SEROEPIDEMIOLOGICAL AND MOLECULAR IDENTIFICATION OF *Neospora caninum* IN CATTLE IN WASIT PROVINCE

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ABSTRACT

The main aim of present study was to identify the seroprevalence of *N. caninum* infections in cattle of some districts in Wasit province using a serological test (indirect-ELISA), and confirmation of seropositive results by a molecular PCR. In this study, the blood samples and epidemiological required data were collected from 327 animals during a period of September 2015 to May 2016. The overall results were revealed that 27.22% and 12.36% of study's cattle were positives with indirect ELISA and PCR, respectively.

Regarding to the epidemiological risk factors submitted for this study; the prevalence of seropositive rates was reported a statistically variable results. Among district factor, the seropositive results were 36.28%, 27.88%, 17.31% and 26.53% in Al-Azizyah, Al-Numaniyah, Al-Kut and Al-Hay districts, respectively. According to age factor, it was 18.58% and 31.78% in < 3 years and ≥ 3years groups, respectively; while in sex factor, males were reported 24.53% and females 27.74%. Relating to reproductive statement factor, the positive infections were 30.99% and 21.97% in aborted and non-aborted cows, respectively. Whereas in productivity nature factor; it was 38.24% and 19.37% in dairy and beef cattle, respectively.

INTRODUCTION

Neospora caninum is an obligate intracellular parasite of Apicomplexa phylum, which first discovered in dogs that have neurological complications in 1984 by Bjerkas *et al.*, (1) in Norway. Then, it's described in aborted cattle and isolated in cell culture in 1988 by Dubey *et al.*, (2). The parasite was demonstrated to affect on other species including

sheep, goat, camel, water buffalo, cat, deer and monkey. This parasite reported, worldwide, in Europe, Americas, Australia and Asia (3). In cattle, the parasite can be transmitted by consumption of contaminated foods with oocysts that excreted by final host, and via transplacental route during consecutive pregnancies to birth a congenitally infected newborn (4).

Although, neosporosis is generally latent, asymptomatic, or related to repeated abortions and stillbirth at full time, cow can birth a clinically healthy calve but persistently infected with pathogen (5). Bovine abortion, caused by *N. caninum*, can be occur sporadically especially in endemic areas or as epidemic pattern to resulting in a significant economic losses in cattle husbandry (6). As reported in several studies, the external source and the prior or congenitally infected animals are most likely the cause of abortion outbreaks (7, 8). Many direct and indirect economic losses for cattle neosporosis are showed, such as the costs of fetus's losses, decrease in milk production and weight gain, time of rebreeding, health and culling losses (9).

N. caninum is diagnosed, usually, through depending on clinical signs, pathological findings, immune-histochemical methods, tissue culture, serological and molecular techniques (10). Recently, different serological methods were applied directly or indirectly for detecting of infection (11). The indirect ELISA test has more advantages than other methods as the reaction is registered, objectively, and the results can read automatedly. Also, it is considered as one of the most suitable techniques in processing large number of samples (12). Molecularly, PCR was used to detect of *N. caninum* DNA in lung and/or brain of aborted or suspected neosporosis animals. However, (13) was inferred to that possibility of presence the circulating tachyzooites in blood of infected animals, which demonstrated by (14) in experimentally oral infected ewes. Then, the DNA of *N. caninum* was detected, successfully, in semen of naturally infected bulls by (15), and in blood of naturally infected heifers by (16).

The objectives of present study were to identify the seroprevalence of IgG *N. Caninum* antibodies in cattle of some districts in Wasit province by application of a commercially available indirect-ELISA and using PCR test. In addition, the study was directed toward

determining of relationships between the seropositivity with some epidemiological factors (districts, age, sex, reproductive statement and productivity nature).

MATERIALS AND METHODS

During of September 2015 to May 2016, a total of 327 cattle were selected randomly from some areas related to main districts (Al-Azizyah, Al-Numaniyah, Al-Kut and Al-Hay) in Wasit province. Blood samples were taken via jugular vein puncture under a septic condition using disposable syringes, transported into anticoagulant-free tubes, and then centrifuged at 4000 rpm for 15 minutes (**17**). The obtaining serums were installed into 1 ml micro-tubes and saved under -20°C until used, whereas, the tubes of whole blood samples were kept at 4°C for DNA extraction. In addition, the required data were obtained by clinical examination with assistance of the owners.

According to manufacturer's instructions, serum samples were analyzed using a commercially available *N. caninum* indirect ELISA kit (*Svanova Biotech AB, Sweden*). The DNA was isolated from whole blood samples of seropositive samples according to manufacturer's instruction of a DNAeasy blood kit (*Qiagen, Germany*). As described by (16), a pair of Np6 / Np21 primers {(5′ GGGTGTGCGTCCAATCCTGTAAC 3′) and (5′ CTCGCCAGTCAACCTACGTCTTCT 3′)} were used for amplification the DNA fragment at 357 bp. The negative and positive controls were introduced in all PCR reactions, and the amplification of products was stained by ethidium bromide, separated with 2% of agarose-gel electrophoresis, and viewed under Ultra-violet light.

Statistically, all data were tabled and analysed using of two computerized programs, Microsoft Office Excel (2013) and IBM SPSS (*v.23*). The significant differences of seropositive results and their association with data of risk factors were tested by an application of descriptive statistics at a level of $P \leq 0.05$ (18).

RESULTS

Out of (327) cattle tested by indirect-ELISA, the overall positive seroprevalence of cattle *N. caninum* infections were 89 (27.22 %), (Table 1).

Table (1): Total seroprevalence results of indirect-ELISA in 327 cattle

Total No.	Seropositives	Seronegatives
327	89 **(27.22 %)**	238 (72.78 %)

Among all seropositive samples were submitted for molecular examination by PCR technique that revealed on 11 (12.36%) positive samples, (Table 2).

Table (2): Total results of PCR technique in 89 seropositive cattle

Total No.	Positives PCR	Negatives PCR
89	11 **(12.36%)**	78 (87.64%)

Dealt with the positive samples that detected by application of PCR test in an overall 89 seropositive samples, which isolated by agarose-gel electrophoresis. The Lane M was showed the DNA maker (100-2000 bp), whereas, the Lanes (1-11) were represented the positive samples at 357 bp PCR product size of 2% agarose, 100 Volt and 80 Am for 1 hour, (Figure 1).

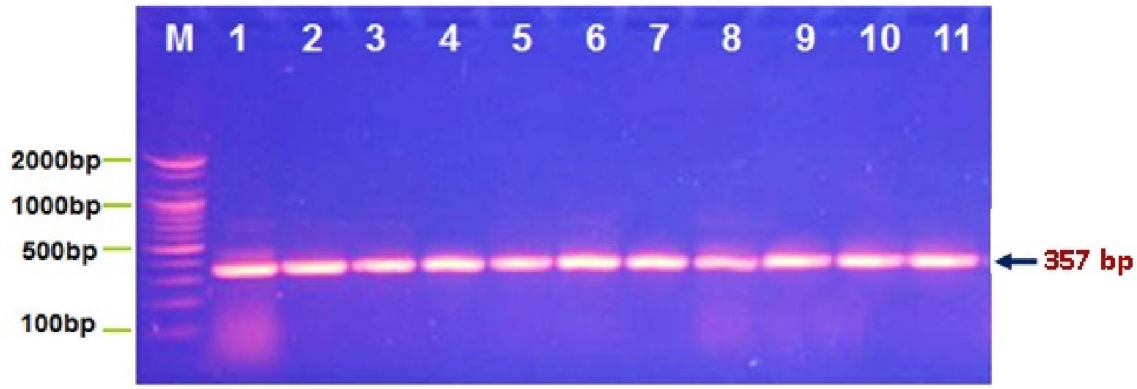

Figure (1): Agarose-gel electrophoresis of positive *N. caninum* samples

In present study, the epidemiological risk factors were studied to evaluate their associations with seropositive results. Among regions of samples collection, the results were revealed on 41/113 (36.28%), 17/61 (27.88%), 18/104 (17.31%) and 13/49 (26.53%) seropositive cattle in Al-Azizyah, Al-Numaniyah, Al-Kut and Al-Hay; respectively. According to age factor, the examined cattle were divided into < and ≥3 years groups that appeared on 21/113 (18.58%) and 68/214 (31.78%) seropositive cattle,

respectively. In sex factor, the males and females cattle were having 13/53 (24.53%) and 76/274 (27.74%) seropositives, respectively.

Regarding to adult cows only, the factor of reproductive statement were discussed in a total of 203 cows and the results reported that 22/71 (30.99%) and 29/132 (21.97%) of aborted and non-aborted cows, respectively, were seropositives. Relating to factor of productivity nature, seropositive neosporosis infections were detected in 52/136 (38.24%) and 37/191 (19.37%) of dairy and beef cattle, respectively.

Table (3): Association of epidemiological factors to seropositive results

Epidemiological risk factor		Total No.	Seropositives		Seronegatives
			No.	%	
District	Al-Azizyah	113	41	36.28 [a]	72 (63.72%)
	Al-Numaniyah	61	17	27.88 [b]	44 (72.13%)
	Al-Kut	104	18	17.31 [c]	86 (82.69%)
	Al-Hay	49	13	26.53 [b]	36 (73.47 %)
Age/Year	< 3	113	21	18.58 [b]	44 (88%)
	≥ 3	214	68	31.78 [a]	32 (64%)
Sex	Males	53	13	24.53 [a]	40 (75.47%)
	Females	274	76	27.74 [a]	198 (72.26%)
Reproductive Statement	Aborted	71	22	30.99 [a]	49 (69.01 %)
	Not-Aborted	132	29	21.97 [b]	103 (78.03 %)
Productivity Nature	Dairy	136	52	38.24 [a]	84 (61.76%)
	Beef	191	37	19.37 [b]	154 (80.63 %)

Within each factor, difference in small letters, vertically, refer to significant difference

DISCUSSION

Globally, several and various studies were carried out to detect the prevalence of bovine *N. caninum*, using different diagnostic tests that revealed on great variation in their prevalence between countries, regions as well as between herds (19). However, the total seropositive result of this study was higher than those reported by (20) and (21).

In addition, the PCR reaction and subsequent sequence analysis was clearly demonstrate the presence of DNA *N. caninum* in whole blood samples for first time in Iraq. This successful in detection of DNA was expected because of the blood was seemed to provide a transport media for *Neospora* tachyzoites between body tissues (16). Among

most diagnostic techniques, PCR is more sensitivity and specificity than other tests and less to be affected by autolysis or postmortem changes. In addition, it can be apply for identification of *N. caninum* DNA in blood, semen, brain, spinal cord, different fetal fluids, embryonic tissues, and even oocysts in feces of final host (16, 22, 23). The current results were demonstrated that in Iraq, the seroprevalence of neosporosis has a wide range in infection rates with presence of contrast between study's districts. This finding might be attributed to inequality of applied techniques and/or their cut-offs, origin of evaluated herds and probability of frequent exposing to sources of infection (24). As well as, the increasing of seroprevalence could be occur because discrepancy of animal housing or management, herds that involved in a study, increasing exposure to definitive host or intermediate hosts, and the contact, directly or indirectly, to adjacent endemic areas (25, 26).

The risk of cattle for being seropositive might be increase, decrease or had not effect with advancing of age or gestation. The current study was showed a marked rising in seropositivity with an increasing of age. This finding was in agreement with those reported by (27) and (28), and disagreement with (3). Recently, European study observed that the seropositivity has an increased in certain life age and decreased in other; while in another, it's revealed that the effect of age on seropositivity may vary in different study areas (19). While, (29) and (30) were detected that, for most herds, the levels of *N. caninum* seroprevalence were in equal across all age groups.

The significant differences in seroprevalence of antibodies against *N. caninum* in both sexes were similar. It might be indicating for expositing of both sexes to infected parasite at the same level. Also, the low number of study's males submitted for indirect-ELISA testing could be played role in received results.

In this study, the higher seroprevalence of *N. caninum* antibodies in adult cows with a history of abortion compared to those without abortion referred to that *N. caninum* might play an important role in occurrence of abortion in cows. Although, (31) and (32) suggested that the probability of abortion in seropositive cows had an increased for several folds in mid or late stages of pregnancy but not in seronegatives or early infected cows. Whereas, (33) was observed a marked increasing in abortion risk during the early

stage of it. However, sero-epidemiological studies agreed that most seropositive cows might be at high risk for early embryonic death, stillbirth or birth of a feeble abnormal calf, birth of a normally infected calf with no obvious effect, culling as well abortions in both dairy and beef cattle (34, 35).

The current results showed that the seropositivity in dairies was more than in beeves cattle. This finding is in agreement with (36, 37). The differences, in seroprevalence, observed between dairy and beef cattle might be caused by the stress of high milk production that accompanied by a normally depression in immunity especially during mid-gestation and an elevating of dairy's age (38, 39). As well as, the differences in management practice between both types that included variation in feeding or grazing system, frequent regular herd movement, and high herding density (40, 41).

In conclusion, the results of present study were exhibited on high prevalence of *N. caninum* in cattle of some districts in Wasit province. In addition, both applied diagnostic methods, indirect-ELISA and PCR, were showed a reasonable efficacy in detection of specific-species *Neospora*-IgG antibodies and DNA in blood samples. Although, association of some epidemiological factors was discussed in this study, further investigations are required to evaluate the definite role of *N. caninum* in other herds, regions or provinces.

الكشف المصلي الوبائي والجزيئي للنيوسبورا الكلابية في الابقار في محافظة واسط

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الخلاصة

يعد الهدف الرئيسي من الدراسة الحالية الى تحديد الانتشار المصلي لاصابات النيوسبورا الكلابية في الابقار في بعض المناطق التابعة لمحافظة واسط / العراق ، باستعمال اختبار المصلي (الاليزا غير المباشر) وتثبيت النتائج الموجبة مصليا بواسطة تفاعل السلسلة متعدد البلمرة . في هذه الدراسة ، تم جمع عينات الدم والبيانات الوبائية المطلوبة من ٣٢٧ حيوان خلال الفترة من ايلول ٢٠١٥ الى ايار ٢٠١٦ . كشفت النتائج الكلية ان ٢٧.٢٢% و

١٢.٣٦% من ابقار الدراسة كانت موجبة مع اختباري الاليزا غير المباشر وتفاعل السلسلة متعدد البلمرة ، على التوالي .

مايخص عوامل الخطر الوبائية الخاضعة لهذه الدراسة ، سجلت معدلات الانتشار الموجب مصليا نتائج احصائية متباينة . في عامل المنطقة ، بلغت النتائج المصلية الموجبة ٣٦.٢٨% و ٢٧.٨٨% و ١٧.٣١% و ٢٦.٥٣% في مدن العزيزية والنعمانية والكوت والحي ، على التوالي . اعتمادا على عامل العمر ، فقد بلغت ١٨.٥٨% و ٣١.٧٨% في مجموعتي > ٣ سنوات و ≤ ٣ سنوات ، على التوالي ؛ بينما في عامل الجنس ، فقد سجلت الذكور ٢٤.٥٣% والاناث ٢٧.٧٤% . مايتعلق بعامل الحالة التكاثرية ، فقد بلغت الاصابات الموجبة ٣٠.٩٩% و ٢١.٩٧% في الابقار المجهضة وغير المجهضة ، على التوالي . بينما بلغت اعتمادا على عامل طبيعة الانتاج ٣٨.٢٤% و ١٩.٣٧% في ابقار الحليب واللحم ، على التوالي .

REFERENCES

1. Reichel MP., Ayanegui-Alcérreca MA., Gondim LF., and Ellis, JT. (2013): What is the global economic impact of *Neospora caninum* in cattle - the billion dollar question. Int. J. for Parasitol, 43(2), 133-142.
2. Dubey JP., Barr BC, Barta JR., Bjerkås I., Björkman C., Blagburn BL., Bowman DD., Howe DK., Jenkins MC., Kobayashi Y., Koudela B., Marsh AE., Mattsson JG., Speer CA., Trees AJ., Uggla A., Upton SJ., Williams DJ., and Lindsay, DS. (2002): Redescription of *Neospora caninum* and its differentiation from related coccidia. Int. J. Parasitol. 32, 929-946.
3. Bartels CJ., Arnaiz-Seco JI., and Ortega-Mora, LM. (2006): Supranational comparison of *Neospora caninum* seroprevalences in cattle in Germany, The Netherlands, Spain and Sweden. Vet. Parasitol., 137(1), 17-27.
4. Buxton D., McAllister M.M., and Dubey, JP. (2002): The comparative pathogenesis of neosporosis. Trends in parasitology, 18(12), 546-552.
5. Gharekhani J., and Tavoosidana, G. (2013): Serological survey of *Neospora caninum* (Sarcocystidae) infection in beef cattle from western Iran: a serological study. Scientia Parasitologica, 14, 95-98.
6. Atkinson RA., Cook RW, Reddacliff LA., Broady KW., Harper PW., and Ellis, JT. (2000): Seroprevalence of *Neospora caninum* infection following an abortion outbreak in a dairy cattle herd. Aust. Vet. J. 78, 262-266.
7. Thilsted JP., and Dubey, JP. (1989): Neosporosis-like abortions in a herd of dairy cattle. J. Vet. Diagn. Invest 1, 205-209.

8. Anderson ML., Blanchard PC., Dubey JP., Hoffman RL., and Conrad, PA. (1991): *Neospora* - like protozoan infection as a major cause of abortion in California dairy cattle, J. Am. Vet. Med. Assoc. 198, 241-244.

9. Trees AJ., Davison HC., Innes EA., and Wastling, JM. (1999): Towards evaluating the economic impact of bovine neosporosis. Int. J. for Parasitol., 29(8), 1195-1200.

10. Pereira-Bueno J., Quintanilla-Gozalo A., Alvarez-Garcıa G., Collantes-Fernández E., and Ortega-Mora, LM. (2003): Evaluation by different diagnostic techniques of bovine abortion associated with *Neospora caninum* in Spain. Vet. Parasitol., 111(2), 143-152.

11. Ortega-Mora LM., Fernández-García A., and Gómez-Bautista, M. (2006): Diagnosis of bovine neosporosis: recent advances and perspectives. Acta Parasitol., 51(1), 1-14.

12. Aguado-Martínez A., Álvarez-García G., Innes E., and Ortega-Mora, LM. (2005): Use of avidity enzyme linked immunosorbent assay and avidity western blot to discriminate between acute and chronic *Neospora caninum* infection in cattle. J. of Vet. Diag. Invest., 17, 442-450.

13. Williams DJ., Guy CS., Smith RF., Guy F., Mc Garry JW., McKay JS., and Trees, AJ. (2003). First demonstration of protective immunity against foetopathy in cattle with latent *Neospora caninum* infection. International Journal for Parasitology, 33 (10), 1059-1065.

14. O'Handley R., Liddell S., Parker C., Jenkins MC., and Dubey, JP. (2002). Experimental infection of sheep with *Neospora caninum* oocysts. Journal of Parasitology, 88(6), 1120-1123.

15. Ortega-Mora LM., Ferre I., Caetano-da-Silva A., Collantes-Fernández E., Regidor Cerrillo J., and Aduriz, G. (2003). Detection of *Neospora caninum* in semen of bulls. Veterinary Parasitology, 117(4), 301-308.

16. Okeoma CM., Williamson NB., Stowell KM., and Gillespie, L. (2004). The use of PCR to detect Neospora caninum DNA in the blood of naturally infected cows. Veterinary parasitology, 122(4), 307-315.

17. Duncan JR., and Prasse, KW. (2003): Veterinary Laboratory Medicine: Clinical Pathology, 4th edition Ames: Blackwell: 3-45.

18. Petrie A., and Watson, P. (2006). Statistics for Veterinary and Animal Science, Second Edition. Ames: Blackwell Publishing, Pp: 24-112.

19. Dubey JP., Schares G., and Ortega-Mora, LM. (2007): Epidemiology and control of neosporosis and *Neospora caninum*. Clin. Rev, 20(2), 323-367.

20. Alhindawe, AJ. (2010): Seroprevalence of *Neospora caninum* in cattle in some provinces in Iraq, 4th Sci. Egypt. Soc. Anim. Manag. 25, 189-200.

21. Mallah MO., Dawood KA., and Alrodhan, MA. (2012): Seroepidemiological study for the prevalence of *Neospora caninum* in Dairy & Beef cattle in some Iraqi provinces. AL-Qadisiya J. Vet. Med. Sci., 11 (1), 103-110.

22. Razmi, G. (2009). Fecal and molecular survey of *Neospora caninum* in farm and household dogs in Mashhad area, Khorasan province, Iran. The Korean journal of parasitology, 47 (4), 417.

23. Kamali A., Razmi GR., and Naseri, Z. (2014). Histopathological and molecular study of *Neospora caninum* infection in bovine aborted fetuses. Asian Pacific Journal of Tropical Biomedicine, 4 (12), 990-994.

24. Moura AB., Souza AP., Sartor AA., and Heusser Junior, A. (2011): *Neospora caninum* antibodies and risk factors in dogs from Lages and Balneário Camboriú, SC. Arqu. Brasil de Med. Vet. Zoo, 63(1), 262-265.

25. Hässig M., and Gottstein, B. (2002): Epidemiological investigations of abortions due to *N. caninum* on Swiss dairy farms. Vet. 150, 538-542.

26. Celik HA., Kozan E., Eser M., Yilmaz O., and Sarimehmetoğlu, HO. (2013): A research on seroprevalence of *Neospora caninum* in cattle. Ankara Üniversitesi Veteriner Fakültesi Dergisi, 60 (2), 99 -102.

27. Davison HC., French NP., and Trees, AJ. (1999): Herd-specific and age-specific seroprevalence of *Neospora caninum* in 14 British dairy herds. The Veterinary Record, 144(20), 547-550.

28. Wanha K., Edelhofer R., Gabler-Eduardo C., and Prosl, H. (2005): Prevalence of antibodies against *Neospora caninum* and *Toxoplasma gondii* in dogs and foxes in Austria. Vet. Parasitol., 128(3), 189-193.

29. Sadre-bazzaz AH., Esmailnia H., Habibi K., and Hashemi fesharaki, RM. (2004): Serological prevalence of *Neospora caninum* in healthy and aborted dairy cattle in Mashhad, Iran. Vet. Parasitol., 124, 201-204.

30. Sánchez GF., Morales SE., Martínez MJ., and Trigo, JF. (2003): Determination and correlation of anti *Neospora caninum* antibodies in dogs and cattle from Mexico. Can Vet. J., 67(2), 142.

31. Pare´ J., Thurmond MC., and Hietala, SK. (1997): *Neospora caninum* antibodies in cows during pregnancy as a predictor of congenital infection and abortion. J. Parasitol. 83,82-87.

32. Lo´pez-Gatius .F, Pabo´n M., and Almerı´a, S. (2004): *Neospora caninum* infection does not affect early pregnancy in dairy cattle. Theriogenology 62, 606-613.

33. Pérez E., Gonzalez O., Barr B., and Conrad, PA. (1998): First report of bovine neosporosis in dairy cattle in Costa Rica. Vet. Rec. 142, 520-521.

34. Romero-Salas D., Garcia-Vazquez Z., and Cruz-Vazquez, C. (2010): Seroprevalence of *Neospora caninum* antibodies in cattle in Veracruz, Mexico. J. of Animal and Vet. Advances, 9(10), 1445-1451.

35. Fort M., Edelsten M., and Innes, E. (2015): Seroepidemiological study of *Neospora caninu*m in beef and dairy cattle in La Pampa, Argentina. Acta Parasitologica, 60(2), 275-282.

36. Haddad JPA., Dohoo IR., and VanLeewen, JA. (2005): A review of *Neospora caninum* in dairy and beef cattle - a Canadian perspective. Can Vet J, 46(3), 230-243.

37. Gharekhani J., and Bahonar, A. (2014): Prevalence of immunoglobulin G (IgG) antibody to *Neospora caninum* in dairy cattle of Hamedan province, west of Iran. In Veterinary research forum: Faculty of Veterinary Medicine, Urmia University, Urmia, Iran. Int. quarterly J., 5, (2), p. 149).

38. Moore DP., Campero CM., Posso MA., Cano D., Leunda MR., and Späth, E. (2002): Seroepidemiology of beef and dairy herds and fetal study of *Neospora caninum* in Argentina. Vet. Parasitol, 107(4), 303-316.

39. Koiwai M., Hamaoka T., Shimizu S., Tsutsui T., Eto M., and Yamane, I. (2005): Seroprevalence of *Neospora caninum* in dairy and beef cattle with reproductive disorders in Japan. Vet. parasitol., 130(1), 15-18.

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

41. Mondragón-Zavala K., Cruz-Vázquez C., Medina-Esparza L., Ramos-Parra M., and García -Vázquez, Z. (2011): *Neospora caninum* infection in beef cattle reared under grazing conditions in north-central Mexico. Revista MVZ Córdoba, 16(2), 2484-2490.