Review Article

Neosporosis in Iran; recent evidences and perspectives

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Summary

Neospora caninum is considered as a cyst forming coccidian parasite nearly related to Toxoplasma gondii. Shortly after discovery of N. caninum, neosporosis has identified as a notable infectious disease of both cattle and dogs worldwide, which it frequently leads to clinical infections in warm-blooded animals such as horses, goats, sheep, camels and deer. More importantly, in cattle industry, it is mentioned one of the important causes of abortion in too many countries. Economic losses from N. caninum infection are associated with abortion, stillbirth, neonatal mortality, increased culling and reduced milk yield in cattle industry in the world. Different diagnostic tools can be used for detection of N. caninum infection including histology, polymerase chain reaction and serology. Because of the intimately biologic relationship of N. caninum to Toxoplasma gondii and since non-human primates had been experimentally infected, an issue of concern is that N. caninum might be zoonotic. Previously, some researchers successfully infected two rhesus monkeys (Macaca mulata) with N. caninum experimentally, which reinforces the concern about the zoonotic potential of this disease. In the one last decade, N. caninum has been extensively investigated in Iran. In this sense, the present paper reviews recent knowledge on biology, life cycle, transmission and zoonotic aspects of N. caninum. Attention is also paid to presence of N. caninum infection in the last decade in Iran.

Keywords: Neospora caninum; neosporosis; cattle; dog; Iran

Introduction

In 1984 in Norway was observed an encephalomyelitis and myositis in dogs (Bjerkås et al., 1984) and later in calves with myeloencephalitis (Parish et al., 1987) due to unidentified protozoan parasite which it simulated Toxoplasma gondii but did not respond to the antibodies of T. gondii. The parasite was named and described later as a new discovered genus and species Neospora caninum, which has been ordered in the family Sarcocystidae as a sister group to Toxoplasma...
in the phylum Apicomplexa (Dubey et al., 2007). As results of many studies that had been conducted on *N. caninum* in the past two decades on warm-blooded animals, including many domestic and wildlife species, now it is investigated as a cause of severe canine neuromuscular disease, and neonatal mortality and abortion in cattle, leading to expanding economic losses to the dairy industries (Dubey et al., 2007). Moreover, clinical neosporosis has been known in goats, sheep, white-tailed deer, rhinoceros, water buffaloes, llamas, alpacas, and horses. *N. caninum* antibodies have also been demonstrated in the serum samples of raccoons, camels, pigs, horses, foxes, coyotes, and felids (Dubey et al., 2002). Previous studies proposed that neosporosis had been presented as a critical disease of dogs and cattle worldwide (Dubey et al., 2007). For this reason, in the last decade, much research has been performed on *N. caninum* because of its emphasis as a veterinary pathogen in Iran like many countries. Therefore, the present review describes the present knowledge on the biology, life cycle, transmission, and zoonotic aspects of *N. caninum* and, with especial attention, summarizes the studies of presence specific antibodies, DNA detection, species affected, and its geographical distribution in the last decade in Iran.

**Life cycle, biology and transmission of *N. caninum***

Among various hosts of *N. caninum* as previously mentioned, dogs are assessed as both the intermediate and definitive host for this parasite (Dubey et al., 2007). Previously, grey wolves (*Canis lupus*) were also affirmed to be natural definitive hosts of *N. caninum* by shedding of lasting *N. caninum* oocysts in their feces (Dubey et al., 2011). Besides, coyotes (*Canis latrans*) (Gondim et al., 2004) and Australian dingoes (*Canis lupus dingo*) (King et al., 2010), and grey wolves (*Canis lupus lupus*) (Dubey et al., 2011) have also been experimentally recognized as definitive hosts of *N. caninum*. Cattle are interestingly the most prevalent intermediate (middle) host of *N. caninum*; however, a large number of other warm-blooded animals may act as intermediate hosts (fig. 1). Importantly, the presence of birds on dairy farms mentioned as a notable risk factor for this infection and has been related to spread of abortion (Donahoe et al., 2015). Indeed, it has been indicated that chickens may be an admissible intermediate host for *N. caninum* since parasite DNA was revealed in tissue specimens of outdoor birds (Costa et al., 2008). Recent documents demonstrated high prevalence of *N. caninum* infection in pigeons and also in free ranging chickens in Iran and thereupon it seems that soil contamination because of the shedding *N.
caninum oocysts, since the birds feed from the ground, and determined that the meat from these birds can be a main source for this infection in dogs (Sayari et al., 2014; Bahrami et al., 2016). Moreover, several studies demonstrated susceptibility of different embryonated eggs of domestic birds to N. caninum infection and for this reason, at present, these extensively use for experimental studies on N. caninum (Furuta et al., 2007; Namavari et al., 2011; Mansourian et al., 2015). Tissues of infected animals or feed and water contaminated by these oocysts can infect the intermediate hosts (Donahoe et al., 2015). While definitive hosts become infected by ingesting contaminated tissues of intermediate hosts and can shed oocysts via their faeces (Donahoe et al., 2015; Dubey et al., 2007).

Tachyzoites, bradyzoites (tissue cysts) and oocysts have been identified as the infective stages of the parasite (Dubey et al., 2007). All three mentioned infectious stages are implicated in the transmission of the parasite. Tissue cysts and tachyzoites are asexual stages of the parasite, which found in different cell types and organs of infected hosts (intermediate and definitive host), frequently in the spinal cord and brain (Dubey et al., 2007).

Tachyzoites had also been indicated in the placenta of pregnant cattle. Those are lunate-shaped, show a central nucleus without amylopectin granules and measure approximately 2×6 µm. They propagate rapidly within cells and can contaminate various cell types, such as neural cells, myocytes, renal cells, vascular endothelial cells, dust cells, hepatocytes, and placental trophoblasts (Dubey et al., 2007). Tissue cysts can differ substantially in size, belong to the number of bradyzoites within them. Tissue cysts were found in dogs up to 4 µm thick with a cyst wall up to 107 µm in diameter (Dubey et al., 2007). Bradyzoites replicate slowly (unlike tachyzoites) encysted stages of the parasite, which are slender, have a terminally placed nucleus, and measure approximately 6.5×1.5 µm, and possess a few amylopectin granules, which react with the periodic acid Schiff (PAS) and stain red (Dubey et al., 2004). Dogs as definitive hosts excrete N. caninum oocysts in the unsporulated form in their faeces, which measure approximately 10×12 µm. After sporulation, each oocyst comprises two sporocysts, each of which includes four sporozoites, exclusively 6.5×2 µm (Dubey et al., 2007).
*N. caninum* can be transmitted horizontally (also termed postnatally or laterally) and also can be transmitted vertically (also termed transplacentally or congenitally). Two forms of vertical transmission were previously indicated: exogenous transplacental transmission and endogenous transplacental transmission (Williams et al., 2009). Horizontal transmission results through eating of tissues contain tachyzoites and/or tissue cysts (bradyzoites) or by consumption of food or drinking water contaminated with sporulated oocysts. While vertical transmission happens when tachyzoites from the dam pass the placenta (Dubey et al., 2007), which preserve spreading in a herd for several years. Exogenous transplacental transmission occurs subsequent eating of sporulated oocysts by ordinary cattle and is related with epizootic abortion storms within a herd (Williams et al., 2009). Endogenous transplacental transmission accompanies recrudescence.
infection in a persistently contaminated cow during pregnancy. Horizontal transmission of neonatal animals after birth is significantly needed to retain infection within a herd. While vertical transmission alone cannot sustain infection within herds, which is suggested the main route of transmission in cattle and other domesticated Bovidae species such as the water buffalo (*Bubalus bubalis*) (Chryssafidis et al., 2011). Domestic dogs and some wild canids, as the only known definitive host of *N. caninum*, become infected by consuming tissues or placenta from infected cattle with *N. caninum* and shed the unsporulated oocysts in their faeces during two weeks after that, and sporulate outside the host within 24 hours (Dubey et al., 2007; Gondim et al., 2004).

**Economic impact**

In general, less is known about economic losses of neosporosis in cattle industry in the world but losses are computed in milliards of dollars. Reported rates of congenital neosporosis differ, with report of 40.7% up to 95% (Reichel et al., 2013). A previous study indicated a congenital infection rate in heifers, in second, third and fourth parity cows 80%, 71%, 67%, 66%, respectively (Dijkstra et al., 2003). It is believed that dairy cattle generally show a higher rate of infection with *N. caninum* than beef cattle (Reichel et al., 2013).

Therefore, the economic losses will link to the direct price and cost of fetuses lost which is variable accordingly to the age and genetic potential of the dam and also the productive potential of the progeny (Dubey et al., 2007). Moreover, the diagnostic methods of neosporosis-associated abortions are tough and costly (Ortega-Mora et al., 2006). Indirect costs additionally involve professional price and costs related with rebreeding, feasible loss of milk production, and renewal costs of aborted cows (Dijkstra et al., 2003). Neosporosis can result of other economic losses, such as stillbirth or birth of weak calves (Trees et al., 1999). Regarding to the lack of clinical neosporosis in calves more than two months of age, to date, there is no clear document of *N. caninum*-related incidence in adult cows (Dubey et al., 2007). In Iran, seroepidemiological reports have shown the high prevalence of neospora infection, especially in dairy cattle (33%, 37%, 46%) (Namavari et al., 2010; Hajikolaei et al., 2007; Razmi et al., 2006), and dogs (Malmasi et al., 2006; Haddadzadeh et al., 2007; Khordadmehr et al., 2012). Also, *N. caninum* infection was detected as a notable causative agent of bovine abortion in dairy farms in Iran (Razmi et al., 2006; Sadreazzaz et al., 2007; Salehi et al., 2009; Nematollahi et al., 2013; Gharekhani and Yakhchali, 2019). As indicated recently, the transplacental transmission rate of neosporosis infection in dairy cattle had
estimated as 52% in Iran (Mashhad area- north east of Iran) (Razmi et al., 2013). Therefore, it seems that there are large direct and indirect economic losses (such as congenital infection, abortion, stillbirth or birth of weak calves, loss of milk production, and substitution costs for culled aborted cows) due to neosporosis to cattle industry in Iran. Although, the economic important of the infection has not been established in Iran yet.

**Zoonotic Aspects of N. caninum**

Until 1988, most of the neosporosis infection had been misdiagnosed as toxoplasmosis (Dubey et al., 2007). Later, major differences were subsequently indicated that investigate the two parasites regarding to their natural host, virulence factors, antigenicity, and pathogenicity (Dubey et al., 2007). Application of comparative genomics and transcriptomic analyses had also been proposed for differential diagnosis of these two similar parasites (Reichel et al., 2013). In comparison between neosporosis and toxoplasmosis, *T. gondii* is known as a main disease of sheep and humans, and not of cattle, but neosporosis is considered as a severe disease in cattle, not of sheep, and to date, there is no strong reports for human infection. Previously, some researchers successfully infected the rhesus monkeys (*Macaca mulata*) with *N. caninum* experimentally (Barr et al., 1994), which reinforces the concern about the zoonotic potential of this disease. However, only low levels of antibodies have been observed (particularly in immunocompromised populations), and neither the parasite nor its DNA were observed in human tissues. Seroprevalences findings of *N. caninum* in humans are summarized in Table 1. Although, these results are not frequently comparable because of various serologic assays and different cut-off values used. Recently, immunoglobulin G antibodies to *N. caninum* was predominantly determined in patients with HIV infection (38%) and patients with neurological disorders (18%), while newborns (5%) and healthy persons (6%) presented lower seropositivity rates. Apparently, seropositivity to *N. caninum* was markedly related with seropositivity to *T. gondii* in both HIV-infected patients and patients with neurological illnesses (Lobato et al., 2006). Older literature reported low level IFAT antibodies in sera from blood donors in California and people (women with repeated abortions and farm workers) in England (6.7% and 0.4%, respectively) (Tranas et al., 1999; Trees and Williams, 2000). Currently, nothing is recognized about the seroprevalences of *N. caninum* in humans in Iran.
Table 1 Worldwide seroprevalence of *N. caninum* in humans.

<table>
<thead>
<tr>
<th>Country</th>
<th>Source of sample</th>
<th>No. tested</th>
<th>Test</th>
<th>% positive</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Korea</td>
<td>Blood donors</td>
<td>172</td>
<td>IFAT</td>
<td>6.7</td>
<td>Nam et al., 1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ELISA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Denmark</td>
<td>Repeated miscarriage</td>
<td>76</td>
<td>IFAT</td>
<td>0</td>
<td>Petersen et al., 1999</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ELISA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>North of Ireland</td>
<td>Blood donors</td>
<td>247</td>
<td>IFAT</td>
<td>8</td>
<td>Graham et al., 1999</td>
</tr>
<tr>
<td>United States</td>
<td>Blood donors</td>
<td>1029</td>
<td>IFAT</td>
<td>6.7</td>
<td>Tranas et al., 1999</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IB</td>
<td>1.55</td>
<td></td>
</tr>
<tr>
<td>United Kingdom</td>
<td>Farm workers and women</td>
<td>500</td>
<td>IFAT</td>
<td>0</td>
<td>Trees et al and Williams, 2000</td>
</tr>
<tr>
<td></td>
<td>with miscarriage</td>
<td></td>
<td></td>
<td></td>
<td>McCann et al., 2008</td>
</tr>
<tr>
<td></td>
<td>Farm workers</td>
<td>518</td>
<td>ELISA</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>General population</td>
<td>3232</td>
<td></td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td>Brazil</td>
<td>AIDS</td>
<td>61</td>
<td>ELISA, IFAT, IB</td>
<td>38</td>
<td>Lobato et al., 2006</td>
</tr>
<tr>
<td></td>
<td>Neurologic disorder</td>
<td>50</td>
<td>ELISA, IFAT, IB</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Newborns</td>
<td>91</td>
<td>ELISA, IFAT, IB</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Egypt</td>
<td>Pregnant women</td>
<td>101</td>
<td>ELISA</td>
<td>7.92</td>
<td>Ibrahim et al., 2009</td>
</tr>
</tbody>
</table>

Seroprevalence, Prevalence, and Isolation Studies of *Neospora caninum* in Iran

In 2006, seroepidemiology of *N. caninum* infection was reported in dairy cattle herds in Iran (Khorasan Province, Mashhad area) using ELISA and interestingly, 46% of the examined animals were seropositive for neosporosis infection (Razmi et al., 2006). They believed that abortion was remarkably associated with seropositivity of cattle. Also, their results indicated that neospora infection is widespread in Iran like as many countries. After that, many studies have been conducted on *N. caninum* to date especially in dairy cattle (because of economic important) and dogs (as a confirmed definitive host) and are summarized in Table 2 and Table 3. Recorded rates of infection vary with observation of 7.8% (Heidari et al., 2014) up to 33.3% (Namavari et al., 2012) in cattle by ELISA. While, infection rates of the dogs are 10.6% (Khanmohammadi et al., 2011) up to 44.4% (Khordadmehr et al., 2012) by IFAT and NAT, respectively. In the most reports, no sex predisposition was detected in the examined animals, but it seems age and living places are the important risk factors for *N. caninum* infections. For example, significant difference was detected regarding infection in industrial (43.9%) and rural cattle (25.8%) (Youssefi et al., 2009). Another study reported that house hold dogs had a lower rate of infection (8.65%) than stray and shepherd dogs (43.35%) (Hosseininejad and Hosseini, 2011). Although, in horse, it was found higher in
riding club samples (42.2%) rather rural samples (40%) (Gharekhani et al., 2013). In 2007, *N. caninum* associated bovine abortion was identified in Iran, which was primarily diagnosed by PCR and then confirmed by histopathology and IHC methods. These Iranian researchers observed a thick-walled (2µm) cyst of *N. caninum* with 50 µm diameter in one of the IHC-positive brain. Therefore, based on their findings, they stated that neosporosis is a main cause of abortion in dairy cattle of Iran (Razmi et al., 2007). Later, *N. caninum* was isolated from an aborted fetus in seropositive cattle, which was determined as Nc-Iran that recorded under the accession number FJ655914 in the GenBank database (Salehi et al., 2012). Recently, Pouramini et al. reported the presence of *N. caninum* in CSF (26.2%), brain (19%), and skeletal muscle (13.42%) of asymptomatic infected stray dogs in Tehran, Iran (Pouramini et al., 2017). Importantly, it was also proposed that close contact to infected farm dogs, carnivores, rodents and poultry could be important risk factors for the occurrence of *N. caninum*-associated abortion in dairy cattle (Gharekhani and Yakhchali, 2019).

Table 2. Seroprevalence, prevalence, and isolation studies of *Neospora caninum* in dogs in Iran.

<table>
<thead>
<tr>
<th>Location (Province)</th>
<th>No. tested</th>
<th>Tissue/source</th>
<th>Test</th>
<th>%positive</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tehran</td>
<td>100</td>
<td>Serum</td>
<td>ELISA</td>
<td>33</td>
<td>Malmasi et al., 2006</td>
</tr>
<tr>
<td>Tehran</td>
<td>103</td>
<td>Serum</td>
<td>IFAT</td>
<td>19.4</td>
<td>Haddadzadeh et al., 2007</td>
</tr>
<tr>
<td>Tehran</td>
<td></td>
<td>CSF Brain Skeletal muscle</td>
<td>PCR</td>
<td>26.2 19 13.42</td>
<td>Pouramini et al., 2017</td>
</tr>
<tr>
<td>Khorasan</td>
<td>174</td>
<td>Feces</td>
<td>PCR</td>
<td>1.1</td>
<td>Razmi, 2009</td>
</tr>
<tr>
<td>Ardebil (Meshkin-Shahr)</td>
<td>171</td>
<td>Serum</td>
<td>ELISA</td>
<td>30.4</td>
<td>Sharifdini et al., 2011</td>
</tr>
<tr>
<td>Isfahan</td>
<td>248</td>
<td>Serum</td>
<td>ELISA</td>
<td>29</td>
<td>Hosseinnejad et al., 2011</td>
</tr>
<tr>
<td>Khuzestan</td>
<td>200</td>
<td>Serum</td>
<td>ELISA</td>
<td></td>
<td></td>
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<tr>
<td>East Azarbaijan (Sarab district)</td>
<td>384</td>
<td>Serum</td>
<td>IFAT</td>
<td>10.6</td>
<td>Khademamadi et al., 2011</td>
</tr>
<tr>
<td>Fars</td>
<td>180</td>
<td>Serum</td>
<td>ELISA</td>
<td>54.62</td>
<td>Khordadmeh et al., 2012</td>
</tr>
<tr>
<td>Lorestan</td>
<td>428</td>
<td>Feces</td>
<td>PCR</td>
<td>2.1</td>
<td>Dalimi et al., 2014</td>
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<tr>
<td>Hamedan</td>
<td>270</td>
<td>Serum</td>
<td>IFAT</td>
<td>27</td>
<td>Gharekhani et al., 2014</td>
</tr>
<tr>
<td></td>
<td>185</td>
<td>Serum</td>
<td>ELISA</td>
<td>8.65</td>
<td>Gharekhani and Yakhchali, 2019</td>
</tr>
</tbody>
</table>
### Table 3. Seroprevalence, prevalence, and isolation studies of *Neospora caninum* in different intermediate hosts in Iran.

<table>
<thead>
<tr>
<th>Host</th>
<th>Location</th>
<th>No. tested</th>
<th>Tissue/source</th>
<th>Test</th>
<th>% positive</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>Khorasan</td>
<td>337</td>
<td>Serum</td>
<td>ELISA</td>
<td>46</td>
<td>Razmi et al., 2006</td>
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<td></td>
<td>Khorasan</td>
<td>100</td>
<td>Brain of aborted fetuses</td>
<td>PCR</td>
<td>13</td>
<td>Razmi et al., 2007</td>
</tr>
<tr>
<td></td>
<td>Khorasan</td>
<td>12</td>
<td>Brain of aborted fetuses</td>
<td>PCR</td>
<td>33</td>
<td>Sadrebazzaz et al., 2007</td>
</tr>
<tr>
<td></td>
<td>Khorasan</td>
<td>151</td>
<td>Brain of aborted fetuses</td>
<td>PCR</td>
<td>11.9</td>
<td>Razmi et al., 2010</td>
</tr>
<tr>
<td></td>
<td>Tehran</td>
<td>12</td>
<td>Brain of aborted fetuses</td>
<td>PCR</td>
<td>100</td>
<td>Salehi et al., 2009</td>
</tr>
<tr>
<td></td>
<td>Tehran</td>
<td>7</td>
<td>Brain of aborted fetuses</td>
<td>PCR</td>
<td>71.4</td>
<td>Salehi et al., 2009</td>
</tr>
<tr>
<td></td>
<td>Kerman</td>
<td>285</td>
<td>Serum</td>
<td>ELISA</td>
<td>12.6</td>
<td>Nourollahi-Fard et al., 2008</td>
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<td></td>
<td>Mazandaran</td>
<td>237</td>
<td>Serum</td>
<td>ELISA</td>
<td>32</td>
<td>Razmi et al., 2007</td>
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<td></td>
<td>Fars</td>
<td>135</td>
<td>Serum</td>
<td>ELISA</td>
<td>33.3</td>
<td>Namavari et al., 2012</td>
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<td>East Azarbaijan</td>
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<td>ELISA</td>
<td>10.5</td>
<td>Nematollahi et al., 2011</td>
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<td>Serum</td>
<td>ELISA</td>
<td>18.4</td>
<td>Nematollahi et al., 2013</td>
</tr>
<tr>
<td></td>
<td>East Azarbaijan</td>
<td>14</td>
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<td>PCR</td>
<td>42.8</td>
<td>Nematollahi et al., 2013</td>
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<td></td>
<td>Hamedan</td>
<td>1046</td>
<td>Serum</td>
<td>ELISA</td>
<td>17.4</td>
<td>Gharekhani et al., 2014 and Yakhchali, 2019</td>
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<td>ELISA</td>
<td>24.8</td>
<td>Gharekhani et al., 2014 and Yakhchali, 2019</td>
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<td>Kurdistan</td>
<td>368</td>
<td>Serum</td>
<td>ELISA</td>
<td>7.8</td>
<td>Heidari et al., 2014</td>
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<tr>
<td></td>
<td>Various regions</td>
<td>395</td>
<td>Brain of aborted fetuses</td>
<td>PCR</td>
<td>45%</td>
<td>Kamali et al., 2014</td>
</tr>
<tr>
<td></td>
<td>Various regions</td>
<td>175</td>
<td>Semen</td>
<td>PCR</td>
<td>17.14</td>
<td>Shariatzadeh et al., 2012</td>
</tr>
<tr>
<td></td>
<td>Various regions</td>
<td>57</td>
<td>Semen</td>
<td>PCR</td>
<td>10.53</td>
<td>Doosti et al., 2015</td>
</tr>
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<td>Neishabour</td>
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<td>Serum</td>
<td>ELISA</td>
<td>26</td>
<td>Nematollahi-Fard et al., 2017</td>
</tr>
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<td></td>
<td>Sistan</td>
<td>184</td>
<td>Serum</td>
<td>ELISA</td>
<td>3.8</td>
<td>Noori et al., 2019</td>
</tr>
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<td>Water buffalo (<em>Babalus bubalis</em>)</td>
<td>Khuzestan</td>
<td>181</td>
<td>Serum</td>
<td>ELISA</td>
<td>37</td>
<td>Hajikolaei et al., 2007</td>
</tr>
<tr>
<td></td>
<td>West Azarbaijan</td>
<td>83</td>
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Diagnostic assays for identification of *N. caninum* and neosporosis infection

Many diagnostic tools had been used with different rating of consequences for tracing of *N. caninum* and its infection in domestic animals, wildlife species, birds and humans (Dubey et al., 2011).

1) **Serological techniques**

Most of the research had employed serological techniques, which are beneficial diagnostic methods to identified animals for presence of *N. caninum* disposal. In this regard, it was stated that serum samples of inspected cases are the most commonly used specimens for identification of *N. caninum* antibodies in adult animals. Mostly, because of high antibody levels in aborted cows with neospora-infected fetuses (Wouda et al., 1998), identification of antibodies to neospora in the serum of acute abortion cases, particularly when sera taken within two weeks of the abortion, can be a noteworthy diagnostic tool. Moreover, evaluation of fetal serum or fetal body fluid (especially peritoneal fluid) for neospora antibodies can assist in diagnosing the infection in five months and older fetuses (Wouda et al., 1998). Serological test may be used on newborn calves before feeding colostrum to identify whether they are congenitally infected. Evaluation of dams and their offspring seroprevalences are useful to compute the frequency of transplacental transmission of infection (Dubey et al., 2007). In an abortion storm, taking blood samples immediately of all animals can be advisable for detection of endemic infection. Since, most abortions arise several weeks behind an acute infection, it is more helpful when the paired serology samples are taken at abortion and also three weeks later (Wouda et al., 1998). Also, individual and bulk milk samples of dairy cows can be applied as further samples for either screening or diagnosis of the infection (Varcasia et al., 2006).
Some serologic assays can be used to identified *N. caninum* antibodies, such as various types of ELISAs, IFAT (indirect fluorescent antibody test), NAT (neospora agglutination test), and immunoblot (IB) is useful for detecting *N. caninum*-specific antigen/antibody with a high sensitivity and specificity. Besides, IgG avidity ELISAs had been considered to differ between chronic and acute neosporosis (Björkman et al., 2005). It is believed that the seroprevalence results are not analogous between different studies because of the use of various techniques, variation in research object, methodology, sample size, samples source, and data commentary (Dubey et al., 2011). In serological examinations, titer and absorbance values are dependent on some factors, such as antigen composition, secondary antibodies, and other using reagents. Additionally, cut-off levels may be indiscriminately designated to assign sensitivity and specificity demanded for a special application (Dubey et al., 2007).

In the recent years, numerous determination of *N. caninum* seroprevalence have been performed in domestic animals and birds in Iran (Table 2, 3). In these studies, the most frequent used serological methods were ELISA and NAT. Recently, a disperse dye immunoassay method (DDIA) was provided and evaluated for rapid detection of antibodies against *N. caninum* in cattle and no marked differences were found between DDIA and ELISA, which provides an economic, modest, rapid, and authentic test for diagnosis of infection in cattle (Selahi et al., 2013). Moreover, Iranian researchers developed an indirect ELISA assay using *N. caninum* surface antigen (P38) for the sensitive and specific detection of infection in dog colonies. Their findings demonstrated that a favorable sensitivity (100%) and specificity (97.9%) were assessed for SI, cut-off point of 0.23 (Hosseininejad et al., 2010). Also, it was shown that two protein bands with 45 and 41 kDa molecular weight are the most important antigens investigated in Western blotting, in seropositive aborted cows (Nematollahi et al., 2010). Another research also indicated that the LAT with recombinant *N. caninum* surface antigen 1 (rNcSAG1) might be a rapid, easy, relatively inexpensive, and adequate diagnostic test for detection of specific antibodies under field conditions (Moraveji et al., 2012). In addition, they believed that sanitation of rNcSAG1 purification may decrease possible false positive results and so enhance the agreement rating between the LAT and ELISA. The results of another study after cloning and expression of *N. caninum* dense granule protein 7 (NcGRA7) in *E. coli* approved that recombinant NcGRA7 with pMAL-c2X vector might be appropriate for expanding of diagnostic procedures (Kefayat et al., 2012). New literature demonstrated that NcGRA7-based ELISA suggesting utilized a
novel fragment of genomic DNA is a suitable tool for epidemiological and screening purposes on cattle and water buffaloes herds (Hamidinejat et al., 2015).

2) Polymerase chain reaction (PCR)

It is presented as a reliable sensitive and specific laboratory technique for identification of *N. caninum* DNA in a variety of tissues from aborted bovine fetuses, such as liver, heart, spinal cord, brain, placentas, and amniotic fluid of infected cattle (Salehi et al., 2009; Nematollahi et al., 2013; Salehi et al., 2012). Moreover, PCR had been used to determined oocysts in faeces of dogs (Gondim et al., 2004; Razmi, 2009; Dalimi et al., 2014). *N. caninum* DNA can be surprisingly identified through PCR in formalin fixed and paraffin-embedded aborted brain tissue (Dubey et al., 2011). The Nc5 gene and ITS1 region (the internal transcribed spacer 1) of the rRNA gene of the parasite are the most frequent markers used for common PCR-based *N. caninum* finding (Dubey et al., 2011). Recently, quantitative PCR (qPCR) is used in academic research on *N. caninum* (Collantes-Fernandez et al., 2009), which has greater sensitivity and authorizes both discovery and quantitative assessment of the parasite in biological specimens in comparison with conventional and nested PCR.

3) Histopathological examinations and immunohistochemistry (IHC)

Histopathologic evaluation of the bovine aborted fetus is essential for a deterministic diagnosis and fetal brain is the most systematically affected organs. Since most aborted fetuses might be autolyzed at the time of sampling, even autolyzed semi-liquid brain tissue could be fixed in buffered neutral formalin for histopathologic diagnosis of H&E (hematoxylin and eosin) stained sections (Dubey et al., 2007). The most important feature of brain lesion is focal non-suppurative encephalitis associated with liquefactive necrosis (Nematollahi et al., 2013). Recent study reported the lesions of the brains and spinal cords of aborted fetuses of dairy cattle which included severe congestion, perivascular and perineuronal edema, status spongiosis, perivascular cuffing, focal gliosis, neurophagy, and focal necrosis (Nematollahi et al., 2013; Kamali et al., 2014). In most aborted fetus extensive cellular infiltrations and focal necrosis are also found in the heart, liver, and skeletal muscle. Moreover, lesions can be observed in the placenta, which are of little diagnostic value (Dubey et al., 2011). In placentas, severe congestion, vascular thrombosis, perivascular infiltration of mononuclear cells, focal placentitis, and necrotic foci in cotyledons were observed recently (Nematollahi et al., 2013). To date, most researchers believe that histopathology remains an extremely valuable diagnostic tool, and the sensitivity and specificity of fetal
histopathology is high. However, immunohistochemistry (IHC) is required because there are generally a few parasites present in autolyzed tissues that frequently not visible in common H&E stained sections. So, demonstration of *N. caninum* by immunohistochemical method in tissue lesions is the best deposition for etiology of abortion presently. A preference of IHC is the presence of the parasites can be linked to the lesions; but, this method is effortful and proportionally insensitive. However, a recent experimental study indicated a reliable compromise between PCR and IHC in discrimination of neospora antigen in the affected tissues (Khodakaram-Tafti et al., 2012).

**Novel Experimental Studies on *N. caninum* in Iran**
Confirmed recognition of intermediate host species to neospora infection implicates isolation of viable parasites via bioassays in cell culture and/or animal models. In recent years, some interesting studies have been carried out by Iranian researchers on cell culture and animal models for isolation of lasting parasites.

![Fig. 2. Diagnostic assays for identification of *N. caninum* and neosporosis infection.](image)

1) *N. caninum and cell culture*
Up to now, numerous host cells have been hopefully suggested for the laboratory preservation, multiplication and passage of *N. caninum* tachyzoites, such as Vero cell (Cadore et al., 2009), bovine mononuclear cell (Tuo et al., 2005), cat and dog fibroblast cell (Lei et al., 2005), cat kidney cell (Lei et al., 2005), rat astrocytes (Pinheiro et al., 2006), human cancer cell lines such as MCF-7 (Lv et
al. 2010), trophoblastic (BeWo) and uterine cervical (HeLa) cells (Carvalho et al. 2010). Among these, the Vero cell line is the most commonly used for the propagation of the parasite in vitro in an attachment surface. Previous data described that MA-104 (African green monkey kidney epithelial-like cell) and SW742 (human colorectal epithelial-like cell) cells display convenience susceptibility to N. caninum in comparison with Vero cells (Khordadmehr et al. 2014). Moreover, it has been stated that Theileria lestoquardi and Theileria annulata infected lymphoblastoid cell lines as suspension cell culture are liable to Nc-1 tachyzoites and could be used as a suitable host cell line for tachyzoites culture in vitro conditions (Khordadmehr et al., 2014; Kargar et al., 2013; Khordadmehr et al., 2012b). Interestingly, it has been reported that the culture of tachyzoites in J774 cell resulted in a significant increase in the number of multiplicated tachyzoites and led to rapid attenuation of tachyzoites in comparison with Vero cell line which can be used as an appropriate in vitro model to produce of live attenuated vaccine. These findings, for the first time, represented the marked impact of host cell on virulence of N. caninum tachyzoites (Khordadmehr et al., 2013).

2) N. caninum and animal models

Previous studies have been described that some species of gerbils (Meriones unguiculatus and Meriones tristrami) and sand rats (Psammoomys ubsesus) are sentient to N. caninum tachyzoites infection (Dubey et al., 2007; Pipano et al., 2002). Mostly, bioassays implementation in these models are expensive associated with regarding ethical considerations and need populations of immunosuppressed species, such as cortisone-treated outbred mice or IFN-γ gene knockout mice (Dubey et al., 2011). In these senses, embryonated eggs have recently been suggested and approved as a laboratory animal model for experimental infection (Furuta et al., 2007; Khodakaram-Tafti et al., 2012; Khordadmehr et al., 2013; Mansourian et al., 2017) and also for assessment of the virulence of N. caninum tachyzoites (Namavari et al., 2011). In a separate study, experimental N. caninum infection was performed in quail, partridge, broiler and laying chicken embryonated eggs. These findings interestingly showed that among various animal models, the lowest LD50 was belonged to the broiler chickens, which suggested the broiler chicken embryonated egg as the best animal model for experimental neosporosis. Surprisingly, partridge is known as the most susceptible bird to N. caninum infection. Also, these results reinforced that there is genetic sensitivity to N. caninum in chickens like mice (Mansourian et al., 2015). Another recent publication suggests that pigeon embryos may be a suitable choice for the biologic studies and acute infection of N. caninum in living
organisms (Bahrami et al., 2016). The results of these studies suggest new insights into application of the inexpensive and available animal models for further N. caninum research.

**Conclusion**

Neosporosis has identified as a notable infectious disease of both cattle and dogs worldwide, which it frequently leads to clinical infections in warm-blooded animals. Because of the intimately biologic relationship of N. caninum to Toxoplasma gondii and since non-human primates had been experimentally infected, an issue of concern is that N. caninum might be zoonotic. On the other hand, it seems that there are large direct and indirect economic losses (such as congenital infection, abortion, stillbirth or birth of weak calves, loss of milk production and substitution costs for culled aborted cows) due to neosporosis to cattle industry in Iran.

**Acknowledgments**

No applicable

**Conflict of interest statement**

There is no conflict of interest.

**Ethical approval**

No applicable

**References**


latrans) are definitive hosts of Neospora caninum. *International Journal of Parasitology*, 34, pp. 159-161.


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