogenized tissues utilizing CinnaGen DNA extraction kit. Probe and primers were created for amplifying an 85 bp segment of Nc5. Purified forward primer (5´-CTGTGCTCGCTGGGACTTC-

3´), reverse primer (5´-CGATTTACGACATACGGTGTTCA-3´), and probe (5´(FAM)CATCGGAGGACATCGCTCACTGA CTG(BHQ1)-3´) were used (19). The procedure was carried out with 400 nM of each primer, 120 nM probe, and 5 µL of nucleic acid template. The reactions were conducted on a 7500 Fast Real-Time PCR System under the specified thermal cycling conditions: 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 sec and 60 °C for 45 sec.

Results

Poor hygiene practices and the presence of stray dogs with unrestricted access to different parts of the farm were management defects of the studied cattle. Three cattle had previous abortion history while others experienced their first abortion. In one of the seropositive cattle, placental retention was observed. One of the aborted cattle which was positive in real-time PCR test, showed a fever of 39.3 ºC. None of the aborted fetuses had visible gross lesions (Fig. 1). Gelatinous edema was seen in one of the collected placentas. The serum samples were collected from all the aborted cattle and seven fetuses were analyzed since others were either not found or scavenged by stray dogs.

Seven cattle (33.3%) were identified positive in the serological assessment. From seven aborted fetuses, three were confirmed positive using Real-time PCR. *N. caninum* wasn't detected in one of the placental samples. All the samples which were positive in the molecular method belonged to the seropositive cattle.

Fig. 1: Fresh aborted fetus with *Neospora* positive result using real-time PCR

Discussion

Neospora caninum serves as a significant abortifacient agent in dairy cattle. The detection of 33.3% seropositive animals within the studied herd indicates a high prevalence of neosporosis, consistent with previous reports from Iran (17, 19). Several epidemiological studies in Iran have documented substantial infection rates of *N. caninum* in cattle. Furthermore, the molecular confirmation obtained in this study via real-time PCR strongly reinforces the etiological role of *N. caninum* in abortion cases. Detection of the parasite's DNA in three aborted fetuses, all from seropositive dams, provides direct molecular proof of infection and validates the diagnostic accuracy of PCR for detecting active infections in fetuses. Amouei et al. conducted PCR analysis on 70 aborted fetuses (bovine, ovine, and caprine) in Mazandaran province, detecting *N. caninum* in 22.2% of cases (17). Further supporting these observations, a three-year investigation in Khorasan Razavi Province reported *N. caninum* in 19.8% of the brain samples belonged to aborted bovine fetuses, employing PCR for detection (19).

In the present study, all the positive fetuses originated from seropositive dams, a finding consistent with established epidemiological patterns. Similar observations were reported in South Africa, where *N. caninum*-associated abortion rates were significantly elevated in seropositive cattle (20). This correlation is further supported by another prospective study in Iran (2017), which documented a 30.40% seroprevalence of *N. caninum*, with abortion incidence in seropositive animals (20.80%) being 2.4-fold higher than in seronegative counterparts (8.50%) (21).

Cattle seropositive for *N. caninum* antibodies pose a significant risk of vertical transmission to their offspring. Because all PCR-positive fetuses were derived from seropositive dams, vertical transmission, defined as transplacental infection from dam to fetus, can

be inferred as the predominant route of transmission within herds (9). Similar results were reported by Bernardes et al., indicating a higher risk of vertical transmission in *N. caninum* seropositive cattle than in seronegative ones, highlighting the importance of vertical transmission in dairy cattle (22). Gharekhani and Yakhchali confirmed vertical transmission as a key mechanism of parasite persistence across generations (23). This vertical route enables the parasite to persist endemically even without continuous horizontal exposure, representing a key epidemiological challenge in control programs. Culling seropositive animals constitutes an effective control strategy to interrupt disease transmission (24), and breeding should ideally be restricted to seronegative cattle. This approach often proves economically impractical in endemic areas. The persistent presence of stray dogs – definitive hosts that maintain the parasite's life cycle – renders culling strategies particularly ineffective for long-term herd protection.

In the current studied herd, stray dogs had easy accessibility to different parts of cattle farm including milking parlor, cattle shed and food storage and one of the reasons of difficult access to aborted fetuses and placenta was snatching them by dogs. An epidemiological investigation in Tabriz Province, Iran in 2021 examined *Neospora caninum* prevalence in canine populations by analyzing fecal samples from 100 indoor and outdoor dogs. Light microscopy detected oocysts in 45% of samples, with subsequent PCR confirmation of *N. caninum* in 21% of oocyst-positive cases. The study revealed differential infection rates among dog populations: 2% in pet dogs, 8% in stray dogs, 6% in shelter dogs, and 5% in rural dogs, demonstrating varying exposure risks based on living conditions (25). Another study used serological methods to identify the role of stray and domestic dogs in the epidemiological cycle of neosporrosis and dissemination of *N. caninum* infection among animals in Babylon Province, Iraq. The study reported a significantly higher seroprevalence in stray dogs (36.8%) compared to domestic dogs (15.7%), highlighting their greater potential for disseminating *N. caninum* infection (26).

In consideration of high prevalence of *N. caninum* infection in stray dogs, preventative strategies must be taken seriously in dairy cattle farms. Uncontrollable reproduction of stray dogs has become a major worldwide issue leading to various negative consequences. This unrestrained breeding can result in increased risks diseases transmitted by dogs including neosporosis (26). As a result, regular control of dog's population in dairy herds by strategies such as avoiding feeding them and fencing around the cattle farm, impact on *N. caninum* controlling plane.

Approximately 30% of bovine abortions can be prevented through effective control of *Neospora caninum* infection (21, 25, 27). In dairy cattle operations, multiple management strategies can mitigate *N. caninum*-associated abortions, including: 1) maintaining stringent hygiene protocols during calving, 2) identifying and culling seropositive animals from breeding stock, 3) proper disposal of placental tissues and aborted fetuses to prevent canine access, and 4) implementing biosecurity measures to protect feed and water sources from canine fecal contamination. Currently, no commercially available vaccines or therapeutic agents have been approved for the prevention or treatment of neosporosis in either cattle or dogs. Accordingly, in areas with high prevalence of the parasite which eradication is not practical, early detection of infected animals, improving farms hygiene levels and controlling dogs are recommended.

Conclusion

The present study provides substantive epidemiological evidence establishing *Neospora caninum* as a significant abortifacient pathogen in dairy cattle operations. While vertical transmission from infected dams to their offspring represents the predominant route of

infection, our findings highlight the critical role of environmental reservoirs - particularly stray dogs maintaining the parasite's life cycle - in perpetuating high infection rates within cattle herds.

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Conflict of interest

The authors declare that there is no conflict of interests.

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