



## 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

*Neospora caninum*, 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. This study aimed to identify *Neospora caninum* infection in aborted cattle and their fetuses, at a dairy cattle farm. Twenty-one cattle showed abortion at 2, 4, 5, and 7 months of pregnancy. Serum samples were collected from each of the aborted dams. Seven aborted fetuses, along with two placentas, were collected for the molecular detection of *N. caninum*. Anti-*Neospora* antibodies were detected in the sera of 7 out of 21 cattle, corresponding to a seroprevalence rate of 33.3%. Three out of seven aborted fetuses tested positive for *Neospora* via real-time PCR, all of which were derived from seropositive cattle. *Neospora caninum* was not detected in any of the placental samples examined. The findings of the current research demonstrate that *N. caninum* significantly influences the occurrence of abortions in dairy cattle. The proximity of stray dogs to cattle herds plays a key role in increasing number of infected cases.

**Keywords:** Abortion, Dairy cattle, Dogs, *Neospora caninum*, Neosporosis, Real-time PCR

### 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 ELISA and IFA diagnostic techniques respectively [4]. According to the study of Nicolino et al. in 2015, 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 in 2020 estimated the seroprevalence of the infection in Iranian dairy cattle as 23.6% [7]. Gharekhani and Heidari in 2014, 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]. In a study carried out in 2017, 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, which are 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.

## Material and methods

### The study design and sample collection

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'-CTGTGCTCGCTGGACTTC-3'), reverse primer (5'-CGATTTACGACATACGGTGTTC-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 minutes, followed by 40 cycles of 95 °C for 15 seconds and 60 °C for 45 seconds.

## 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° C. None of the aborted fetuses had visible gross lesions (Figure 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.

A total of 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.

## Discussion

The findings of this study demonstrate that *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. Several epidemiological studies in Iran have documented substantial infection rates of *N. caninum* in cattle. Amouei et al. (2019) 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 [19].

In the present study, all the positive fetuses originated from seropositive dams, a finding consistent with established epidemiological patterns. Similar observations were reported by Tagwireyi et al. (2024) 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. Vertical transmission, defined as transplacental infection from dam to fetus, represents the parasite's primary transmission route [9]. While culling seropositive animals constitutes an effective control strategy to interrupt disease transmission [22], 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 [23]. Another study in 2025, used serological methods to identify the role of stray and domestic dogs in the epidemiological cycle of neosporosis 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 [24].

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 [24]. 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.

Studies have demonstrated that approximately 30% of bovine abortions can be prevented through effective control of *Neospora caninum* infection [21, 23, 25]. In dairy cattle operations, multiple management strategies can mitigate *N. caninum*-associated abortions, including: (1) maintaining stringent hygiene protocols during calving, (3) identifying and culling seropositive animals from breeding stock, (4) proper disposal of placental tissues and aborted fetuses to prevent canine access, and (3) 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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