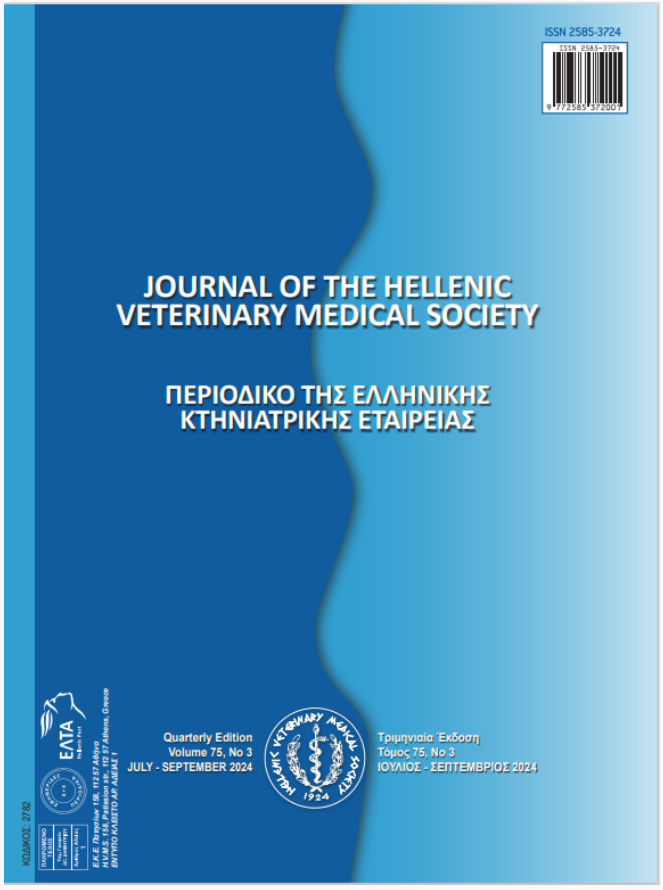
Journal of the Hellenic Veterinary Medical Society

Vol 75, No 3 (2024)



An estimation of vertical transmission of Neospora caninum in dairy cattle, in Khorasan Razavi Province, Iran

M Shahidi, Mohsen Maleki, G Razmi

doi: 10.12681/jhvms.31252

To cite this article:

Shahidi, M., Maleki, M., & Razmi, G. (2024). An estimation of vertical transmission of Neospora caninum in dairy cattle, in Khorasan Razavi Province, Iran . *Journal of the Hellenic Veterinary Medical Society*, *75*(3), 7635–7640. https://doi.org/10.12681/jhvms.31252

J HELLENIC VET MED SOC 2024, 75 (3): 7635-7640
ΠΕΚΕ 2024, 75 (3): 7635-7640

Research article
Ερευνητικό άρθρο

An estimation of vertical transmission of *Neospora caninum* in dairy cattle, in Khorasan Razavi Province, Iran

M. Shahidi[1], M. Maleki[2], G.R. Razmi[3,4]*

[1]*Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran*

[2]*Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran*

[3]*Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran*

[4]*Center of Excellence in Ruminant Abortion and Neonatal Mortality, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran*

ABSTRACT: *Neospora caninum* is an important agent of abortion and reproductive loss in dairy cattle. The objective of thi study was to determine the rate of vertical transmission of *N. caninum* in dairy cattle, Khorasan Razavi province, Iran. Two dairy farms with history of *Neospora* abortion were selected and two hundred -eighty blood samples of dairy cattle in the third trimester of gestation were collected from January 2019 to April 2020. The serum samples of each dairy cattle were examined by an indirect ELISA. At the calving, the colostrum samples of seropositive dams with blood samples of their precolostral calves were collected and again examined by the ELISA method. The seroprevalence of *Neospora* infection was determined by 16.59% (41/247) in dairy farm 1 and 12.12% (4/33) in dairy farm 2 (P>0.05). In the present study, 100% of precolostral calves born of seropositive dairy cattle had antibodies against *Neospora caninum* before colostrum ingestion. Based on the seroprevalence of dam and daughter, the vertical transmission was estimated at the highest rate (100%). In conclusion, the results are shown the importance of vertical transmission to remain and contribute to *N. caninum* infection in two dairy farms.

Keywords: *Neospora; Vertical transmission; Dairy cattle; Iran*

Corresponding Author:
Gholamreza Razmi, Ferdowsi University of Mashhad, Department of Pathobiology, Faculty of Veterinary Medicine, P.O. Box: 91775-1793 Mashhad, Iran
E-mail address: razmi@um.ac.ir

Date of initial submission: 01-09-202
Date of acceptance: 27-08-2024

INTRODUCTION

Neospora caninum has been known as the most significant cause of abortion in cattle all over the world (Dubey et al., 2007). There are two transmission routes for *N. caninum* in cattle. The main route of infection is usually described as a vertical, or congenital or endogenous transmission that occurs when the bardyzoites of cysts in dam's tissues are reactivated and differentiated to tachyzoites which spread across the placenta and into the fetus. The minor route of infection is called horizontal or post-natal infection, that occurs when the cattle ingest sporulated *N. caninun* oocysts, the sporozoites differentiate into tachyzoites which spread probably via the circulation in cells of the mononuclear phagocytic system and may be infected the fetus by transplacental transmission (Conraths and Gottstein, 2007). The endogenous (vertical) transmission could occur in approximately up to 95% of infected dairy cattle (Dubey et al., 2007; Wouda, 2000). Most calves congenitally infected with *Neospora* appear healthy and carry the infection. (Dubey et al., 2007). The presence seropositive dairy cattle mainly indicate the *Neospora* infection in the farm, but this cannot be directly related to the cause of abortion in animals. Seroepidemiology studies were shown that a *Neospora* seropositive dam have 4 to 8 fold higher risk of abortion than a seronegative dairy cattle (Davison et al., 1999 a; Sager et al., 2001; Václavek et al., 2003). Because the mother's serum IgG antibody is not transferred to the fetus through the placenta and the bovine fetus develops the antibodies during the fifth month of gestation, therefore, testing the calf's serum before eating colostrum is an important indicator in the diagnosis of congenital infection of the calf. (Bartley et al., 2004; Gracia-Alvares, et al., 2007), whereas the maternal antibodies in milk could be affected the results of postcolostral serology (Staubli, et al. 2006). The serologic status of cow-calf pairs can also be established to assess the main transmission pattern within the herd (Dubey et al., 2007; Conraths and Gottstein, 2007)

The high seroprevalence of *Neospora* infection has been shown in dairy cattle in Iran (Ansari-Lari, 2020; Gharekhani et al., 2020; Darijani et al.,2021). Thus, *N. caninum* infection was also determined as an agent of abortion in dairy cattle (Razmi et al., 2007; Salehi et al., 2009; Ansari-Lari 2021; Saleh et al., 2021; Gharekhani et al., 2022). However, there is little information about the rate of vertical transmission of *Neospora* in Iranian dairy farms. The aim of the study was to estimate the rate of vertical transmission of *Neospora* infection in dairy farms of Torbat-e Heidareih area, Iran.

MATERIALS AND METHODS

Study area

This study was performed over a one year in two Holstein-Friesian dairy farms from 2019-2020. The farms are located in Torbat-e- Heidareih area, the Khorasan Razvi Province at 35.17˚N, 59.12˚E. The climate is semi-arid with hot and dry summer and cold winter.

Sampling

Iranian dairy farms vary in scale from small farms with less than 100 cows to large farms with 7,000 cows, with an overall average herd size of 680 cows (Sadeghi-Sefidmazgi et al., 2012). The conventional dairy cattle farms were classified by the number of cows into 4 herd size classes, small (<100 cows), Medium (100-299 cows), large (300-499 cows), and very large (≥500 cows) based on the Welfare Quality Assessment protocol for dairy cattle (Gieseke, et al., 2018). In this study, the first farm as very large size (up to 2000 animals) and the second farm as small size (less than 100 animals) were selected based on known history of *N. caninum* infection. All dairy cattle were bred under an intensive system and were fertilized by artificial insemination. They were vaccinated against infectious diseases annually and were free from tuberculosis and brucellosis, as shown by yearly tests. Both farms had no dogs.

Blood and colostrum sampling

Two hundred and eighty blood samples were collected from pregnant dairy cattle. After birth, the colostrum of seropositive dams and blood samples of their calves were immediately collected before colostrum ingestion. The collected blood samples were centrifuged at 2000 rpm for 10 min, and sera of them were stored at −20 °C until serological examination. The colostrum samples were also centrifuged at 2000 rpm for 10 min, then the fat was removed from the top tube, and the lower liquid was stored at −20 °C until used.

Serology

The sera and colostrum samples were examined to

detect antibody against N. *caninum* by an ELISA kit (ID screen ® *Neospora caninum* indirect Multi-species, ID. vet, Montpellier, France). Briefly, blood and colostrum serum samples were diluted at 1:10 and 1:2, respectively. The positive and negative controls provided with the test kit were used as controls at the 1:40 dilution as recommended by the manufacturer. Serological results of were recorded at 450 nm using a microplate reader. (ELX800 absorbance reader, Bio TeK, USA). Test results were validated if: the mean value of the positive control optical density (OD) is greater than 0.350 and the ratio of the mean OD values of the positive and negative control is greater than 3. The S/P ratio was defined as the optical density (OD) of the sample (S) minus the OD of the negative control (NC), all divided by the OD of the positive control (PC) minus the OD of the negative control ([S-NC]/[PC-NC]). For blood sera: a cut-off S/P ratio of≤0.40% was defined as negative, an S/P ratio of 0.40-50% as Doubtful and an S/P ratio ≥50 as positive.

For colostrum sera: a cut-off S/P ratio of≤0.25% was defined as negative, an S/P ratio of 0.25-29% as Doubtful and an S/P ratio≥30 as positive.

Statistics analysis

Chi- square test was used to analyze the association between aerostats of pregnant dairy cattle and some risk factors. The vertical transmission rate was estimated based on the proportion of seropositive precolostrum calves born from seropositive mothers (More, et al, 2009).

RESULTS

The serological findings related to *Neospora* infection in a very large dairy farm and a small dairy farm are presented in Table 1. The seroprevalence of *N.caninum* infection in pregnant dairy cattle associated with size farm, age and the number of birth were non- significant (p>0.05). Based on the serologic status of dam and daughters, the rate of vertical transmission in infected cattle was calculated by %100 (45/45). Six colostrum samples of seropositive dams were also seropositive (Table 2).

DISCUSSION

In this study, *N. caninum* antibodies were found in 16.07% pregnant dairy cattle. The sero prevalence of *Neospora* infection has been reported from 3.8% to

Table. 1. The frequency of antibodies against Neospora infection in two dairy farms by different risk factors

Rick factors	n	Seropositive	%	95% CI	p-value
Age					P=0.40*
2-3	89	15	16.8	9.43 - 27.80	
4-3	71	8	11.2	4.86 - 22.20	
4-5	61	9	14.7	6.75 - 28.01	
>5	59	13	22	11.73 - 37.68	
Herd size					P=0.51*
Very Large farm	247	41	16.59	11.91 - 22.52	
Small farm	33	4	12.12	0.33 - 31.04	
Number of calving					P=0.10*
First	95	19	20	12.04 - 31.23	
Second	73	9	12.3	5.64 - 23.40	
Third	59	4	6.7	1.847 - 17.35	
Fourth	53	13	24.5	13.06 - 41.94	
Total	280	45	16.07	11.72 - 21.50	

not significant=*

Table2. The frequency of antibodies against of N.caninum infection in precolostral calves ser and colostrum in 45 seropositive dams not significant

Samples	n	Seropositive	%	95% CI
Precolostral calves	45	45	100	72.94 - 100
Colostrum sera	45	6	13.3	4.89 - 29.02

72% in Iranian dairy cattle of different provinces (Ansari-Lari, 2020; Gharekhani et al., 2020). The seroprevalence of Neospora infection in every dairy farm could be affected by some risk factors such as host, methods of serology, climate and herd health (Wouda , 2007). The seroprevalence of *N.caninum* infection did not show any significant differences in relation to age group and number of pregnancies as risk factors in this study. These finding of the preset study are in accordance with other studies. (Nazir et al., 2013; Sadrebazzaz et al., 2004; Wouda et al., 1998; Yu et al., 2007; Darijani et al., 2021). Despite that, many studies found significant association between age, number of pregnancies and seropositivity for *N. caninum* in cattle (Bartels et al., 2006; Gharekhani et al., 2021; Guimarães et al., 2004; Jensen et al., 1999; Klauck et al., 2016; Razmi et al., 2006). They suggested that the results may be related to increased chances of *Neospora* infection in horizontal transmission or more antigenic stimulation after repeated reactivation of infection in vertical transmission in older dairy cattle.

Our study showed non-significant association between seroprevalence of N. *caninum* infection and herd size of dairy farms. Other studies also reported similar results in small and large dairy farms (Davison, et al., 1999 b; Paré et al., 1998; Quintanilla-Gozalo et al., 1999). They explained that the results could be related to the maintenance of health management in dairy farms independent herd sizes. In contrast to the above reports, many studies were shown the high prevalence of *N.caninum* infection in large dairy farms (Gharekhani et al., 2021; Klauck et al., 2016; Klun et al., 2019; Otranto et al., 2003; Razmi et al., 2006; Schares et al., 2004). They showed that the chance of acquiring *Neospora* infection is increased in large farms due to the purchases of external heifer replacement and challenging to do hygienic measures to prevent to dogs from feeding placenta and other infectious materials.

In the present study, all seropositive dairy cattle gave birth to infected precolostral calves. Similar results with the highest vertical transmission rate of *Neospora* (100%) were obtained in the USA (Anderson et al., 1997), the Netherland (Wouda et al., 1998), Argentina (Campero et al., 2003; Rodriguez et al., 2016) and Brazil (de Magalhães et al., 2014). The results indicated that endogenous transmission is the main route of *Neospora* infection in dairy farms studied. So far, the estimated rate of vertical transmission was reported from 81.1% to 100 % in the USA (Anderson et al., 1997; Pare et al., 1996; Paré et al., 1997), from 68 to 100% in the Netherland (Bartels et al., 2007; Wouda et al., 1998), 95.2% in the UK (Davison, et al., 1999 b), from 40 .7% to 75% in Canada (Baillargeon et al., 2001; Bergeron et al., 2000), 64 % to 70% in Costraica (Romero & Frankena, 2003), 90.6 in Spain (López-Gatius et al., 2004), 58 % in Thailand (Chanlun et al., 2007); 61.6 % in Israel (Mazuz et al., 2014), 9% to 100% in Brazil (de Magalhães et al., 2014; do Nascimento et al., 2014; Santos et al., 2012), 14 to 100% in Argentina (Moré et al., 2009; Rodriguez et al., 2016) and 13 to 52% in Iran (Gharekhani & Yakhchali, 2020; Razmi et al., 2013). It seems that the difference in the reported vertical transmission rate has directly correlated to the high proportion of active versus latent infection in dairy farms (Moingeon et al., 1986).

The small number of colostrum samples that contain *Neospora* antibodies in the current study could be linked to the levels of serum immunoglobulins found in the blood of the maternal cow. Investigations have revealed that a low titer of *Neospora* antibodies in the maternal blood leads to a negative outcome in the serological assessment of colostrum for these antibodies (Cardoso et al., 2008; Bezerra et al., 2022). The detection of antibodies against *Neospora* in Precolostral calves is not associated with the serum of the mother. Instead, the serum response is attributed to the calf's immune reaction resulting from the vertical transmission of *Neospora* via the placenta to the newborn calf.

In conclusion, this study demonstrated that the maternal and precolostral calves' serology occur an appropriate indicator for the study of *Neospora* infection associated with dairy farms. Based on the serologic results, the rate of vertical transmission was the highest rate in dairy farms in this area.

ACKNOWLEDGEMENTS

We are very grateful to Mr. H. Eshrati for their technical assistance.

FUNDING INFORMATION

This study was supported by grant **No: 3/43234** from the Vice President Research and Technology of Ferdowsi University of Mashhad, Iran. We are very grateful to Hamid Eshrati for his technical assistance.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Anderson, M. L., Reynolds, J. P., Rowe, J. D., Sverlow, K. W., Packham, A. E., Barr, B. C., & Conrad, P. A. (1997). Evidence of vertical transmission of *Neospora* sp infection in dairy cattle. J AmVet Med Asso 210(8): 1169-1172.

Ansari-Lari, M. (2020). Bovine neosporosis in Iran: A systematic review and meta-analysis. Pre Vet Med 176: 104913. https://doi.org/10.1016/j.prevetmed.

Ansari-Lari, M. (2021). *Neospora caninum* in aborted bovine fetuses in Iran: a systematic review and meta-analysis. Ann Parasitol 67(3): 357-366.

Baillargeon, P., Fecteau, G., Paré, J., Lamothe, P., & Sauvé, R. (2001). Evaluation of the embryo transfer procedure proposed by the International Embryo Transfer Society as a method of controlling vertical transmission of *Neospora caninum* in cattle. J AmVet Med Asso 218(11): 1803-1806.

Bartels, C., Arnaiz-Seco, J., Ruiz-Santa-Quitera, A., Björkman, C., Frössling, J., Von Blumröder, D., Conraths, F., Schares, G., Van Maanen, C., & Wouda, W. (2006). Supranational comparison of *Neospora caninum* seroprevalences in cattle in Germany, The Netherlands, Spain and Sweden. Vet Parasitol 137(1-2):17-27.

Bartels, C. J., Huinink, I., Beiboer, M. L., van Schaik, G., Wouda, W., Dijkstra, T., & Stegeman, A. (2007). Quantification of vertical and horizontal transmission of *Neospora caninum* infection in Dutch dairy herds. Vet Parasitol 148(2): 83-92.

Bartley, P.M., Kirvar, E., Wright, S., Swales, C., Esteban-Redondo, I., Buxton, D., Maley, S.W., Schock, A., Rae, A.G., Hamilton, C. & Innes, E.A. (2004). Maternal and fetal immune responses of cattle inoculated with *Neospora caninum* at mid-gestation. J Comp Pathol 130(2-3): 81-91.

Bergeron, N., Fecteau, G., Pare, J., Martineau, R., & Villeneuve, A. (2000). Vertical and horizontal transmission of *Neospora caninum* in dairy herds in Québec. Can Vet J 41(6): 464.

Bezerra, R. A., Lima, B. A., Alvares, F. B. V., Rossi, G. A. M., Braga, F. R., de Melo, R. P. B., & Feitosa, T. F. (2022). Detection of Anti-*Neospora caninum* IgG in blood serum and colostrum samples in naturally infected sheep and in their newborn offspring. Pathogens 11(11), 1263: ; https://doi.org/10.3390/pathogens11111263

Campero, C., Moore, D., Lagomarsino, H., Odeón, A., Castro, M., & Visca, H. (2003). Serological status and abortion rate in progeny obtained by natural service or embryo transfer from *Neospora caninum*-seropositive cows. J Vet Med, Series B 50(9):458-460.

Cardoso, J. M. S., Funada, M. R., Soares, R. M., & Gennari, S. M. (2008). Perfil sorológico dos anticorpos colostrais para *Neospora caninum* em bezerros livres da infecção. *Brazilian* Braz J Vet Res Anim Sci *45*(5): 379-384.

Chanlun, A., Emanuelson, U., Frössling, J., Aiumlamai, S., & Björkman, C. (2007). A longitudinal study of seroprevalence and seroconversion of *Neospora caninum* infection in dairy cattle in northeast Thailand. Vet Parasitol 146(3-4): 242-248.

Conraths, J. and Gottstein, B., 2007. Neosporosis: General considerations. In: L.M.Ortega-Mora., B. Gottstein., and F.J Conraths. and D. Buxton (Eds.) ,Protozoal abortion in farm ruminants CAB international, Wallingford, UK: pp 42-45.

Darijani, A., Arefkhah, N., Shahriarirad, S., Zoghi, S., Namavari, M., Moshfe, A., & Sarkari, B. (2021). *Neospora caninum* infection in cattle in the Province of Kohgiluyeh and Boyer Ahmad, Southwest of Iran: Seroprevalence and Molecular Assessment. J Parasitol Res *2021*(1), 4258513.

Davison, H., French, N., & Trees, A. 1999a. Herd-specific and age-specific seroprevalence of *Neospora caninum* in 14 British dairy herds. Vet Rec 144(20): 547-550.

Davison, H., Otter, A., & Trees, A. 1999b. Estimation of vertical and horizontal transmission parameters of *Neospora caninum* infections in dairy cattle. Inter J Parasitol 29(10):1683-1689.

de Magalhães, V. C. S., de Oliveira, U. V., Costa, S. C. L., dos Anjos Santos, I., Pereira, M. J. S., & Munhoz, A. D. (2014). Transmission paths of *Neospora caninum* in a dairy herd of crossbred cattle in the northeast of Brazil. Vet Parasitol 202(3-4):257-264.

do Nascimento, E. E., Sammi, A. S., Dos Santos, J. R., Nino, B. d. S. L., Bogado, A. L. G., Taroda, A., Vidotto, O., & Garcia, J. L. (2014). Anti-*Neospora caninum* antibody detection and vertical transmission rate in pregnant zebu beef cows (Bos indicus): Neospora caninum in pregnant beef cows (Bos indicus). Comp Immunol Microbiol Infect Dis 37(4) :267-270.

Dubey, J., Schares, G., & Ortega-Mora, L. (2007). Epidemiology and control of neosporosis and Neospora caninum. Clin Microbiol Rev 20(2): 323-367.

Gharekhani, J., Yakhchali, M., & Berahmat, R. (2020). *Neospora caninum* infection in Iran (2004-2020): A review. J Parasitol Dis 44(4): 671-686.

Gharekhani, J., & Yakhchali, M. (2020). Vertical transmission of *Neospora caninum* in Iranian dairy cattle. Ann Parasitol *66*(4): 495-500

Gharekhani, J., Yakhchali, M., Afshari, A., & Adabi, M. (2021). Herd-level contamination of *Neospora caninum*, *Toxoplasma gondii* and *Brucella* in milk of Iranian dairy farms. Food Microbiol 100: 103873.

Gharekhani, J., Yakhchali, M., & Heidari, R. (2022). Molecular detection and phylogenetic analysis of *Neospora caninum* in various hosts from Iran. Comp Immunol Microbiol Infect Dis *80*:101737. 101737. https://doi.org/10.1016/j.cimid.2021.101737

Gracia-Alvares, G., Ortega-Mora, L.M., Dijkstra, T., Wouda, W. (2007). Diagnostic application and recommended diagnostic schemes In: L.M.Ortega-Mora., B. Gottstein., and F.J Conraths. And D. Buxton (Eds). protozoal abortion in farm ruminants, CAB international, Wallingford, UK: pp:89-113

Gieseke, D., Lambertz, C., & Gauly, M. (2018). Relationship between herd size and measures of animal welfare on dairy cattle farms with freestall housing in Germany. J Dairy Sci *101*(8):7397-7411.

Guimarães Jr, J., Souza, S. L. P. d., Bergamaschi, D. P., & Gennari, S. M. (2004). Prevalence of *Neospora caninum* antibodies and factors associated with their presence in dairy cattle of the north of Paraná state, Brazil. Vet Parasitol 124(1-2):1-8.

Jensen, A. M., Björkman, C., Kjeldsen, A., Wedderkopp, A., Willadsen, C., Uggla, A., & Lind, P. (1999). Associations of *Neospora caninum* seropositivity with gestation number and pregnancy outcome in Danish dairy herds. Pre Vet Med 40(3-4):151-163.

Klauck, V., Machado, G., Pazinato, R., Radavelli, W. M., Santos, D. S., Berwaguer, J. C., Braunig, P., Vogel, F. F., & Da Silva, A. S. (2016). Relation between *Neospora caninum* and abortion in dairy cows: Risk factors and pathogenesis of disease. Microb Pathogen 92:46-49.

Klun, I., Ćirković, V., Maletić, M., Bradonjić, S., & Djurković-Djaković, O. (2019). Seroprevalence of *Neospora caninum* infection and associated risk factors in dairy cattle in Serbia. Parasitol Res118(6): 1875-1883.

López-Gatius, F., López-Béjar, M., Murugavel, K., Pabón, M., Ferrer, D., & Almería, S. (2004). *Neospora*-associated abortion episode over a 1-year period in a dairy herd in north-east Spain. J Vet Med Series B 51(7):348-352.

Mazuz, M. L., Fish, L., Reznikov, D., Wolkomirsky, R., Leibovitz, B., Savitzky, I., Golenser, J., & Shkap, V. (2014). Neosporosis in naturally infected pregnant dairy cattle. Vet Parasitol 205(1-2):85-91.

Moré, G., Bacigalupe, D., Basso, W., Rambeaud, M., Beltrame, F., Ramirez, B., Venturini, M., & Venturini, L. (2009). Frequency of horizontal and vertical transmission for *Sarcocystis cruzi* and *Neospora caninum* in dairy cattle. Vet Parasitol 160(1-2): 51-54.

Nazir, M. M., Maqbool, A., Khan, M. S., Sajjid, A., & Lindsay, D. S. (2013). Effects of Age and Breed on the Prevalence of *Neospora caninum* in Commercial Dairy Cattle from Pakistan. J Parasitol 99(2):368-370, 363.

Otranto, D., Llazari, A., Testini, G., Traversa, D., di Regalbono, A. F., Badan, M., & Capelli, G. (2003). Seroprevalence and associated risk factors of neosporosis in beef and dairy cattle in Italy. Vet Parasitol

118(1-2): 7-18.

Pare, J., Thurmond, M. C., & Hietala, S. K. (1996). Congenital *Neospora caninum* infection in dairy cattle and associated calfhood mortality. Can J Vet Res 60(2):133. 139

Paré, J., Thurmond, M. C., & Hietala, S. K. (1997). *Neospora caninum* antibodies in cows during pregnancy as a predictor of congenital infection and abortion. J Parasitol 83(1): 82-87.

Paré, J., Fecteau, G., Fortin, M., & Marsolais, G. (1998). Seroepidemiologic study of *Neospora caninum* in dairy herds. J AmVet Med Asso 213(11): 1595-1598.

Quintanilla-Gozalo, A., Pereira-Bueno, J., Tabares, E., Innes, E., González-Paniello, R., & Ortega-Mora, L. (1999). Seroprevalence of *Neospora caninum* infection in dairy and beef cattle in Spain. Inter J Parasitol 29(8):1201-1208.

Razmi, G. R., Mohammadi, G. R., Garrosi, T., Farzaneh, N., Fallah, A., & Maleki, M. (2006). Seroepidemiology of *Neospora caninum* infection in dairy cattle herds in Mashhad area, Iran. Vet Parasitol 135(2): 187-189.

Razmi, G. R., Maleki, M., Farzaneh, N., Talebkhan Garoussi, M., & Fallah, A. (2007). First report of *Neospora caninum*-associated bovine abortion in Mashhad area, Iran. Parasitol Res 100(4): 755-757.

Razmi, G., Zarae, H., Norbakhsh, M. F., & Naseri, Z. (2013). Estimating the rate of transplacental transmission of *Neospora caninum* to aborted fetuses in seropositive dams in Mashhad area, Iran. Iran J Vet Med 7(4): 253-256.

Rodriguez, A. M., Maresca, S., Cano, D. B., Armendano, J. I., Combessies, G., Lopéz-Valiente, S., Odriozola, E. R., Späth, E., Odeón, A. C., & Campero, C. M. (2016). Frequency of *Neospora caninum* infections in beef cow-calf operations under extensive management. Vet Parasitol 219: 40-43.

Romero, J., & Frankena, K. (2003). The effect of the dam-calf relationship on serostatus to *Neospora caninum* on 20 Costa Rican dairy farms. Vet Parasitol 114(3): 159-171.

Sadeghi-Sefidmazgi, A., Moradi-Shahrbabak, M., Nejati-Javaremi, A., Miraei-Ashtiani, S. R., & Amer, P. R. (2012). Breeding objectives for Holstein dairy cattle in Iran. J Dairy Sci 95(6):3406-3418.

Sadrebazzaz, A., Haddadzadeh, H., Esmailnia, K., Habibi, G., Vojgani, M., & Hashemifesharaki, R. (2004). Serological prevalence of *Neospora caninum* in healthy and aborted dairy cattle in Mashhad, Iran. Vet Parasitol 124(3-4):201-204.

Sager, H., Fischer, I., Furrer, K., Strasser, M., Waldvogel, A., Boerlin, P., Audigé, L., & Gottstein, B. (2001). A Swiss case-control study to assess *Neospora caninum*-associated bovine abortions by PCR, histopathology and serology. Vet Parasitol 102(1-2): 1-15.

Salehi, B., Amouei, A., Dodangeh, S., Daryani, A., Sarvi, S., Safari-Kharyeki, M. R., & Hosseininejad, Z. (2021). Molecular identification of *Neospora caninum* infection in aborted fetuses of sheep, cattle, and goats in Mazandaran Province, Northern Iran. Iran J Parasitol *16*(3):483-489

Salehi, N., Haddadzadeh, H., Ashrafihelan, J., Shayan, P., & Sadrebazzaz, A. (2009). Molecular and pathological study of bovine aborted fetuses and placenta from *Neospora caninum* infected dairy cattle. Iran J Parasitol 4(3):40-51.

Santos, R. R. D., Rocha, C. M. B. M. d., Gonçalves, T. d. M., & Guimarães, A. M. (2012). Quantification of vertical transmission of *Neospora caninum* in dairy cows in Minas Gerais, Brazil. Rev Bras ParasitolVet 21:294-297.

Schares, G., Bärwald, A., Staubach, C., Ziller, M., Klöss, D., Schröder, R., Labohm, R., Dräger, K., Fasen, W., & Hess, R. (2004). Potential risk factors for bovine *Neospora caninum* infection in Germany are not under the control of the farmers. Parasitol 129(3): 301-309.

Staubli, D., Sager, H., Haerdi, C., Haessig, M., & Gottstein, B. 2006. Precolostral serology in calves born from *Neospora*-seropositive mothers. Parasitol Res 99(4): 398-404.

Václavek, P., Koudela, B., Modrý, D., & Sedlák, K. (2003). Seroprevalence of *Neospora caninum* in aborting dairy cattle in the Czech Republic. Vet Parasitol 115(3): 239-245.

Wouda, W., Moen, A., & Schukken, Y. (1998). Abortion risk in progeny of cows after a *Neospora caninum* epidemic. Theriogenol 49(7):1311-1316.

Wouda, W. (2000). Diagnosis and epidemiology of bovine neosporosis: a review. Vet Quarter 22(2):71-74.

Wouda, W., 2007. Biology, Transmission and Clinical signs. In: L.M.Ortega-Mora., B. Gottstein., and F.J Conraths. And D. Buxton (Eds) , *Protozoal abortion in farm ruminants*(PP: 46-53). CAB international, Wallingford, UK.

Yu, J., Xia, Z., Liu, Q., Liu, J., Ding, J., & Zhang, W. (2007). Seroepidemiology of *Neospora caninum* and *Toxoplasma gondii* in cattle and water buffaloes (Bubalus bubalis) in the People's Republic of China. Vet Parasitol 143(1): 79-85.