



Neospora caninum serostatus is affected by age and species variables in cohabiting water buffaloes and beef cattle



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ABSTRACT

The aim of this study was to investigate how *Neospora caninum* serostatus may be affected by variables such as host species (water buffaloes or cattle) and age in animals cohabiting in the same ranch. A convenience cross-sectional study was performed on four ranches in the Northeast of Argentina, where water buffalo are cohabiting with beef cattle. Blood samples were collected from 1350 female water buffaloes (*Bubalus bubalis*) and 880 female beef cattle (*Bos taurus* and *Bos indicus* crossbreeds) from four ranches. Calving and weaning percentages at herd level for each ranch were also recorded. *N. caninum* antibody levels were measured by an indirect fluorescent antibody test (IFAT) (reciprocal antibody titers ≥ 100). Serological results were classified into 2 categories (0: negative; 1: positive). A logistic regression model was used to describe the relationship between *N. caninum* serostatus and specie (water buffalo or cattle), age or ranch and their interactions. Likelihood ratio tests were used to assess the significance of the model and their terms. Odds ratios were estimated and 95% profile likelihood (LR) and Wald confidence intervals (CI) obtained. Overall, specific antibody titers were found in 43.3% (584/1350) of water buffaloes and 28.6% (252/880) of cattle. Seropositive water buffaloes and cattle were observed on all ranches. Age was statistically significant ($p = 0.01$) with an overall estimate of logit (log odds) of age of 0.03 for both species. This indicates that for every one year increase in age, the expected change in log odds of being seropositive increased by 0.03. On three of four ranches a water buffalo was 4.48, 1.54 and 2.25 times more likely to be seropositive than cattle for animals of the same age. The *N. caninum* serostatus was affected by age in the first place, but also by species on at least three of the four ranches. Calving and weaning percentages were higher in water buffaloes than in beef cattle ($p < 0.05$). Even though the low pathogenicity that *N. caninum* seems to have in water buffaloes, this study reinforces the importance of this specie as maintenance of the disease.

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1. Introduction

Neospora caninum is a coccidian parasite that causes costly disease in cattle (Reichel et al., 2013) and dogs worldwide (Dubey et al., 2007). Transplacental transmission appears to be the main mechanism by which the

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parasite persists in cattle (Anderson et al., 2000). Nevertheless, after recognizing the dog as the definitive host of the parasite (McAllister et al., 1998), epidemiological work has also established the association between the presence of dogs and the disease in cattle (Dubey et al., 2007). In addition, it has been established that intensive herd management may be associated with increased seroprevalence to *N. caninum* (Sanderson et al., 2000; Barling et al., 2000; Dubey et al., 2007). Many advances in the understanding of the epidemiology of *N. caninum* have been achieved in the last decades (Dubey et al., 2007), nevertheless, the frequency of postnatal transmission is not fully understood.

Evidence of *Neospora*-infections has been reported for many domestic and wild species including water buffaloes (*Bubalus bubalis*) (Dubey et al., 1998; Huong et al., 1998; Meenakshi et al., 2007; Nasir et al., 2011) where vertical transmission also occurs naturally (Chrysafidis et al., 2011). An increasing prevalence of antibodies to *N. caninum* with age was found in water buffalo populations from Argentina (Campero et al., 2007), southeastern Brazil (Fujii et al., 2001), southern Italy (Guarino et al., 2000) and Southern Peninsular India (Sengupta et al., 2012). It has also been demonstrated that this species is susceptible to experimental *Neospora*-infection causing fetal death (Konrad et al., 2012) and the occurrence of spontaneous abortions caused by *N. caninum* buffaloes has been recently reported (Auriemma et al., 2014).

Water buffaloes are better adapted to the wet tropical and subtropical environment than cattle and the water buffalo population is growing in many areas of the world (Food and Agriculture Organization, 2012) including the North East of Argentina (NEA) (Campero et al., 2007). Although a high seroprevalence to *N. caninum* in water buffaloes was previously described in the NEA (Campero et al., 2007), it is still uncertain whether neosporosis is a serious cause of reproductive failure in this species. The aim of this study was to investigate how *N. caninum* serostatus may be affected by variables such as species (water buffaloes or cattle) and age in animals cohabiting the same area. Also reproductive and productive parameters for water buffaloes and beef cattle at herd level are described.

2. Material and methods

2.1. Animals and sampling

A convenience cross-sectional study was performed on four ranches in the NEA, where water buffalo are cohabiting with beef cattle. Descriptive data about area, total number of animals and reproductive parameters including calving and weaning percentages at herd level for each ranch were recorded.

Blood samples were collected from a jugular vein from 1355 female water buffaloes (*B. bubalis*) and 880 female beef cattle (*Bos taurus* and *Bos indicus* crossbreeds). Samples were obtained at the end of the calving season and before the breeding season starts; March–April for water buffaloes and October–November for beef cattle. The age of the animals was also recorded at the moment of sampling

and ranged from 2 to 19 and 3 to 8 years old for water buffaloes and beef cattle, respectively. The serum samples were kept at -20°C until analysis.

2.2. Serological analysis

2.2.1. Parasites and antigen slide preparation

Antigen slides were prepared using tachyzoites of the NC-1 *N. caninum* strain kindly provided by Dr MC Venturini, La Plata Veterinary College, Argentina. They were grown continuously in stationary monolayer cultures of VERO cells as described previously (Konrad et al., 2012). Culture medium consisted of Dulbecco's Minimum Essential Medium (DMEM) supplemented with 10% (v/v) heat-inactivated adult equine serum, 2 mM-glutamine, 50 U/ml penicillin, and 50 $\mu\text{g}/\text{ml}$ streptomycin (DMEM-HS). Parasite-infected cultures were maintained in 75 cm^2 flasks incubated at 37°C in an atmosphere of 5% CO_2 . Parasites were harvested for antigen preparation when 80% of the VERO cells in the culture flask were infected with clusters of tachyzoites. The infected monolayer was removed from the flask by scraping into the medium and then passed 3 times through a 25-ga needle to disrupt the cells. The suspension was passed through a 5- μm filter to remove cellular debris, and tachyzoites were pelleted by centrifugation at 1300 g for 10 min. After removing the supernatant, the pellet was washed twice in sterile phosphate-buffered saline (PBS) (pH 7.2) and then resuspended in a modified PBS saline (137 mM NaCl, 3 mM KCl, 3 mM $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7\cdot 2\text{H}_2\text{O}$, 0.4 mM $\text{NaH}_2\text{PO}_4\cdot \text{H}_2\text{O}$, 12 mM NaHCO_3 , 6 mM glucose) to a final concentration of approximately 10^7 tachyzoites/ml. Aliquots of 10 μl of tachyzoite suspension were dispensed into each 4-mm well on 12-well heavy-teflon-coated antigen slides. Slides were air dried at room temperature (RT) and stored at -20°C .

2.2.2. Indirect fluorescent antibody test (IFAT)

N. caninum antibody levels either from water buffaloes or beef cattle were assayed by IFAT as previously mentioned (Fujii et al., 2001). Briefly, antigen slides and sera were thawed at RT prior to use. The serum samples were tested at a dilution of 1:100 (Rodrigues et al., 2004). Ten microliters of diluted test or control sera were placed in separate wells on the antigen slides. Positive and negative control sera from buffalo were provided by Dr. S.M. Genari, Veterinary College, São Paulo University, Brazil. Slides were incubated at 37°C for 1 h in a moist chamber, washed 3 times for 5 min each in PBS, and then tapped gently to remove excess PBS. A polyclonal rabbit anti-bovine IgG labeled with fluorescein isothiocyanate (Sigma, St. Louis, MO) diluted 1:200 in PBS was added in 10 μl aliquots to each well. Slides were incubated at 37°C for 30 min, washed 3 times with PBS for 5 min each, tapped to remove excess PBS, cover-slipped with buffered glycerol (25% [w/v] glycerine in Tris-HCl [pH 9.0]), and examined at 200x using a fluorescence microscope. The IFAT was considered to be positive when the typical peripheral staining pattern of the tachyzoites was observed.

Table 1Numbers and proportions of *N. caninum* seropositive water buffalo and bovine females on four ranches in the Northeast region of Argentina tested by IFAT.

| Ranch | <i>Bubalus bubalis</i> | | | <i>Bos taurus</i> and <i>Bos indicus</i> crossbreeds | | |
|-------|------------------------|-----------------|------------------------|--|-----------------|------------------------|
| | Stock n | Tested n (%) | Positive n (%) | Stock n (%) | Tested n (%) | Positive n (%) |
| 1 | 355* | 133(37.5) | 81(60.9) ^a | 780 | 73(9.3) | 9(12.3) ^b |
| 2 | 1100 | 720(65.5) | 378(52.5) ^a | 7000 | 507(7.2) | 202(39.8) ^b |
| 3 | 450 | 233(51.7) | 54(23.2) ^a | 530 | 65(12.3) | 8(12.3) ^a |
| 4 | 450 | 264(58.6) | 71(26.9) ^a | 2500 | 235(9.4) | 33(14) ^b |
| Total | 2355 | 1350(57.3) | 584(43.3) | 10,810 | 880(8.1) | 252(28.6) |

* Water buffalo dairy farm.

Different superscript letters within the same row represent statistical differences ($p < 0.05$) between proportions of seropositive water buffaloes and beef cattle.

2.3. Statistical analysis

Serological results were classified into 2 categories (0: negative; 1: positive) according to the absence or presence of specific antibodies. A logistic regression model was used to describe the relationship between *N. caninum* serostatus and variables like specie (water buffaloes or cattle) and age and their interactions. Likelihood ratio tests were used to assess the significance of the model and their terms. From the resulting logistic model, odds ratios were estimated and 95% profile likelihood (LR) and Wald confidence intervals (CI) obtained. All analyses were done in R version 2.14.1 (R-Development Core Team, 2011). Proportions for reproductive and productive parameters were compared between water buffaloes and cattle in each ranch. All analyses were done in R version 2.14.1 (R-Development Core Team, 2011) and a significance level of 0.05 was used.

3. Results

Overall, specific antibody titers were found in 43.3% (584/1350) of water buffaloes and 28.6% (252/880) of cattle. Seropositive water buffaloes and beef cattle were observed on all ranches. Data about animal stock, number of tested animals and proportions of seropositive animals for both water buffaloes and beef bovine females per each ranch are presented in Table 1. Data regarding the number of mated cows, calving and weaning percentages at herd level are shown in Table 2.

The distributions of *N. caninum* seropositive animals by ranch, species and age are shown in Fig. 1. From the logistic model, neither triple nor double interactions with age ($p > 0.05$) were detected. In a reduced model without those

interactions, age was statistically significant ($p = 0.01$) with an overall estimate of logit (log odds) of age of 0.03. This indicates that for every one year increase in age, the expected change in log odds of being seropositive (vs non-positive) increase by 0.03. Moreover, for every one year increase in age, the estimated odds of being seropositive is 1.035 (holding ranch and species fixed) indicating that odds of being serologically positive to *N. caninum* increased 3.5% per year, independent of species and ranch. Both, LR and Wald 95% CI for the odds are (1.006, 1.065).

It is noteworthy that on 3 of 4 ranches, a water buffalo was 4.48, 1.54 and 2.25 times more likely to be seropositive than cattle, for animals of the same age. The LR and Wald 95% CI for these odds ratios are shown in Fig. 1. Unexpectedly in ranch #3 there was no association between serological status and species (holding age fixed), i.e. the odds of being serologically positive to *N. caninum* is the same for water buffaloes and cattle, when animals have the same age. In particular, odds ratio did not differ from 1 (LR 95% CI = (0.87, 4.5), Wald 95% CI = (0.83, 4.21)).

4. Discussion

In agreement with the results obtained in this study, most literature shows a higher prevalence for *N. caninum* in water buffaloes than in cattle (Guarino et al., 2000; Fujii et al., 2001; Gennari et al., 2005; Dubey et al., 2007; Nasir et al., 2011). In the present study, specific antibody titers were detected in 43.3% and 28.6% of water buffaloes and cattle cohabiting in the same area. It is noteworthy that, despite the high prevalence of antibodies against *N. caninum*, reproductive and productive parameters in the water buffaloes from all ranches evaluated here were

Table 2

Reproductive and productive parameters in water buffalo and beef bovine females at herd level in four ranches at the Northeast of Argentina.

| Ranch (hectares) | <i>Bubalus bubalis</i> | | | <i>Bos taurus</i> and <i>Bos indicus</i> crossbreeds | | |
|------------------|------------------------|------------------------|----------------------|--|-------------------------|------------------------|
| | Impregnated n | Calving n (%) | Weaning n (%) | Impregnated n | Calving n (%) | Weaning n (%) |
| 1 (2300) | 220* | 172(78.2) ^a | 165(75) ^a | 520 | 322(62) ^b | 286(55) ^b |
| 2 (17,000) | 825 | 625(75.8) ^a | 619(75) ^a | 4000 | 2360(59) ^b | 2200(55) ^b |
| 3 (5200) | 320 | 230(71.8) ^a | 224(70) ^a | 400 | 248(62) ^b | 228(57) ^b |
| 4 (4500) | 250 | 200(80.0) ^a | 190(76) ^a | 1400 | 1029(73.5) ^b | 981(70.1) ^a |
| Total | 1615 | 1227(76.0) | 1198(74.2) | 6320 | 3959(62.6) | 3695(58.5) |

* Water buffalo dairy ranch.

Different superscript letters within the same row represent statistical differences ($p < 0.05$) between calving or weaning for water buffaloes and beef cattle, respectively.

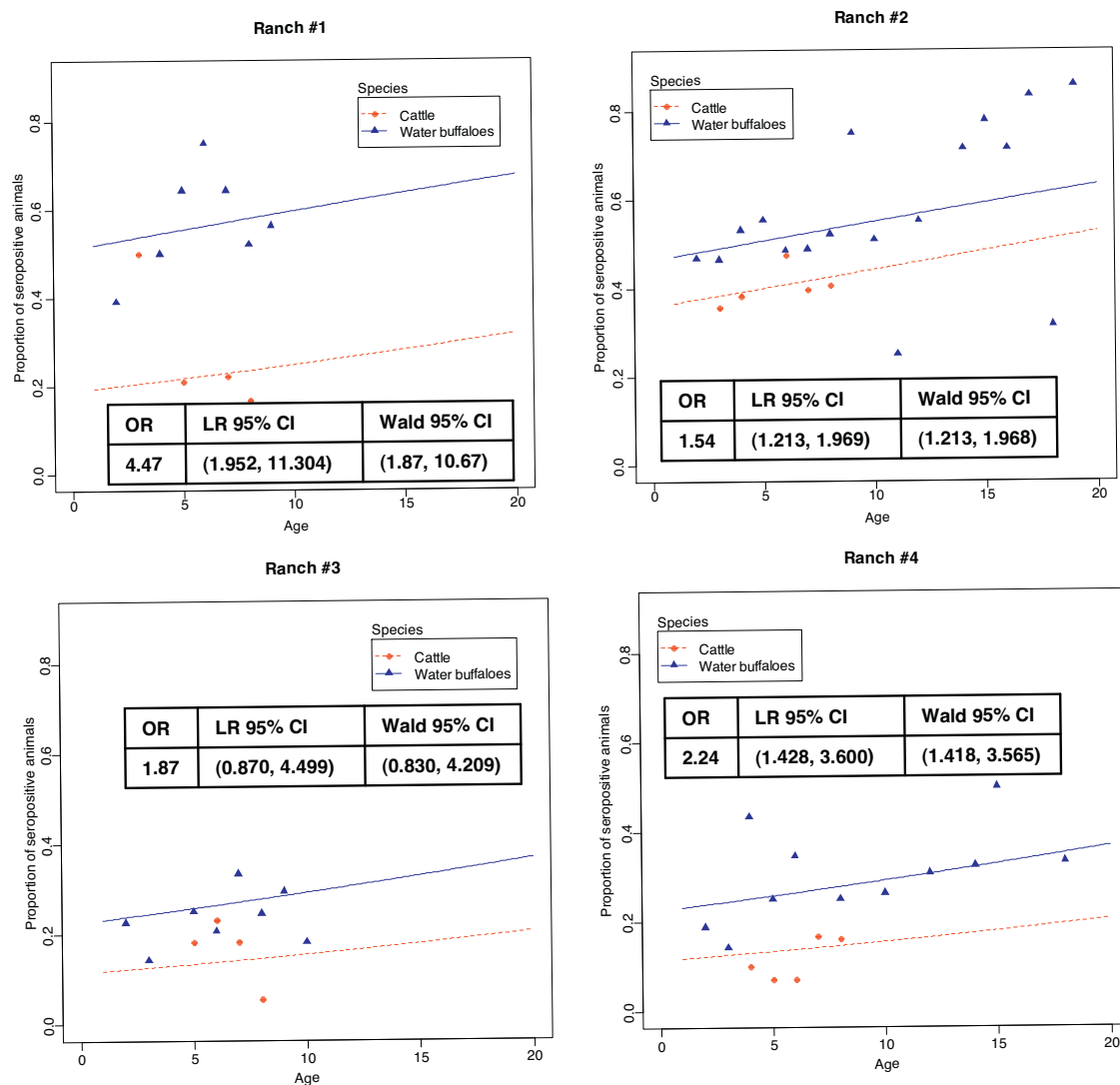


Fig. 1. Observed and estimated probability of *N. caninum* seropositive animals for each species, age and ranch obtained from the logistic model. Odds ratio (OR) estimates and profile likelihood (LR) and Wald 95% confidence intervals for OR of being serologically positive to *N. caninum* between water buffaloes and beef cattle.

reported to be normal. *Neospora*-like cysts in two aborted buffalo fetuses showing non-suppurative encephalitis and myocarditis have been reported (Guarino et al., 2000). Vertical transmission appears to occur naturally in water buffaloes (Chrysafidis et al., 2011) and this species is susceptible to experimental *Neospora*-infection causing fetal death (Konrad et al., 2012). Abortions caused by *N. caninum* have been recently reported in water buffaloes (Auriemma et al., 2014). Also, Nasir et al. (2011) report a significantly higher sero-prevalence in aborting buffalo in Pakistan over non-aborting ones. *Neospora*-exposure was tested in dairy cattle and water buffaloes in Southern Peninsular India but an association between reproductive failure and presence of antibodies against to *N. caninum* was only mentioned for cattle (Sengupta et al., 2012).

Considering that isolation of *N. caninum* by dog bioassay was possible in 5 of 6 water buffaloes that were seropositive at a serum dilution of 1:100 (Rodrigues et al.,

2004), that serological dilution was used in the present work. Prevalence rates obtained by different assays are not comparable but they varied from zero when using an enzyme-linked immunosorbent assay (ELISA) in water buffaloes in the People's Republic of China (Yu et al., 2007) or 1.5% of 200 animals by IFAT in Vietnam (Huong et al., 1998) to 9.9% of 341 and 54.7% of 300 water buffaloes by a competitive ELISA in Southern Peninsular India (Sengupta et al., 2012) and the Lahore District of Punjab Province, Pakistan (Nasir et al., 2011), respectively. Whether the diagnostic sensitivity and specificity of the IFAT at a dilution of 1:100 varies depending on the species tested (water buffaloes or cattle), remains to be clarified.

The *N. caninum* serostatus was primarily affected by age, but also by species on at least three of the four ranches investigated. The odds of being serologically positive to *N. caninum* increased by 3.5% per year in the populations of

buffaloes and cattle assessed in this study. Older animals can be expected to have a higher probability of having been exposed to oocysts at least once during their lifespan compared with younger animals. An association between the presence of antibodies to *N. caninum* and age has been reported from buffalo populations in southern Italy (Guarino et al., 2000) and southeastern Brazil (Fujii et al., 2001). More over, this finding is in agreement with that reported by Sengupta et al. (2012), who mentioned that the highest seroprevalence was recorded in dairy cattle and water buffaloes of ≥ 4 years old. Nasir et al. (2011) also reported that the highest sero-prevalence was observed in buffaloes older than 3–5 years of age, and those with contact to farm dogs. On the other hand, such association with age was not found in water buffaloes from northern Brazil (Gennari et al., 2005). Which route of infection (vertical or post-natal transmission) is the more frequent in water buffaloes remains to be studied. On the other hand the present study also provides evidence of increased exposure to this coccidian parasite in older beef cattle. Postnatal transmission occurs in dairy cattle in Argentina (Moré et al., 2009, Moore et al., 2009) but a higher risk of being seropositive in older beef cattle is reported here for the first time. Evidence of postnatal point-source exposure to *N. caninum* and protective immunity in previously infected beef cows having antibodies with high avidity have been well documented (McAllister et al., 2000).

Although *N. caninum* does not appear to be a frequent abortifacient in water buffaloes, more studies need to be performed in order to know all the consequences of *Neospora*-infections in this species. On the other hand, the epidemiological role of infected buffaloes and the consequences for cattle cohabiting the same ranch should be also considered when performing management control of bovine neosporosis.

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References

- Anderson, M.L., Andrianarivo, A.G., Conrad, P.A., 2000. Neosporosis in cattle. *Anim. Reprod. Sci.* 60–61, 417–431.
- Auriemma, C., Lucibelli, M.G., Borriello, G., De Carlo, E., Martucciello, A., Schiavo, L., Gallo, A., Bove, F., Corrado, F., Girardi, S., Amoroso, M.G., Degli Uberti, B., Galiero, G., 2014. PCR detection of *Neospora caninum* in water buffalo foetal tissues. *Acta Parasitol.* 59, 1–4.
- Barling, K.S., Sherman, M., Peterson, M.J., Thompson, J.A., McNeill, J.W., Craig, T.M., Garry Adams, L., 2000. Spatial associations among density of cattle, abundance of wild canids, and seroprevalence to *Neospora caninum* in a population of beef calves. *J. Am. Vet. Med. Assoc.* 217, 1361–1365.
- Campero, C.M., Pérez, A., Moore, D.P., Crudeli, G., Benitez, D., Draghi, M.G., Cano, D., Konrad, J.L., Odeón, A.C., 2007. Occurrence of antibodies against *Neospora caninum* in water buffaloes (*Bubalus bubalis*) on four ranches in Corrientes province, Argentina. *Vet. Parasitol.* 150, 155–158.
- Chrysafidis, A.L., Soares, R.M., Rodrigues, A.A., Carvalho, N.A., Gennari, S.M., 2011. Evidence of congenital transmission of *Neospora caninum* in naturally infected water buffalo (*Bubalus bubalis*) fetus from Brazil. *Parasitol. Res.* 108, 741–743.
- Dubey, J.P., Romand, S., Hilali, M., Kwok, O.C.H., Thullies, P., 1998. Sero-prevalence of antibodies to *Neospora caninum* and *Toxoplasma gondii* in water buffaloes (*Bubalus bubalis*) from Egypt. *Int. J. Parasitol.* 28, 527–529.
- Dubey, J.P., Schares, G., Ortega-Mora, L.M., 2007. Epidemiology and control of neosporosis and *Neospora caninum*. *Clin. Microbiol. Rev.* 20, 323–367.
- Food and Agriculture Organization, 2012. www.fao.org
- Fujii, T.U., Kasai, N., Nishi, S.M., Dubey, J.P., Gennari, S.M., 2001. Sero-prevalence of *Neospora caninum* in female water buffaloes (*Bubalus bubalis*) from the southeastern region of Brazil. *Vet. Parasitol.* 99, 331–334.
- Gennari, S.M., Rodrigues, A.A.R., Viana, R.B., Cardoso, E.C., 2005. Occurrence of anti-*Neospora caninum* antibodies in water buffaloes (*Bubalus bubalis*) from the Northern region of Brazil. *Vet. Parasitol.* 134, 169–171.
- Guarino, A., Fusco, G., Savini, G., Di Francesco, G., Cringoli, G., 2000. Neosporosis in water buffalo (*Bubalus bubalis*) in southern Italy. *Vet. Parasitol.* 91, 15–21.
- Huong, L.T.T., Ljungstrom, B.L., Ugjala, A., Bjorkman, C., 1998. Prevalence of antibodies to *Neospora caninum* and *Toxoplasma gondii* in cattle and water buffaloes in southern Vietnam. *Vet. Parasitol.* 75, 53–57.
- Konrad, J.L., Moore, D.P., Crudeli, G., Caspe, S.G., Cano, D.B., Leunda, M.R., Lischinsky, L., Regidor-Cerrillo, J., Odeón, A.C., Ortega-Mora, L.M., Echaide, I., Campero, C.M., 2012. Experimental inoculation of *Neospora caninum* in pregnant water buffalo. *Vet. Parasitol.* 187, 72–78.
- McAllister, M.M., Dubey, J.P., Lindsay, D.S., Jolley, W.R., Wills, R.A., McGuire, A.M., 1998. Dogs are definitive host of *Neospora caninum*. *Int. J. Parasitol.* 28, 1473–1478.
- McAllister, M.M., Björkman, C., Anderson-Sprecher, R., Rogers, D.G., 2000. Evidence of point-source exposure to *Neospora caninum* and protective immunity in a herd of beef cows. *J. Am. Vet. Med. Assoc.* 217, 881–887.
- Med Calc., 1993. Version 4.16b – Windows 95 Copyright©.
- Meenakshi, Sandhu, K.S., Ball, M.S., Kumar, H., Sharma, S., Sidhu, P.K., Sreekumar, C., Dubey, J.P., 2007. Seroprevalence of *Neospora caninum* antibodies in cattle and water buffaloes in India. *J. Parasitol.* 93, 1374–1377.
- Moore, D.P., Pérez, A., Agliano, S., Brace, M., Cantón, G., Cano, D., Leunda, M.R., Odeón, A.C., Odriozola, E., Campero, C.M., 2009. Risk factors associated with *Neospora caninum* infections in cattle in Argentina. *Vet. Parasitol.* 61, 122–125.
- Moré, G., Bacigalupe, D., Basso, W., Rambeaud, M., Beltrame, F., Ramirez, B., Venturini, M.C., Venturini, L., 2009. Frequency of horizontal and vertical transmission for *Sarcocystis cruzi* and *Neospora caninum* in dairy cattle. *Vet. Parasitol.* 160, 51–54.
- Nasir, A., Ashraf, M., Khan, M.S., Yaqub, T., Javeed, A., Avais, M., Akhtar, F., 2011. Seroprevalence of *Neospora caninum* in dairy buffaloes in Lahore district, Pakistan. *J. Parasitol.* 97, 541–543.
- R-Development Core Team, 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, ISBN 3-900051-07-0, www.R-project.org/.
- Reichel, M.P., Ayanegui-Alcérrec, M.A., Gondim, L.F.P., Ellis, J.T., 2013. What is the global economic impact of *Neospora caninum* in cattle – the billion dollar question. *Int. J. Parasitol.* 43, 133–142.
- Rodrigues, A.A.R., Gennari, S.M., Aguiar, D.M., Sreekumar, C., Hill, D.E., Miska, K., Vianna, M.C.B., Dubey, J.P., 2004. Shedding of *Neospora caninum* oocysts by dogs fed tissues from naturally infected water buffaloes (*Bubalus bubalis*) from Brazil. *Vet. Parasitol.* 124, 139–150.
- Sanderson, M.W., Gay, J.M., Baszler, T.V., 2000. *Neospora caninum* seroprevalencia and associated risk factors in beef cattle in the north-western United States. *Vet. Parasitol.* 90, 15–24.
- Sengupta, P.P., Balumahendiran, M., Raghavendra, A.G., Honnappa, T.G., Gajendragad, M.R., Prabhudas, K., 2012. Prevalence of *Neospora caninum* antibodies in dairy cattle and water buffaloes and associated abortions in the plateau of Southern Peninsular India. *Trop. Anim. Health Prod.* 45, 205–210.
- 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, 79–85.