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REVIEW ARTICLE

# A Mini Review on *Neospora Caninum:* Updates and Epidemiology in Iran

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**Abstract**

*Neospora caninum* is a globally distributed parasite affecting animals, especially dogs and cattle. Its pathogenesis depends on the balance between the parasite's ability to invade and multiply within cells and the host's capacity to limit infection. Encephalomyelitis and polymyositis are the most significant pathological findings. The tachyzoites multiply inside cells, causing necrosis and cell death. Clinical signs such as abortion and neuromuscular paralysis are predominant in cattle and dogs, respectively. The most common histopathological features include necrotic foci in the brain. The overall prevalence of *N. caninum* in dogs and cattle worldwide is 17.1% and 20%, respectively. In Iran, infection rates vary from 3.8% to 76.2% in cattle, zero to 54.6% in dogs, 0.9% to 9.9% in sheep, 6.2% to 10.8% in goats, 19.2% to 55.9% in buffaloes, 20% to 2.42% in horses, 52% in donkeys, 3.2% to 27% in camels, 14% to 19% in cats, zero to 20.4% in rodents, 17.3% in chickens, 9.8% to 30.4% in pigeons, 2.8% to 3.7% in sparrows, and 9.9% in crows. Managing the disease to reduce infection can improve animal productivity and help prevent economic losses. To control the disease on a farm or regionally, it is advisable to start with an initial assessment of the disease's epidemiology and risk factors.

## 1. Introduction

*Neospora caninum* (*N. caninum*) is an obligate apicomplexan parasite (Dubey *et al.,* 2007). A wide range of animals are hosts for this parasite, but the main definitive and intermediate hosts are dogs and cattle, respectively (Dubey and Schares, 2011). Transplacental transmission of *N. caninum* is the primary route of infection spread (up to 95%) in animals; however, horizontal transmission of the infection via the ingestion of oocysts has an important role in causing the disease and stormy abortions (Gharekhani and Yakhchali, 2019, 2020; Gharekhani *et al.,* 2021a). Dogs are the main hosts that have a significant impact on the life cycle of this parasite (Gharekhani *et al.,* 2019; Gharekhani *et al.,* 2020a).

Abortion and neuromuscular disorders are the top clinical manifestations in cattle and dogs, respectively (Gharekhani, 2014; Heidari *et al.,* 2014; Gharekhani *et al.,* 2013a; Gharekhani and Heidari, 2014a). Congenital infections in sheep and goats cause lambs to be born with encephalitis (Gharekhani *et al.,* 2013b, 2016, 2018). *Neospora hughesi* is a species closely related to *N. caninum,* which causes rapid-onset nervous system impairment and paralysis in horses, commonly referred to as Equine Protozoal Myeloencephalitis (EPM) (Gharekhani *et al.,* 2013c). Regarding human neosporosis, studies have shown that there are varying levels of anti-*N. caninum* antibody titers in patients with AIDS and neurological symptoms. This dis-

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ease has not yet been confirmed in humans and requires further investigations (Gharekhani *et al.,* 2021b).

Neosporosis significantly affects the economy, mainly due to abortion (Gharekhani, 2014). The yearly estimated costs vary from a median of US$1.119 million within New Zealand's beef sector to around US$546.3 million in US dairy farms. In just these two nations, the total annual costs surpass US$1 billion (Richel *et al.,* 2013). Different laboratory methods, such as immuno-serology, pathology, bioassay, cell culture and various molecular biology, are used for detecting the infection (Gharekhani *et al.,* 2014, 2021a, 2022, 2023). A precise diagnosis of abortions linked to *Neospora*-infection is attained by evaluating the timing of the abortion, conducting histopathological examinations, employing different laboratory techniques, and eliminating other potential causes of abortion (Gharekhani *et al.,* 2012; Gharekhani and Tavoosidana, 2013; Gharekhani and Heidari, 2014b).

The control of *N. caninum* infection/neosporosis in farms is divided into two main categories. The programs aim to reduce infection in final hosts to limit the horizontal transmission of the parasite, as well as methods to decrease infection in intermediate hosts, prevent vertical transmission, and reduce the economic losses. Vaccination is also a control measure for both groups of hosts (Gharekhani and Yakhchali, 2022).

## 2. Epidemiology in Iran and other Countries

Neosporosis is a highly prevalent disease worldwide (Dubey *et al.,* 2007; Dubey and Schares, 2011; Gharekhani *et al.,* 2020a). There have been varying accounts of *N. caninum* infection from different hosts (Dubey *et al.,* 2007; Dubey and Schares, 2011). In a comprehensive evaluation (Anvari *et al.,* 2020), the pooled prevalence of *N. caninum* in dogs was 17.1%. Moreover, the infection rates were found to be 15.8% for males and 15.1% for females. Regarding different locations, the infection rate was 26.5% in Africa, 21.3% in Australia, 19% in Asia, 17.7% in Europe, and 15% in America. In a similar study, the pooled prevalence in the cattle population has been reported to be 20% (America 20%, Asia 18%, Europe 15%, Africa 13% and Australia 8%). The infection rate was higher in dairy cattle than in beef cattle and also in cows with a history of abortion 1.6 times higher than in others (Ribeiro *et al.,* 2019).

For the first time in Iran, *N. caninum* infection was reported in dairy cattle from Mashhad (Sadrebazzaz *et al.,* 2004). Reports of *Neospora* infection in various hosts, especially cattle and dogs, have been reported in Iran (Gharekhani *et al.,* 2020a). In a meta-analysis study, the seroprevalence of *N. caninum* in cattle was reported to be 23.6%, and a statistically significant association was detected between the infection rate and history of abortion. There was no statistically significant difference among infection, age, and breed (Ansari-Lari, 2020). In a recent report on infection from Iran, this rate was estimated to range from 3.8% to 76.2% in cattle, zero to 54.6% in dogs, 0.9% to 9.9% in sheep, 6.2% to 10.8% in goats, 19.2% to 55.9% in buffaloes, 20% to 2.42% in horses, 52% in donkeys, 3.2% to 27% in camels, 14% to 19% in cats, zero to 20.4% in rodents, 17.3% in chickens, 9.8% to 30.4% in pigeons, 2.8% to 3.7% in sparrows, and 9.9% in crows (Gharekhani *et al.,* 2020a). All studies conducted in Iran are summarized in Tables 1-5.

## 3. Clinical and Pathological Findings

Neuromuscular disorders are dominant in dogs. Motor nerve paralysis and muscle inflammation cause progressive muscle contraction and fibrosis, as well as joint stiffness (Gharekhani *et al.,* 2013a, 2019, 2020b). In addition, diarrhea, multifocal pulmonary consolidation (necrotic and purulent bronchopneumonia), fibrinohemorrhagic enteritis, myocarditis, and nonpurulent encephalitis are common (Dubey and Schares, 2011; Silva and Machado, 2016). In pregnant dogs that are chronically infected, bradyzoites switch back to tachyzoites and cross the placenta, causing infection of the fetus. finally, animals die due to CNS and muscular weakness (Silva and Machado, 2016).

Abortion is the most significant symptom in pregnant cows. The majority of abortions caused by *N. caninum* happen during the 5–6-month period of gestation. In the endemic abortions, about 3 to 5% of the herd aborts annually without any sudden increase, whereas in epidemic outbreaks, more than 12.5% of pregnant animals abort within 2 months (Dubey *et al.,* 2017). Exophthalmia, asymmetrical eyes, CNS disorders such as hydrocephalus, and spinal cord stenosis are rare symptoms (Bowman, 2015). The placental inflammation, focal non-suppurative encephalitis, non-suppurative myocarditis, myositis, and extensive infiltration of non-suppurative inflammatory cells into other tissues are common (Dubey *et al.,* 2017). Cysts containing bradyzoites form mainly in brain tissue. Multifocal necrosis and non-suppurative encephalomyelitis are the main lesions in aborted fetuses. The parasite invades the placental blood vessels, causing placental inflammation and vasculitis in the fetus; extensive placental necrosis is also observed after chorioallantoic degeneration (Sasani *et al.,* 2013).

## 4. Molecular Biology and Pathogenesis

The surface proteins of *N. caninum* play a crucial role in its survival, as they initiate the interactions between the pathogen and the surface molecules of host cells, as well as the host's immune response. The surface of this parasite is coated with a family of glycosylphosphatidylinositol (GPI)-linked proteins (SRSs). Attachment of the parasite to host cells and interaction with the host immune response are mediated by SRS proteins to regulate the virulence of the parasite.

**Table 1**
Frequency of *Neospora caninum* infection in cattle in different regions of Iran.

| Region | Sample type | Diagnostic method | No. of sample | Positive% | References |
|---|---|---|---|---|---|
| | blood | ELISA | 200 | 19 | (Razmi *et al.*, 2013) |
| | Brain of aborted fetus | PCR | 200 | 11.5 | (Razmi *et al.*, 2013) |
| | Blood | ELISA | 116 | 24.3 | (Mikhchi *et al.*, 2013) |
| Neishabour | blood | ELISA | 100 | 26 | (Nourollahifard et al., 2017) |
| Ahvaz | blood | ELISA | 557 | 21 | (Hajikolaei et al., 2008) |
| Golestan province | blood | ELISA | 800 | 13.4 | (Sattari et al., 2011) |
| Babol | blood | ELISA | 237 | 32 | (Youssefi et al., 2009) |
| Garmsar | blood | ELISA | 104 | 38.5 | (Ranjbarbahadori et al., 2010) |
| Arak | Brain of aborted fetus | PCR | 38 | 26.3 | (Khani *et al.*, 2018) |
| Tehran | blood | ELISA | 768 | 38.8 | (Salehi et al., 2010) |
| Kerman | blood | ELISA | 285 | 12.6 | (Nourollahifard et al., 2008) |
| Tabriz | blood | ELISA | 236 | 17.8 | (Gharedaghi, 2012a) |
| | blood | ELISA | 76 | 18.4 | (Nematollahi et al., 2013) |
| Shiraz | blood | ELISA | 253 | 30.4 | (Ansari Lari et al., 2017) |
| | blood | ELISA | 180 | 32.1 | (Tavanaee and Namavari, 2017) |
| Isfahan | blood | ELISA | 611 | 32.1 | (Morovati and Noaman, 2016) |
| | blood | ELISA | 1500 | 26.3 | (Hosseininejad et al., 2017) |
| | blood | ELISA | 216 | 19 | (Noaman and Nabinejad, 2020) |
| Lorestan | blood | ELISA | 347 | 9.8 | (Nayebzadeh *et al.*, 2015) |
| Shahrekord | Brain of aborted fetus | PCR | 100 | 11 | (Rafati and Jafarian, 2014) |
| | milk | PCR | 100 | 24 | (Alipour *et al.*, 2018) |
| Hamedan | blood | ELISA | 400 | 20 | (Gharekhani *et al.*, 2012) |
| | blood | ELISA | 1406 | 17.4 | (Gharekhani *et al.*, 2013a) |
| Sanandaj | blood | ELISA | 336 | 17.6 | (Adhami *et al.*, 2019) |
| Hamedan and Sanandaj | blood | ELISA | 768 | 14.2 | (Gharekhani and Heidari, 2014a) |
| Kurdistan | blood | ELISA | 368 | 7.8 | (Heidari *et al.*, 2014) |
| Sistan | blood | ELISA | 184 | 3.8 | (Noori *et al.*, 2019) |

ELISA: enzyme-linked immunosorbent assay; PCR: polymerase chain reaction.

**Table 2**
Frequency of *Neospora caninum* infection in dogs in different regions of Iran.

| Animals type | Region | Sample type | Diagnostic method | No. of sample | Positive% | References |
|---|---|---|---|---|---|---|
| Stray dogs | Tehran | blood | ELISA | 42 | 2.2 | (Pouramini et al., 2017) |
| | Urmia | blood | IFAT | 135 | 26.6 | (Yakhchali et al., 2010) |
| | Tabriz | blood | IFAT | 100 | 31 | (Gharedaghi, 2012b) |
| | Hamedan | blood | IFAT | 200 | 52.8 | (Gharekhani et al., 2013a) |
| | | blood | ELISA | 180 | 5 | (Gharekhani *et al.*, 2019) |
| Pet dogs | Tehran | Stool | IFAT | 50 | 20 | (Malmasi et al., 2007) |
| | Mashhad | Stool | PCR | 85 | 0 | (Razmi, 2009) |
| | Hamedan | blood | ELISA | 184 | 4.9 | (Gharekhani *et al.*, 2020b) |
| Farm dogs | Tehran | blood | IFAT | 50 | 28 | (Haddadzadeh et al., 2007) |
| | Mashhad | Stool | PCR | 89 | 2.2 | (Razmi, 2009) |
| | Lorestan | Stool | PCR | 428 | 2.1 | (Dalimi et al., 2014) |

ELISA: enzyme-linked immunosorbent assay; IFAT: indirect immunofluorescence antibody test; PCR: polymerase chain reaction.

**Table 3**
Frequency of *Neospora caninum* infection in sheep and goats in different regions of Iran.

| Animals type | Region | Sample type | Diagnostic method | No. of sample | Positive% | References |
|---|---|---|---|---|---|---|
| Sheep | Lorestan | blood | ELISA | 586 | 1.5 | (Ezatpour et al., 2013) |
| | Tabriz | brain tissue | PCR | 70 | 8.5 | (Asadpour et al., 2013) |
| | Tehran | brain tissue | PCR | 330 | 3.9 | (Arbabi et al., 2016) |
| | Hamedan | blood | ELISA | 358 | 2.2 | (Gharekhani *et al.,* 2013b) |
| | Mashhad | brain tissue | PCR | 71 | 9.9 | (Razmi and Naseri, 2017) |
| | Khuzestan | blood | ELISA | 550 | 6.8 | (Gharekhani *et al.,* 2018) |
| Goat | Hamedan | blood | ELISA | 450 | 6.2 | (Gharekhani *et al.*, 2016) |
| | Khuzestan | blood | ELISA | 158 | 10.8 | (Gharekhani *et al.,* 2018) |

ELISA: enzyme-linked immunosorbent assay; PCR: polymerase chain reaction.

**Table 4**
Frequency of *Neospora caninum* infection in cats, camels, buffaloes, equids, and rodents in different regions of Iran.

| Animals type | Region | Sample type | Diagnostic method | No. of sample | Positive% | References |
|---|---|---|---|---|---|---|
| Cat | Ahvaz | blood | DAT | 100 | 14 | (Hamidinejat *et al.,* 2011b) |
| Rodents | Ahvaz | blood | DAT | 150 | 6 | (Mosallanejad *et al.,* 2018b) |
| | Meshkin Shahr | blood | IFAT | 157 | 20.4 | (Nazari *et al.*, 2020) |
| Camel | Mashhad | blood | IFAT | 120 | 5.8 | (Sadrebazzaz et al., 2006) |
| | Esfahan | blood | IFAT | 310 | 3.2 | (Hosseininejad et al., 2009) |
| | Yazd | blood | DAT | 254 | 3.9 | (Hamidinejat et al., 2013) |
| Buffaloe | Ahvaz | blood | ELISA | 188 | 55.9 | (Pourmahdi-Borujeni *et al.*, 2015) |
| | Urmia | blood | ELISA | 83 | 19.2 | (Rezvan et *al.*, 2019) |
| Horse | Tabriz | blood | DAT | 100 | 28 | (Gharedaghi, 2011) |
| | Shiraz | blood | DAT | 200 | 40 | (Moraveji et al., 2011) |
| | Mashhad | blood | DAT | 150 | 30 | (Hosseini *et al.*, 2011) |
| | Hamedan | blood | DAT | 120 | 40.8 | (Gharekhani et al., 2013c) |
| | Ahvaz | blood | DAT | 235 | 20 | (Tavalla *et al.,* 2015) |
| Donkey | Hamedan | blood | DAT | 100 | 52 | (Gharekhani et al., 2013c) |

DAT: direct agglutination test; IFAT: indirect immunofluorescence antibody test; ELISA: enzyme-linked immunosorbent assay.

**Table 5**
Frequency of *Neospora caninum* infection in birds in different regions of Iran.

| Birds type | Region | Sample type | Diagnostic method | No. of sample | Positive% | References |
|---|---|---|---|---|---|---|
| Chicken[a] | Shiraz | blood | DAT | 150 | 17.3 | (Sayari et al., 2016) |
| Pigeon[b] | Ahvaz | brain tissue | PCR | 102 | 9.8 | (Bahrami *et al.,* 2016) |
| Sparrow[c] | Ahvaz | brain tissue | PCR | 210 | 2.8 | (Bahrami *et al.,* 2015) |
| | Tehran | brain tissue | PCR | 217 | 3.7 | (Abdoli *et al.,* 2015) |
| Crow[d] | Tehran | brain tissue | PCR | 55 | 9.9 | (Abdoli *et al.,* 2018) |

*a=Gallus domesticus; b=Columba livia; c=Passer domesticus; d=Corvus cornix*
DAT: direct agglutination test; PCR: polymerase chain reaction.

Proteins linked to the surface, micronemes, rhoptries, and dense granules play crucial roles during the intracellular lytic cycle of *N. caninum.* Due to their role in host-parasite dissemination, these elements could constitute potential drug targets and vaccine candidates (Soltani *et al.,* 2013). There are biological differences in some strains. Nc-Liverpool has a high pathogenicity and causes fetal death in pregnant cows. However, Nc-Nowra is used for attenuated vaccines due to its low virulence. Nc-1 causes very severe clinical signs such as fetal death in pregnant cows, as well as granulomatous polymyositis and polyradiculoneuritis in dogs (Calarco *et al.,* 2018).

The ability of pathogenic protozoa to persist and spread relies on their capacity to escape or undermine the immune responses of their host, both innate and adaptive. Also, hormonal alterations during gestation may trigger reactivation of the parasite (Lopes *et* *al.,* 2012). The transmission rate has a direct connection with gestational age, perhaps related to placental vascularization because the placenta seems to be more permeable in the last trimester (Dubey *et al.,* 2017; Gharekhani and Yakhchali, 2020). The primary placental damage causes the release of maternal prostaglandins that, in turn, cause luteolysis and abortion. Additionally, the development of fetal lesions could result from the proliferation of *N. caninum* in the fetus or due to insufficient oxygen/nutrition (Dubey and Schares, 2011). The balance between the capacity of the tachyzoite to penetrate and multiply inside the cells has a positive impact on the parasite's pathogenesis. The cell-invasion process has two different steps: the adhesion to the host-cell surface and penetration into the cell. Invasion is an active process that demands metabolic energy exclusively from the parasite (Silva *et al.,* 2016). The inhibitor of aspartyl protease in *Neospora* known as pepstatin, plays an important role in assembling and trafficking the MIC and ROP proteins into host cells, and has a significant impact on parasite invasion. (Khan *et al.,* 2020). NC-P43 is one of the most important and largest surface proteins that play a key role in parasite adhesion and invasion into host cells (Dubey *et al.,* 2017). Interferon-gamma (IFN-$\gamma$), IL-12, and Th1 lymphocyte responses play a prominent role in the control of acute *Neospora*-infection. Increased IFN-$\gamma$, by decreasing BCL-2 expression, causes apoptosis of infected cells. Activation of macrophages by IFN-$\gamma$ leads to inhibition and killing of *Neospora* through the production of nitric oxide (Dubey *et al.,* 2017). *N. caninum* causes necrotic lesions and cell death by multiplying tachyzoites, and also causes neuromuscular symptoms in the host by destroying nerve cells. In the CNS lesions, the highest parasite invasion has been observed in astrocytes (Khan *et al.,* 2020).

## 5. Conclusion

Various researchers have frequently detected traces of this infection in different animal species across various regions of the world using multiple diagnostic methods (Dubey *et al.,* 2007). Many risk factors contribute to the occurrence and spread of this infection on farms. Due to their abundance, livestock farms are usually not free of these factors (Gharekhani and Yackchali, 2019). *Neospora* has various mechanisms that promote pathogenicity and tissue lesions in the host, thereby supporting the parasite's survival and transmission cycle (Dubey *et al.,* 2017). Infected animals are usually asymptomatic. Additionally, the disease has been recognized as one of the main causes of economic losses in the livestock industry (Reichel *et al.,* 2013). Understanding the epidemiology of the disease in a region and its transmission methods helps determine the most appropriate strategy for controlling and preventing the parasite (Riberio *et al.,* 2019). Certainly, using multiple methods simultaneously and addressing risk factors will be more effective in managing the disease on a livestock farm or within an area. Managing the disease to reduce infections will increase animal production and offset economic losses. To control the disease on a farm or within a region, it is recommended to begin with an initial assessment of the disease epidemiology and risk factors.

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