

Survey of *Neospora Caninum* Antibody in Aborting Cattle from Three Climate Regions of Iran

¹M.R. Youssefi, ²S. Ebrahimpour and ³B. Esfandiari

¹Department of Veterinary Parasitology, Islamic Azad University, Babol Branch, Iran

²Islamic Azad University, Garmsar, Iran

³DVM, Head of Pasteur Institute of Iran, Amol Research Center, Iran

Abstract: *Neospora caninum* is an intracellular parasite which causes abortion in cattle worldwide. This descriptive, cross-sectional study was conducted during 2009 to investigate the sero-prevalence of *Neospora Caninum* infection among Cattle from three climate zones of Iran. Female animals that had abortion history were sampled. Totally, 226 serum samples were collected from cattle herds living in Ardebil (46), Garmsar (84) and Babol (96) regions, representatives of three cold, warm and dry and mild temperate climate zones of Iran, respectively. Herd's abortion history, age and gestation numbers were assessed by questionnaires. 1:100 serum dilutions were analyzed for *Neospora caninum* antibodies by commercially available ELISA kit. Optical absorbencies (OD) were above 0.5 was considered as positive for NC infection. R² test, linear and logistic regressions were also done. Seroprevalences of 7%, 45.2% and 57.3% were observed for cattle herds from Ardebil, Garmsar and Babol regions, respectively. Gestation number, abortion number and age of cattle had specific effects on *Neospora caninum* seroprevalence, so that, age group of 4 through 5 years with history of 1 abortion and gestation, had highest seroprevalences almost among all three main groups. Cold temperate climate region had significantly reduced rate of *Neospora caninum* seropositivity.

Key words: *Neospora caninum* • Cattle • Seroprevalence • Climate • Iran

INTRODUCTION

Neospora caninum is a *Toxoplasma gondii*-like parasite that was found for the first time in the cerebral tissue of dogs in 1984 [1]. Dogs and coyotes are definitive hosts for the parasite and cattle as an intermediate host can acquire the infection through ingestion of oocysts that referred to as horizontal or postnatal transmission route. Vertical or transplacental transmission of the parasite is also highly efficient route of infection that is common in cattle [2, 3]. Calves born carrying the infection are capable of infecting their offspring when they start to breed [4, 5]. *N. caninum* infection is a major cause of abortion in cattle and is associated with considerable economic and reproductive losses. Prevalence of *N. caninum* antibodies and their relation to abortion rates in cattle were studied in few regions of Iran [6-9], but there are no comparable data about climate changes as a risk factor for the infection.

Our preliminary study was designed to evaluate the potential of climate changes as a risk factor for *N. caninum* infection and to detect correlation between seroprevalences and 3 additional variables: abortion number, gestation number and animal age. For this purpose blood samples were collected from cattle herds living in three different climate zones of Iran: Garmsar, Babol and Ardebil regions, representatives of warm and dry, mild and cold temperate climate zones. Herd's abortion history, age and gestation numbers were assessed by a questionnaire. Only female animals that have some history of abortion were sampled. Our findings showed that *N. caninum* infection is common in mild or warm temperate zones while is rare in cold climates.

MATERIALS AND METHODS

Populations Studied: Totally 226 blood samples including, 46, 84 and 96 samples from cattle living in Ardebil, Garmsar and Babol regions, respectively were collected

during summer in 2009. All studied animals had some history of abortion, based on questionnaire assessed. Blood sera were collected, transported and assessed in the laboratory of Parasitology Department of Islamic Azad University of Babol Branch.

Procedures: All serum samples were tested in duplicate for *N. caninum* antibodies using a commercially available ELISA assay kit (HerdCheck®, IDEXX). Only one dilution of sera, 1:100 was used, according to the manual. If the average of three measurements was higher than 0.5, the result was considered as positive.

Statistical Analysis: Data analysis was carried out using SPSS 16 statistical software. Chi square test was used for prevalence differences, linear and logistic regressions were done for relative risk and OR estimation. T test was used to elucidating any differences between main groups and subgroups with regards to optical density of ELISA tests.

RESULTS

Collected data from ELISA-reader and questionnaires was imported to SPSS statistical software for analysis. Average of triplicate measurements were used to descriptive statistics. If optical density was higher than 0.5 the result was considered as positive value for *Neospora caninum* antibodies, according to the manual of kit. Five gestation number (1-5), four abortion number (0-3) and three different ranges of age were used for further sub grouping of the animals (Table2).

Climate as a Risk Factor: Seroprevalence of *Neospora caninum* antibodies were 7%, 57.3% and 45.2% for Ardebil, Babol and Garmsar regions, respectively. It seems that *N. caninum* infection is rare among cattle from cold climate region (Ardebil) and its role in incidence of abortion in this region should be further investigated. Table 1 represents the results with further details.

Table 1: Seroprevalence of *N.caninum* in cattle from three regions of Iran

Regions	Total	Positive	Prevalence
Ardebil	46	3	7%
Babol	96	55	57.3%
Garmsar	84	38	45.2%

Other Risk Factors: The highest seroprevalence of *Neospora caninum* antibodies was observed among cattle living in the period of first gestation number and had one abortion in their history.

As it can be seen in table 2, cattle during first gestation from Babol and Garmsar regions were 57.3% and 45.2% seropositive for *Neospora caninum* antibodies. The rate was decreased for cattle which had higher gestation number.

DISCUSION

Neosporiasis is the most important cause of abortion in cattle in many geographic regions [10]. But the serologic prevalence of the parasite is associated with considerable differences among countries, within countries, between regions and between beef and dairy cattle [11-13].

Our findings indicate that contribution of *N. caninum* on incidence of abortion in cows specially in cold temperate climate regions (Ardebil) is very little, as we found only 3 positive sera among 46 aborting cows from the regions, (7%), while prevalence of the antibodies in warm and dry and mild climate regions such as Garmsar and Babol regions were 45.2% and 57.3% respectively. This means that life cycle or transmissions of the parasite in the region that usually have very cold winters with mild weather in summers are encountered with problem. Although for supporting the hypothesis the prevalence of parasite in the definitive hosts like dogs and coyotes should also be examined. Inversely the finding shows warm and dry climate like Garmsar or mild climate like Babol regions, could serve as potent risk factors for *N. caninum* infection in cattle.

Table 2: Disturbution of seropositivity based on gestation number, abortion number and age

	Gestation NO.					Abortion NO.				Age (years)		
	1	2	3	4	5	0	1	2	3	≤3	4-5	≥6
Babol%	26(47.2)	15(27.3)	8(14.5)	4(7.2)	2(3.6)	9(15.4)	30(54.5)	13(23.6)	3(5.5)	18(32.7)	31(56.4)	6(10.9)
Garmsar%	17(44.7)	14(36.8)	4(10.5)	2(5.3)	1(2.6)	2(5.3)	28(73.7)	7(18.4)	1(2.6)	10(26.3)	20(52.6)	8(21)
Ardabil%	--	2(66.6)	1(33.3)	--	--	--	--	2(66.6)	1(33.3)	--	2(66.6)	1(33.3)

Whether the parasite life cycle or transmission could be affected by cold climate is not known. Previous reports showed the oocyst stage of the parasite are resistant to environmental conditions and converts to sporulation form after excretion from definitive host in as few as 24 h [14]. Few experimental infections of mice have showed tissue cysts of *N. caninum* were infective even after 13 month of subcutaneous inoculation and were resistant to 4°C for 7-14 days and were killed after 1 day exposure to 20°C [15], but nothing is known about the survival of *N. caninum* oocysts in the cold climate environmental conditions.

Other seropositivity risk factors: In one study, infected cows that had previously aborted had a 5.6-fold higher abortion risk than did congenitally infected cows that had not experienced an abortion before [16]. In the other studies, the risk of being seropositive also be increased with age or gestation number in beef and dairy cattle [17, 18], but we found a negative association between seroprevalence and gestation number. Seroprevalence was significantly higher in cows during first gestation and gradually decreased with elevation of the number. Seropositivity was also highest in cow experienced one abortion and was lowest in cows had 3 abortions in its life.

Murat has not detected association between age and seropositivity to *N. caninum* [19], but in our study we found significant increase in seropositivity in ages ranged from 4-5 years.

ACKNOWLEDGMENT

The authors would like to thank Mr. Hajiesmaili, Mr. Sharifian and Mr. Hasanzadeh for performing the laboratory procedure and Dr. Tavasolian and Mr. Khabaz for collecting the samples in Garmsar and Ardabil.

REFERENCE

- Dubey, J.P., A. Koestner and R.C. Piper, 1990. Repeated transplacental transmission of *Neospora caninum* in dogs. *J. Am. Vet. Med. Assoc.*, 197(7): 857-60.
- Gondim, L.F., M.M. McAllister, W.C. Pitt and D.E. Zemlicka, 2004. Coyotes (*Canis latrans*) are definitive hosts of *Neospora caninum*. *Int. J. Parasitol.*, 34(2): 159-61.
- Innes, E.A., 2007. The host-parasite relationship in pregnant cattle infected with *Neospora Caninum*. *Parasitol.*, 134(13): 1903-10.
- Williams, D.J., C.S. Hartley, C. Bjorkman and A.J. Trees, 2009. Endogenous and exogenous transplacental transmission of *Neospora caninum* - how the route of transmission impacts on epidemiology and control of disease. *Parasitol.*, 136(14): 1895-900.
- Dijkstra, T., T.J. Lam, C.J. Bartels, M. Eysker and W. Wouda, 2008. Natural postnatal *Neospora caninum* infection in cattle can persist and lead to endogenous transplacental infection. *Vet. Parasitol.*, 152(3-4): 220-5.
- Sadrebazzaz, A., H. Haddadzadeh, K. Esmailnia, G. Habibi, M. Vojgani and R. Hashemifesharaki, 2004. Serological prevalence of *Neospora caninum* in healthy and aborted dairy cattle in Mashhad, Iran. *Vet. Parasitol.*, 124(3-4): 201-4.
- Haddadzadeh, H.R., A. Sadrebazzaz, A. Malmasi, H. Talei Ardakani, P. Khazraii Nia and N. Sadreshirazi, 2007. Seroprevalence of *Neospora caninum* infection in dogs from rural and urban environments in Tehran, Iran. *Parasitol. Res.*, 101(6): 1563-5.
- Razmi, G.R., M. Maleki, N. Farzaneh, M. Talebkhan Garoussi and A.H. Fallah, 2007. First report of *Neospora caninum*-associated bovine abortion in Mashhad area, Iran. *Parasitol. Res.*, 100(4): 755-7.
- Malmasi, A., M. Hosseinienejad, H. Haddadzadeh, A. Badii and A. Bamonar, 2007. Serologic study of anti-*Neospora caninum* antibodies in household dogs and dogs living in dairy and beef cattle farms in Tehran, Iran. *Parasitol. Res.*, 100(5): 1143-5.
- Jenkins, M.C., J.A. Caver, C. Bjorkman, T.C. Anderson, S. Romand, B. Vinyard, A. Ugglä, P. Thulliez and J.P. Dubey, 2000. Serological investigation of an outbreak of *Neospora caninum*-associated abortion in a dairy herd in southeastern United States. *Vet. Parasitol.*, 94(1-2): 17-26.
- De Meerschman, F., C. Focant, J. Detry, C. Rettigner, D. Cassart and B. Losson, 2005. Clinical, pathological and diagnostic aspects of congenital neosporosis in a series of naturally infected calves. *Vet. Rec.*, 157(4): 115-8.
- Koiwai, M., T. Hamaoka, M. Haritani, S. Shimizu, T. Tsutsui and M. Eto, 2005. Seroprevalence of *Neospora caninum* in dairy and beef cattle with reproductive disorders in Japan. *Vet. Parasitol.*, 130(1-2): 15-8.

13. Bartels, C.J., J.I. Arnaiz-Seco, A. Ruiz-Santa-Quitera, C. Bjorkman, J. Frossling and D. von Blumroder, 2006. Supranational comparison of *Neospora caninum* seroprevalences in cattle in Germany, The Netherlands, Spain and Sweden. *Vet. Parasitol.*, 137(1-2): 17-27.
14. Lindsay, D.S., J.P. Dubey and R.B. Duncan, 1999. Confirmation that the dog is a definitive host for *Neospora caninum*. *Vet. Parasitol.*, 82(4): 327-33.
15. Lindsay, D.S., B.L. Blagburn and J.P. Dubey, 1992. Factors affecting the survival of *Neospora caninum* bradyzoites in murine tissues. *J. Parasitol.*, 78(1): 70-2.
16. Thurmond, M.C. and S.K. Hietala, 1997. Effect of congenitally acquired *Neospora caninum* infection on risk of abortion and subsequent abortions in dairy cattle. *Am. J. Vet. Res.*, 58(12): 1381-5.
17. Dyer, R.M., M.C. Jenkins, O.C. Kwok, L.W. Douglas and J.P. Dubey, 2000. Serologic survey of *Neospora caninum* infection in a closed dairy cattle herd in Maryland: risk of serologic reactivity by production groups. *Vet. Parasitol.*, 90(3): 171-81.
18. Rinaldi, L., G. Fusco, V. Musella, V. Veneziano, A. Guarino and R. Taddei, 2005. *Neospora caninum* in pastured cattle: determination of climatic, environmental, farm management and individual animal risk factors using remote sensing and geographical information systems. *Vet. Parasitol.*, 128(3-4): 219-30.
19. Murat, S. and G. Mehtap, 2003. Seroprevalence of *Neospora caninum* in Cattle in the Province of panlyurfa. *Turk. J. Vet. Anim. Sci.*, 29: 127-130.