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# *Neospora caninum* infection in dairy farms with history of abortion in West of Iran

Jamal Gharekhani, Mohammad Yakhchali*

*Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Nazlu campus, Sero road, 5756151818, Urmia, Iran*

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ABSTRACT

*Neospora caninum* is a major cause of abortion and economic losses among dairy farms in Iran and other countries. The main goal of current investigation was to evaluate the presence of antibodies against *N. caninum* and associated risk factors in dairy herds with history of abortion in Hamedan province of West Iran. A total numbers of 476 and 185 blood samples of pregnant cattle and farm dogs from 10 dairy farms with history of abortion were randomly collected. Bulk milk sample was taken from each farm. All samples were subjected for detection of IgG antibody against *N. caninum* using ELISA technique. Of all examined animals, 24.8% of cattle and 8.65% of dogs were seropositive to *N. caninum*. The seroprevalence had significant differences with abortion, stillbirth, metritis, breed, close contact to dogs, wild carnivores, rodents, poultry, and pregnancy using artificial insemination method. There were no significant differences among seroprevalence and different age groups, number and stage of gestation, and herd population. In all investigated farms, bulk milk examination was positive. In examined dogs, there was significant difference between seroprevalence and sex. It was concluded that *N. caninum* infection may be responsible for abortion and economic losses in dairy farms of the region. This was also the first comprehensive report on associated risk factors to *N. caninum* infection in dairy farms in the region.

## 1. Introduction

Neosporosis is caused by *Neospora caninum* (Apicomplexa: Sarcocystidae), an intracellular heterogeneous cyst-forming protozoan, is a parasitic disease with global distribution (Dubey, 2003). *Neospora caninum* was reported in puppies with congenital encephalomyelitis from Norway in 1984 at the first time (Bjerkas, Mohn & Presthus, 1984). Domestic dogs (*Canis familiaris*), coyotes (*Canis latrans*), dingoes (*Canis lupus dingo*), and gray wolves (*Canis lupus*) are definitive hosts for *N. caninum* (Dubey, 2003, 2005). A wide-range of herbivore animals are intermediate hosts which causes abortion and economic losses especially in cattle (Dubey, Schares & Ortegamora, 2007).

Neuromuscular neurologic disorders are the most common clinical signs in young infected dogs (Yakhchali, Javadi & Morshedi, 2010). Puppies usually born with no clinical signs; however, they typically show signs progressing toward ascending hind limb paralysis in three weeks after birth (Barber & Trees, 1996). *Neospora caninum* was considered to be transmitted from the dam to the neonates during terminal stages of gestation or postnatal via milk. In contrast, vertical transmission of *N. caninum* in dogs was considered highly variable. Fecal transmission of *N. caninum* in dogs appears to be less important than herbivores (Dubey & Schares, 2011). The rate of congenital transmission of *N. caninum* may reach 93.7%, and this is the most important infection route in bovines (Nicolino, Oliveira & Lopes, 2017). However, definitive hosts play important role in horizontal transmission and in maintaining infection in dairy herds (Dubey et al., 2005; 2007). Cattle neosporosis has association with abortions, neonatal mortality, stillbirth, genitally tract infection, and decreasing of milk production (Guido, Katzer & Nanjiani, 2016; Salehi, Haddadzadeh & Shayan, 2010). Reichel, Ayanegui-Alcérreca, Gondim and Ellis (2013) reported 46.4 million cattle were at annual risk of abortion in different countries. Cattle neosporosis is also a common parasitic infection in livestock causing significant economic losses due to abortion with annual losses of more than US$1.3 billion worldwide (Reichel et al., 2013; Santos, Simões, Mateus & Lopes, 2016). In developing countries like Argentina, Brazil and Australia, it was US$43.6, US$51.3, and US$100 million, respectively (Dubey & Schares, 2011; Dubey et al., 2007). In immunosuppressive humans, different levels of antibodies against *N. caninum* were reported (Oshiro et al., 2015; Robert-Gangneux & Klein, 2009; Tranas, Heinzen, Weiss & McAllister, 1999). *Neospora caninum* has been successfully cultured in human cell lines, but that zoonotic aspect has not been defined yet. Hence infected dogs and cattle may be

* Corresponding author.
*E-mail address:* m.yakhchali@urmia.ac.ir (M. Yakhchali).

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major risk factors for transmission of the infection to humans especially in farm and slaughterhouse workers (McCann, Vyse & Salmon, 2008).

Several laboratory methods, i.e., histopathology, immunology, molecular procedures and bioassay are now available for detection of *N. caninum* infection in animals. Of those, serologic examinations, i.e., enzyme-linked immunosorbent assay (ELISA), indirect fluorescent antibody test (IFAT), direct agglutination test (DAT) and immunoblotting (IB) were proposed to detect of *N. caninum* antibodies. Among serologic methods, ELISA technique is adequately reliable in terms of defining specific antibodies titers to *N. caninum*. Furthermore, owing to its relatively high speed, it has greater applicability for epidemiologic studies (Dubey et al., 2007; Guido et al., 2016). Schares, Barwald and Staubach (2003) reported the accuracy of bulk milk examination is similar to serologic assays. Some commercial ELISA kits are available for detection of specific antibody to *N. caninum* in bovine milk as well as bulk milk. Bulk milk examination can be therefore performed to evaluate antibodies levels to *N. caninum* in dairy farms (Hurkova, Halova & Modry, 2005).

Seroprevalence of *N. caninum* varies from zero to 100% from different animal species throughout the world (Dubey & Schares, 2011). Different seroepizootological data have been reported from Iran, i.e., 1.5–5.7% in sheep, 6.2% in goats, 15.8–46% in cattle, 10.6–33% in dogs, 37% in water buffaloes, 3.2–5.8% in camels, 28–40.8% in horses, and 52% in donkeys (Gharekhani & Tavoosidana, 2013; Gharekhani, Tavoosidana & Naderisefat, 2013; Gharekhani, Tavoosidana & Akbarein, 2014; Gharekhani, Yakhchali, Esmaeilnejad & Rezaei, 2016; Hajikolaei, Goraninejad & Hamidinejat, 2007; Salehi et al., 2010; Sazmand & Joachim, 2017; Yakhchali et al., 2010; Yakhchali, Bahrami & Asri-Rezaei, 2017). However, before our work there was no comprehensive information on risk factors associated with dairy cattle neosporosis and dogs in Iran. Thus, this study was carried out to evaluate the presence of antibodies against *N. caninum* and associated risk factors in dairy herds with history of abortion in Hamedan Province, West part of Iran.

## 2. Materials and methods

### 2.1. Field of study

Hamedan Province with an area of 19,546 km$^2$ is located in west part of Iran (34.77° N and 48.58° E) where is surrounded by mountains. The average annual temperature is 11.3 °C with mild climate and great potential for agriculture and animal husbandry, particularly for dairy cattle rearing. A total number of 66 dairy farms with estimation cattle population of 19,207 distributed over nine subareas in Hamedan province of West Iran (Fig. 1).

### 2.2. Animals and sera collection

In summer 2017, a total of 476 pregnant cattle (10% (476/4760) of population of each examined farm) and 185 farm dogs in 10 dairy farms (named A–J) with history of abortion were randomly selected. The sample size for determining seroprevalence was estimated based on the formula (expected prevalence 20%, level of confidence 95%, and precision 10%) (Gharekhani & Tavoosidana, 2013; Thrusfield, 2005). For each farm, all data (farm location, management system, age, sex, breed, stage of pregnancy, number of gestation, abortion history, stillbirth history, metritis history, rearing service, contact with birds and wild carnivores like coyote and/or fox) were recorded (Tables 1 and 2). Five ml of blood sample was taken from the coccygeal vein of cattle and saphenous vein of dogs. One bulk milk sample out of each examined dairy farm was also collected.

### 2.3. Serological examination

The sera were removed after centrifugation at 1000 × *g* for 10 min and stored at −20 °C until laboratory analysis. The sera and milks were examined to detect anti-IgG against *N. caninum* using ELISA kit (ID Screen® Neosporosis indirect multi-species; ID-Vet, France). According to the instruction, seropositive animals (Sp) were determined calculating of S/P% (Sample to positive ≥50% was positive for serum samples and ≥30% was positive for milk samples).

### 2.4. Statistical analysis

The non-parametric Chi-square ($\chi^2$) test was used to evaluate association of neosporosis with different risk factors (SPSS 16.0, Chicago, IL, USA). A probability score of $P \leq 0.05$ was regarded as significant.

## 3. Results

The seroprevalence of *N. caninum* and associated risk factors in examined dairy farms are shown in Tables 1 and 2. In examined cattle, overall seroprevalence of *N. caninum* was 24.8% (95% CI: ± 3.8%). In all farms, bulk milk examination was positive to *N. caninum*. The seroprevalence had no significant differences in different age groups ($\chi^2 = 0.006$, $p = 0.999$). The seroprevalence had significant differences with history of abortion ($\chi^2 = 6.686$, $p = 0.009$), stillbirth ($\chi^2 = 5.411$, $p = 0.02$), metritis ($\chi^2 = 8.052$, $p = 0.004$), breed ($\chi^2 = 9.930$, $p = 0.001$), and close contact to dogs ($\chi^2 = 14.423$, $p = 0.0001$), wild carnivores ($\chi^2 = 14.235$, $p = 0.0002$), rodents ($\chi^2 = 11.637$, $p = 0.0006$) and poultry ($\chi^2 = 29.542$, $p < 0.0001$). There were no significant association among seroprevalence and stage of gestation ($\chi^2 = 0.046$, $p = 0.977$), number of gestation ($\chi^2 = 0.026$, $p = 0.986$), and herd population ($\chi^2 = 1.812$, $p = 0.178$). Of all Seropositive (Sp) cattle, 33.7% had artificial insemination (AI) in their records ($p < 0.0001$).

Of all examined dogs, 16 (8.65%, 95% CI: ± 4%) were Sp to *N. caninum*. The highest anti-*IgG* to *N. caninum* was detected in dogs over 2 years old (15.7%). There was no significant difference between seroprevalence and different age groups ($\chi^2 = 0.095$, $p = 0.953$). The serum level of IgG against *N. caninum* was significantly higher in male (17.9%) than female dogs (4.7%) ($\chi^2 = 8.619$, $p = 0.003$).

## 4. Discussion

Evaluating on seroprevalence, and hence the exposure of dairy cattle population of *N. caninum* is essential for investigating the possible transmission ways of the parasite as well as identifying populations in which neosporosis may occur. Knowledge on prevalence and risk factors of cattle neosporosis is also an important part of development and implementing of measures to lunch control programs (Gharekhani, Yakhchali, Esmaeilnejad & Rezaei, 2016; Talafha & Al-Majali, 2013). Based on the present study, this was the first report on the seroprevalence of *N. caninum* in pregnant dairy cattle in West part of Iran. This finding confirms the presence of *Neospora* infection and the important role of associated risk factors in the region. The seroprevalence of *N. caninum* infection in examined pregnant dairy cattle was lesser than of 46.3% in North-East, 38.8% in Center and 30.4% in South of Iran (Ansari-Lari, Rowshan & Jesmani, 2017; Razmi, Mohammadi & Garrosi, 2006; Salehi et al., 2010). The differences on seroprevalence in each part of the country may be as a result of various factors like sampling and investigation methods, farms management, food storage, contact with carnivores, geographic conditions, and temperature effect on viability and sporulation of *N. caninum* oocysts. This finding was also similar to the reports in Kenya, Brazil and Slovakia (Dubey et al., 2007; Okumu, John, Wabacha, Tsuma & VanLeeuwen, 2019; Snak, Garcia, Lara, Jesus Pena & Osaki, 2018).

Bulk milk test can be used routinely to evaluation of antibodies to *N. caninum* in dairy farms due to easily on sampling and rapid findings (Hurkova et al., 2005). In current investigation, all of examined bulk milk was positive. While it was 1% in Czech Republic, 7.9% in


**Fig. 1.** Map of distribution of sampled farms (A–I) in different parts in Hamedan province of West Iran (1. Kaboudarahang, 2. Razan, 3. Bahar, 4. Hamedan, 5. Famenin, 6. Asadabad, 7. Toyserkan, 8. Malayer, 9. Nahavand)

Germany, and 46% in Thailand (Chanlun, Naslund & Aiumlamai, 2002; Hurkova et al., 2005; Schares, Bärwald & Staubach, 2004). These differences might be due to climate variations, study design, sample size, methods of detection, experimental strategies, farm management, and different levels of exposure to risk factors (Atkinson, Cook & Reddacliff, 2000; Dubey et al., 2007). In some regions especially in developing countries, congenital transmission of the infection is predominant. Additionally, type of housing, biosecurity status and animals' density in the farms are different (Dubey et al., 2007; Okumu et al., 2019; Snak et al., 2018).

Age influence on seropositivity was wide according to studied regions (Dubey et al., 2007). In our work, the highest Sp detected in cattle over 4 years old. However, the occurrence of *N. caninum* was no significant in different age groups. This finding was in accordance with other reports (Atkinson et al., 2000; Chanlun et al., 2002; Kyaw, Virakul & Muangyai, 2004; Sadrebazzaz, Haddadzadeh & Esmailnia, 2004). According to Jensen, Bjorkman and Kjeldsen (1999), seropositivity increased with age in Danish dairy herds as a result of increasing the possibility of ingestion of oocysts with animals. Sadrebazzaz et al. (2004) and Wouda, Moen and Schukken (1998) reported equal levels of Sp in different examined age groups. The congenital transmission of *N. caninum* infection decreases as a result of increasing the immunity with age (Salehi et al., 2010).

In this work, the highest Sp dairy cattle reported in second stage of gestation (3–6 month).The seroprevalence had no significant association with gestation and herd population. These findings were in parallel with that from Denmark (Jensen et al., 1999). While, Yaniz, Lopez and Garcia (2010) noted that it was different. The risk of *N. caninum* infection may increase with gestation suggesting that horizontal transmission of *N. caninum* was particular importance in some herds (Dubey et al., 2007). Gharekhani, Tavoosidana and Akbarein (2014) reported *N. caninum* infection in dairy farms with >100 population was 2.7-fold higher than <100 population ($p = 0.0005$). Aguiar, Cavalcant and Rodrigues (2006) reported the seroprevalence in

**Table 1**
The seroprevalence of *Neospora caninum* in different dairy farms in Hamedan province of West Iran ($n = 476$).

| Farms | | Bulk tank | Pregnant cattle | | | Dogs | |
|---|---|---|---|---|---|---|---|
| Name | Population | | No. of sample | No. of seropositive (%) | CI 95% (Min–Max) | No. of sample | No. of seropositive (%) |
| A | 250 | + | 25 | 6 (24) | 8–40 | 39 | 5 (12.8) |
| B | 220 | + | 22 | 4 (18.2) | 2.2–34.2 | 21 | 3 (14.3) |
| C | 500 | + | 50 | 9 (18) | 8–28 | 27 | 1 (3.7) |
| D | 400 | + | 40 | 1 (2.5) | 0–7.3 | 8 | 0 (0) |
| E | 190 | + | 19 | 2 (10.5) | 0–24.2 | 10 | 1 (10) |
| F | 260 | + | 26 | 11 (42.3) | 23.4–61.2 | 11 | 3 (27.3) |
| G | 250 | + | 25 | 8 (32) | 13.8–50.2 | 29 | 2 (6.9) |
| H | 540 | + | 54 | 4 (7.4) | 0.5–14.3 | 11 | 0 (0) |
| I | 2000 | + | 200 | 72 (36) | 29.4–42.6 | 17 | 1 (5.9) |
| J | 150 | + | 15 | 1 (6.7) | 0–19.3 | 12 | 0 (0) |
| Total | 4760 | + | 476 | 118 (24.8) | 21–28.6 | 185 | 16 (8.65)* |

* CI 95% = 4.6–12.6%.

**Table 2**
The seroprevalence and risk factors associated to *Neospora caninum* in dairy cattle herds in Hamedan province of West Iran.

| Risk factors | No. of sample and (seropositive %) in different farms ($n$ = 10) | | | | | | | | | | Total | | Statistical analysis |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A ($n$ = 25) | B ($n$ = 22) | C ($n$ = 50) | D ($n$ = 40) | E ($n$ = 19) | F ($n$ = 26) | G ($n$ = 25) | H ($n$ = 54) | I ($n$ = 200) | J ($n$ = 15) | No. of examined animal | Sp (%) | |
| **Age (year):** | | | | | | | | | | | | | |
| <2 | 2 (50) | 4 (0) | 9 (22.2) | 5 (20) | 0 (0) | 1 (0) | 2 (0) | 8 (12.5) | 19 (15.8) | 0 (0) | 50 | 8 (16) | $\chi^2$ = 0.006, $P$ = 0.999 |
| 2–4 | 1 (0) | 7 (0) | 35 (2.8) | 9 (0) | 8 (12.5) | 10 (10) | 7 (14.3) | 20 (10) | 39 (20.5) | 3 (0) | 139 | 14 (10.1) | |
| >4 | 22 (22.7) | 11 (36.4) | 6 (100) | 26 (0) | 11 (9.1) | 15 (66.7) | 16 (43.7) | 26 (3.8) | 142 (42.9) | 12 (8.3) | 287 | 96 (33.4) | |
| **Stage of pregnancy (month):** | | | | | | | | | | | | | |
| 1–3 | 3 (0) | 1 (0) | 5 (60) | 1 (0) | 0 (0) | 0 (0) | 1 (0) | 15 (26.7) | 12 (25) | 4 (0) | 42 | 10 (23.8) | $\chi^2$ = 0.046, $P$ = 0.977 |
| 3–6 | 17 (35.3) | 19 (21) | 14 (7.1) | 21 (0) | 13 (15.4) | 24 (45.8) | 21 (38.1) | 8 (0) | 54 (53.7) | 10 (10) | 201 | 62 (30.8) | |
| >6 | 5 (0) | 2 (0) | 31 (16.1) | 18 (5.5) | 6 (0) | 2 (0) | 3 (0) | 31 (0) | 134 (29.8) | 1 (0) | 233 | 46 (19.7) | |
| **Number of gestation:** | | | | | | | | | | | | | |
| 1 | 1 (0) | 0 (0) | 8 (12.5) | 0 (0) | 1 (0) | 1 (0) | 0 (0) | 11 (9.1) | 44 (20.4) | 2 (0) | 68 | 11 (16.2) | $\chi^2$ = 0.026, $P$ = 0.986 |
| 2 | 15 (33.3) | 4 (0) | 4 (0) | 7 (0) | 1 (0) | 1 (0) | 10 (0) | 3 (0) | 54 (26) | 4 (0) | 103 | 19 (18.4) | |
| ≥3 | 9 (11.1) | 18 (22.2) | 38 (21) | 33 (3) | 17 (11.8) | 24 (45.8) | 15 (53.3) | 43 (7) | 102 (48) | 9 (11.1) | 305 | 88 (28.9) | |
| **Abortion history:** | | | | | | | | | | | | | |
| + | 5 (40) | 2 (0) | 10 (50) | 3 (0) | 6 (33.3) | 3 (33.3) | 9 (33.3) | 12 (8.3) | 42 (45.2) | 2 (0) | 94 | 33 (35.1) | $\chi^2$ = 6.686, $P$ = 0.009 |
| − | 20 (20) | 20 (20) | 40 (10) | 37 (2.7) | 13 (0) | 23 (43.5) | 16 (31.2) | 42 (7.1) | 158 (33.5) | 13 (7.7) | 382 | 85 (22.3) | |
| **Stillbirth history:** | | | | | | | | | | | | | |
| + | 2 (50) | 0 (0) | 7 (42.8) | 0 (0) | 0 (0) | 0 (0) | 3 (66.7) | 1 (0) | 4 (50) | 2 (50) | 19 | 9 (47.4) | $\chi^2$ = 5.411, $P$ = 0.02 |
| − | 23 (21.7) | 22 (18.2) | 43 (13.9) | 40 (2.5) | 19 (10.5) | 26 (42.3) | 22 (27.3) | 53 (7.5) | 196 (35.7) | 13 (0) | 457 | 109 (23.9) | |
| **Metritis history:** | | | | | | | | | | | | | |
| + | 2 (50) | 1 (0) | 5 (60) | 0 (0) | 0 (0) | 0 (0) | 7 (42.8) | 4 (25) | 17 (47) | 3 (33.3) | 39 | 17 (43.6) | $\chi^2$ = 8.052, $P$ = 0.004 |
| − | 23 (21.7) | 21 (19) | 45 (13.3) | 40 (2.5) | 19 (10.5) | 26 (42.3) | 18 (27.8) | 50 (6) | 183 (35) | 12 (0) | 437 | 101 (23.1) | |
| **Breed:** | | | | | | | | | | | | | |
| Holstein | 20 (25) | 10 (30) | 10 (30) | 20 (0) | 10 (10) | 22 (42.3) | 0 (0) | 54 (7.4) | 200 (36) | 0 (0) | 346 | 99 (28.6) | $\chi^2$ = 9.930, $P$ = 0.001 |
| Crossbred | 5 (20) | 12 (8.3) | 40 (15) | 20 (5) | 9 (11.1) | 4 (0) | 25 (32) | 0 (0) | 0 (0) | 15 (6.7) | 130 | 19 (14.6) | |
| **Rearing service:** | | | | | | | | | | | | | |
| Artificial insemination (AI) | 25 (24) | 0 (0) | 0 (0) | 0 (0) | 19 (10.5) | 26 (42.3) | 0 (0) | 0 (0) | 200 (36) | 0 (0) | 270 | 91 (33.7) | $\chi^2$ = 26.587, $P$ < 0.0001 |
| AI and Bull | 0 (0) | 22 (18.2) | 50 (18) | 40 (2.5) | 0 (0) | 0 (0) | 25 (32) | 54 (7.4) | 0 (0) | 15 (6.7) | 206 | 27 (13.1) | |
| **Contact with dogs:** | | | | | | | | | | | | | |
| + | 25 (24) | 22 (18.2) | 0 (0) | 0 (0) | 19 (10.5) | 0 (0) | 25 (32) | 0 (0) | 200 (36) | 15 (6.7) | 306 | 93 (30.4) | $\chi^2$ = 14.423, $P$ = 0.0001 |
| − | 0 (0) | 0 (0) | 50 (18) | 40 (2.5) | 0 (0) | 26 (42.3) | 0 (0) | 54 (7.4) | 0 (0) | 0 (0) | 170 | 25 (14.7) | |
| **Contact with rodents:** | | | | | | | | | | | | | |
| + | 25 (24) | 22 (18.2) | 50 (18) | 0 (0) | 19 (10.5) | 26 (42.3) | 25 (32) | 54 (7.4) | 200 (36) | 15 (6.7) | 436 | 117 (26.8) | $\chi^2$ = 11.637, $P$ = 0.0006 |
| − | 0 (0) | 0 (0) | 0 (0) | 40 (2.5) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 40 | 1 (2.5) | |
| **Contact with poultry:** | | | | | | | | | | | | | |
| + | 25 (24) | 22 (18.2) | 0 (0) | 0 (0) | 0 (0) | 26 (42.3) | 0 (0) | 0 (0) | 200 (36) | 0 (0) | 273 | 93 (34.1) | $\chi^2$ = 29.542, $P$ < 0.0001 |
| − | 0 (0) | 0 (0) | 50 (18) | 40 (2.5) | 19 (10.5) | 0 (0) | 25 (32) | 54 (7.4) | 0 (0) | 15 (6.7) | 203 | 25 (12.3) | |
| **Contact with wild carnivores:** | | | | | | | | | | | | | |
| + | 25 (24) | 0 (0) | 50 (18) | 0 (0) | 19 (10.5) | 26 (42.3) | 25 (32) | 54 (7.4) | 200 (36) | 0 (0) | 399 | 112 (28.1) | $\chi^2$ = 14.235, $P$ = 0.0002 |
| − | 0 (0) | 22 (18.2) | 0 (0) | 40 (2.5) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 15 (6.7) | 77 | 6 (7.8) | |
| **Herd size:** | | | | | | | | | | | | | |
| ≤250 | 25 (24) | 22 (18.2) | 0 (0) | 0 (0) | 19 (10.5) | 0 (0) | 25 (32) | 0 (0) | 0 (0) | 15 (6.7) | 106 | 21 (19.8) | $\chi^2$ = 1.812, $P$ = 0.178 |
| >250 | 0 (0) | 0 (0) | 50 (18) | 40 (2.5) | 0 (0) | 26 (42.3) | 0 (0) | 54 (7.4) | 200 (36) | 0 (0) | 370 | 97 (26.2) | |

farms with >25 animals were 9.7-fold higher than farms with <25 ($p = 0.0002$). In contrast, it was in farms with <50 population ($p > 0.05$) (Talafha & Al-Majali, 2013). These differences may be due to increasing in number of dogs in each farm (Kyaw et al., 2004; Otranto, Lazari & Testini, 2003). Canada, Meireles and Ferreira (2006) noted that semen has important role in cattle neosporosis. The level of IgG against *N. caninum* in examined pregnant cattle with AI was also higher than those reported from Brazil (2.02-fold higher than other reproductive methods), Spain (7.1%), and Iran (17.1%) (Ortega-Mora, Ferre & Del-Pozo, 2003; Sharifzadeh, Doosti & Ghasemi, 2012; Snak et al., 2018). López-Gatius, Santolaria, Yániz, Garbayo and Almería (2005b) noted that AI of seropositive dairy cattle with semen of beef bulls may have beneficial effect on placental function due to crossbreeding. Okumu et al. (2019) reported abortion was significantly the highest in pregnant cattle with AI but with the lack of a rapid testing for cattle neosporosis on the semen donors ($p = 0.05$).

The main economic losses of cattle neosporosis was reproductive problems (Dubey et al., 2007). In the previous investigations, reproductive problems, i.e., abortion, stillbirth, retained fetal membranes, uterine infection, and metritis were reported as risk factors for seropositivity in herds and/or individual level (Ansari-Lari et al., 2017; Dubey et al., 2007; Okumu et al., 2019). Changoluisa, Rivera-Olivero, Echeverria, Garcia-Bereguiain and De Waard (2019) noted that abortion risk was conformed to different patterns because of differences in virulence of various strains of *N. caninum*. Our findings indicated Sp animals with abortion history were significantly higher than those without the abortion history. This was in parallel to study of Razmi et al. (2006) in northeastern Iran. In earlier reports, abortion risk was 2–26-fold higher in Sp animals than seronegative (Jenkins, Baszler & Bjorkman, 2002; López-Gatius, Santolaria & Almeria, 2005a; Schares et al., 2004; Vaclavek, Koudela & Modry, 2003). *Neospora caninum* mixed infection, especially pathogens associated with abortions; increases the frequency of abortion (Changoluisa et al., 2019; Okumu et al., 2019). In Ecuador, *N. caninum* and *Coxiella burnetii* co-infection reported in 14.7% of cattle with history of abortion (Changoluisa et al., 2019). Okumu et al. (2019) also reported the effect of *N. caninum* and bovine viral diarrhea virus co-infection on the occurrence of abortion in dairy farms of Kenya. Thus, culling of Sp animals with history of abortion to reduce the infection rate and economic losses subsequently was recommended (Ansari-Lari et al., 2017).

In the present study, Holstein dairy cattle susceptibility to *N. caninum* infection was higher than crossbred (OR = 2.3). This finding was in accordance to the reports from Argentina, Venezuela and Ethiopia (Dubey et al., 2007). While, Munhoz, Pereira and Flausino (2009) noted that there was no difference between *N. caninum* infection and breed. This may be related to different production system (Dubey et al., 2007; Moore, Perez & Agliano, 2009).

*Neospora caninum* infection in examined dairy cattle had significant association with close contact to farm dogs, wild carnivores, rodents and poultry similar to Barling, Mc-Neill and Paschal (2001) findings. According to Haddadzadeh, Sadrebazzaz and Malmasi (2007) and Malmasi, Hosseininejad and Haddadzadeh (2007) consumption of the aborted materials with dogs and wild carnivores played an important role in increasing horizontal transmission and spreading the infection to other neighboring farms. Rodents were one of the other known intermediate hosts for *N. caninum* (Jenkins, Parker & Hill, 2007). In earlier studies, *N. caninum* infection was 16.4% in rats from Taiwan, 3% in mice, 4.4% in rats from British dairy farms, and 10% in feral mice in the USA (Huang, Yang & Watanabe, 2004; Hughes, Williams & Morley, 2006). The role of poultry in life cycle of *N. caninum* is still uncertain. However, they might be contributed to transmit *N. caninum* infection from sylvatic cycle as mechanical vectors and/or intermediate hosts (Bartels, Wouda & Schukhen, 1999). Bartels et al. (1999) reported significant association between *N. caninum* infection and presence of poultry in infected dairy farms similar to the current work.

*Neospora caninum* infection in dogs was varied from 1% to 100% throughout the world (Dubey & Schares, 2011). In Iran, it was varied in Center (28–46%) and North-West (12.41–27%) (Haddadzadeh et al., 2007; Malmasi et al., 2007; Yakhchali et al., 2010, 2017). In an investigation by Antony and Williamson (2003), *N. caninum* infection in farm dogs in New Zealand was 74.5%. According to Dubey et al. (2007) the high infection risk was in farm dogs with close contact to Sp animals with secreted materials. The role of age in seropositivity suggested that the most of dogs acquire infection in the postnatal period by means of horizontal transmission. In this work, the highest Sp was in farm dogs over 2 years old. Similar to our finding, the effect of age in seropositivity reported in earlier studies (Haddadzadeh et al., 2007; Malmasi et al., 2007; Yakhchali et al., 2010, 2017). The highest infection rate in older dogs may be also due to more exposure to *N. caninum* infection and the study area (Dubey et al., 2007). There was significant difference in seropositivity between males and females which was in agreement with Malmasi et al. (2007) and Yakhchali et al. (2010). It was not in accordance with Goździk, Wrzesień and Wielgosz-Ostolska (2011).

## 5. Conclusion

Our findings uncovered 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 of the region. Furthermore, this was the first comprehensive report of *N. caninum* infection and associated risk factors in Iranian dairy farms. Further studies recommended investigating sanitary strategies in dairy cattle husbandry and launching control programs in dairy farms in the region.

## Declaration of Competing Interest

The authors report no conflict of interest.

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## Ethics

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

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