Short Communication

Seprevalence and risk factors for *Neospora caninum* in sheep in the state Minas Gerais, southeastern Brazil

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ABSTRACT

The aim of this study was to determine the prevalence and risk factors associated with infection due to *Neospora caninum* in serum samples from 488 sheep originating from 63 farms in 63 municipalities distributed across eight of the twelve mesoregions of the state Minas Gerais, southeastern Brazil. For detection of *N. caninum* the sheep serum samples were subjected to the indirect fluorescence antibody test (IFAT $\geq$ 50). To identify the risk factors associated with infection due to *N. caninum* a questionnaire was filled out for each herd by interviewing, the individual responsible for the herd, demanding information on the general characteristics of the property. Sixty-four sheep sera (13.1%; 95% CI = 10.3–16.4) presented IgG-specific anti-*N. caninum* antibodies with the following titers: 50 (49; 76.6%), 100 (7; 10.9%), 200 (4; 6.2%), 400 (3; 4.7%) and 800 (1; 1.6%). The prevalence of infected sheep per mesoregion ranged from 0 to 28.1%. Out of the 63 farms sampled, 31 (49.2%; 95% CI = 36.4–62.1) presented at least one seropositive sheep. No significant association was found between the presence of anti-*N. caninum* antibodies and the risk factors evaluated on the farms, except for the mesoregion variable (p = 0.004; OR = 0.429; CI95% = 0.182–1.008). These results indicate that there is a need for additional research to define the epidemiological importance of this parasite as a cause of reproductive problems in sheep herds in Minas Gerais.

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1. Introduction

Neospora caninum (Apicomplexa: Sarcocystidae) is an obligate intracellular protozoon that is considered to be the main cause of neonatal mortality and abortion among dairy cattle worldwide, capable of infecting both mammals and birds (Anderson et al., 2000; Dubey, 2003).

The presence of antibodies to *N. caninum* in serum indicates exposure to the parasite and can be identified by serological tests, such as the indirect fluorescence antibody test

(IFAT), considered the gold standard test and immunoenzymatic assay (ELISA) (Dubey, 2003). Prevalence studies on *N. caninum* in sheep are scarce. In Brazil, studies on the seroprevalence (IFAT) of natural infection due to *N. caninum* in sheep have presented a wide range, going from rates of less than 10% (Figliuolo et al., 2004; Machado et al., 2011) to a rate of 30% (Aguiar et al., 2004; Andreotti et al., 2009) in asymptomatic sheep.

The state Minas Gerais is the largest state and has the second biggest sheep population of the four states of southeastern region of Brazil, with 223,434 head (IBGE, 2009), mostly aimed at meat production (Anualpec, 2008). With an area of 588,383.6 km^2, Minas Gerais presents significant climatic and socioeconomic differences. Since 2000, sheep-husbandry has been taking on an important role within livestock farming in Brazil. With acquisition of animals from different regions, commercial meat sheep-rearing has increased considerably in areas with no traditional culture in sheep production, like the central-western-southern region of Minas Gerais.

The aim of this study was to determine the seroprevalence and risk factors associated with seropositivity to *N. caninum* among sheep in commercial herds in the homogenous central-western-southern region of the state Minas Gerais, Brazil. This region has well-defined dry and rainy seasons, mean annual temperature of 21.2 °C and annual rainfall ranging from 1000 to 2000 mm. Sampling was organized at two levels: farms and animals. Based on a combined list of sheep herds from the Association of Sheep and Goat Farmers of the State Minas Gerais (ACCOMIG) and the state government agency for animal health (Instituto Mineiro de Agropecuária, IMA), a non-probabililistic sampling was used to select the farms. Animals were randomly selected and a fixed sampling of eight sheep from each property was used (Bennett et al., 1991). Blood was collected by jugular vein puncture and the serum was separated and stored at −20 °C until used for analysis.

A retrospective cross-sectional study was conducted using a sheep serum bank composed of 488 samples. The serum samples were collected from 63 farms located in 63 municipalities, distributed across eight of the twelve geographical mesoregions of the state Minas Gerais (number of farms/number of serum samples): Metropolitana de Belo Horizonte (14/112), Alto Paranaíba/Triângulo (12/91), Sul-Sudoeste de Minas (9/68), Vale do Rio Doce (9/71), Zona da Mata (15/116), and Central Mineira + Campo das Vertentes + Oeste de Minas (4/30). These last three mesoregions were grouped for analysis purposes because of the small number of farms sampled and small number of serum samples collected.

A previously tested questionnaire (Magalhães and Gouveia, 1985; adapted by Pinheiro et al., 2000) was filled out for each herd by interviewing the individuals responsible for the herd, demanding information on the general characteristics of the property. Both serum collection and farmer interviews were done by trained veterinarians from the state government agency for animal health (IMA).

Out of the 63 farms evaluated in this study, 96.8% (61) were producing sheep for slaughter and 3.2% (2) for milk. According to the interviewees, abortions were occurring among the sheep on 21% (13/63) of the farms, and dogs were present on 26.3% (17/63).

Serum samples were tested by IFAT, as described by Paré et al. (1995). The antigen consisted of slides with tachyzoites of *N. caninum* (Laboratório Imunodot, Jaboticabal, SP, Brazil) and sheep anti-IgG conjugate (whole molecule, Sigma, St. Louis, MO, USA). Considering a cut off value IFAT ≥ 50 (Figliuolo et al., 2004), the positive samples were titrated in sequential double dilutions, and the final antibody titer was established as the inverse of the greatest dilution at which fluorescence was observed.

To identify the factors associated with infection due to *N. caninum*, statistical analysis was conducted using the serological results obtained through IFAT as the dependent variable and the variables gathered at the interviews as independent variables. The prevalence was calculated in terms of numbers of animals and farms (herds). With the aim of investigating the behavior of the dependent variables as a function of the independent variables, a multiple model was constructed by means of logistic regression. For this, the dependent variable was dichotomized (0 = negative; 1 = positive) and univariate analysis was performed by means of the chi-square test (x^2). The variables that showed an association at the level of $p < 0.02$ in the x^2 or Fisher exact test were selected for constructing the multiple model. The risk was calculated by means of odds ratios and their 95% confidence intervals, for the variables that presented significant associations ($p < 0.05$) in the logistic regression. All the analyses were carried out using the PASW 18.0 statistical package.

Out of 488 sheep serum sampled, 73% (356) of animals were females and 70% and the age group distribution was: 6–12 months (30%), 13–24 months (32%), 25–36 months (22%) and >36 months (16%). This is characteristic of farms activity meat sheep herds, the objective of production in 97% farms sampled (61/63). These farms the herds are greater and raised in the field, and the dogs are an important tool for the daily management of the herd.

Seroprevalence studies (IFAT) in Brazil have presented a variety of results according to the region studied. The prevalence of seropositive sheep in the present study (13.1%; 64/488; 95% CI = 10.3–16.4) was next to the positivity rate obtained in serological surveys conducted in sheep farms in two brazilian states and geographical regions (São Paulo, southeastern Brazil and Paraná, southern Brazil): 9.5% in one county of Paraná state (Romanelli et al., 2007) and 9.2% in four counties (Figliuolo et al., 2004), 12.8% in two counties (Langoni et al., 2011) and 8.0% in four counties (Machado et al., 2011) in state São Paulo. In both the climatic and sheep-rearing characteristics are similar to those one in the state Minas Gerais, southeastern Brazil. Similar results were found in other brazilian regions: 9.6% in 23 counties of Alagoas state, northeastern Brazil (Faria et al., 2010) and 8.8% in Federal District, Brazil central region (Ueno et al., 2009). Seroprevalence rates were greater in one county in Rondônia state (30%), northern Brazil (Aguiar et al., 2004) and 30.8% in one county in the state Mato Grosso, western Brazil (Andreotti et al., 2009). These two brazilian geographical regions have in common the characteristic of present high annual rainfall high annual rainfall index.

Out of the 64 positive samples, 56 (87.5%) presented antibody titers ≤ 100, four (6.2%) presented titers of 200, three (4.7%) presented titers of 400 and one (1.6%) presented a titer of 800. A similar result was observed in other studies with predominance of low titers (50, 100 and 200), suggestive of sheep with chronic infection due to *N. caninum* (Figliuolo et al., 2004; Munhóz et al., 2010; Salaberry et al., 2010; Rossi et al., 2011).

Two studies were previously conducted in one county (Uberlândia) of Minas Gerais with a rate of 8.1% (Salaberry et al., 2010) and 47.1% (Rossi et al., 2011). The present work was the first serological–epidemiological study covering a large portion of the state of Minas Gerais, with sampling in 63 municipalities in eight mesoregions of the state. The eight sampled mesoregions homogenous make-up the central-western-southern region of the state of Minas Gerais. This region accounts for 61% of the sheep population in this state (IBGE, 2009).

In 31 (49.2%; 95% CI = 36.4–62.1) of the 63 farms sampled in Minas Gerais state, at least one sheep was identified as seroreactive to *N. caninum*. Varying prevalence have been observed among farms in Brazil, with infection levels ranging from 54 to 87.5% of positive farms (Aguiar et al., 2004; Faria et al., 2010; Figliuolo et al., 2004; Munhóz et al., 2010; Salaberry et al., 2010; Ueno et al., 2009). Index in percentage of positive farms, this result is similar to the ones found in the states of Alagoas (Faria et al., 2010) and São Paulo (Machado et al., 2011), where 53.8% (14/26) and 50.0% (8/16) of the farms presented at least one seropositive sheep, respectively. However, in terms of area covered, the index found in Minas Gerais is indicative of the widespread geographical presence of the sheep farms with seropositive to *N. caninum* as in the present study.

Among the mesoregions, the seroprevalence ranged from 4.8 to 17.5%, taking into consideration the total number of positive farms (49.2%). Prevalence of sheep seropositive to *N. caninum* (percentage in relation to the total number of positive samples), according to mesoregion was: Metropolitana de Belo Horizonte, 28.1%; Alto Paranaíba/Triangulo, 26.6%; Vale do Rio Doce, 21.9%; Sul-Sudoeste de Minas, 17.2%; Zona da Mata, 6.3%; and Central Mineira + Campo das Vertentes + Oeste de Minas, 0%. The differences observed between farms and mesoregions can be attributed to greater opportunities for exposure to different sources of infection due to *N. caninum*, diversity in sanitary management and type of exploitation among the herds. In addition, there may be different climatic conditions, which influence the maintenance and viability of oocysts in the environment (Georgieva et al., 2006).

None of the 14 variables analyzed (sex, age group, breed, mesoregion, presence of installations for food storage, presence of sheepfold, type of floor in sheepfold, type of drinking trough, use of silage, type of exploitation, cases of abortion on the farm, cases of birth of weak or abnormal offspring, water source, and presence of dogs on the farm), showed any significant association with *N. caninum*, except for the variable mesoregion (Metropolitana de Belo Horizonte $P = 0.004$, OR = 0.43, 95% CI = 0.182–1.008).

The lack of association between seropositivity and variables such as breed, sex or age has also been observed in other studies in Brazil (Figliuolo et al., 2004; Romanelli et al., 2007; Rossi et al., 2011; Salaberry et al., 2010; Ueno et al., 2009), except for cases of abortion on the farm. In the municipality of Uberlândia (Alto Paranaíba/Triângulo mesoregion), Minas Gerais, Brazil, Salaberry et al. (2010) found a significant association ($p < 0.05$) between seropositivity and high occurrence of abortion, thus suggesting that infection due to *N. caninum* may be associated with reproductive problems in sheep. Also, Machado et al. (2011) in state São Paulo, Brazil, found a significant association ($p = 0.0031$) of seropositivity with the presence of reproductive problems in sheep. Since sheep-rearing in Minas Gerais focuses on meat production, on pastureland (Carneiro et al., 2009; Guimarães et al., 2009), it is possible to infer that infectious causes may have less importance in relation to abortions, and that the causes would more commonly be poorly dimensioned installations (thereby causing stress and fighting at feeding time), ingestion of toxic plants, nutritional imbalance at the prepartum stage and ketosis in obese animals. There was no association between the presence of dogs and seropositivity for *N. caninum*, and this result was similar to what has been observed in other surveys conducted in Brazil (Figliuolo et al., 2004; Romanelli et al., 2007; Salaberry et al., 2010; Soares et al., 2009), suggesting that this coccid is preferentially transmitted vertically, similar to bovine infections.

The serological evidence found in this study indicates that infection due to *N. caninum* is widely distributed on sheep-rearing farms in the state of Minas Gerais, especially in the mesoregions of Metropolitana de Belo Horizonte (28.1%) and Alto Paranaíba/Triângulo (26.6%), which according to the official agriculture and livestock census (IBGE, 2009), presented increases in sheep populations over the decade 1999–2009 of 249.3% and 64.1%, respectively. Further studies will be needed to determine the impact of *N. caninum* as a cause of reproductive problems in sheep in Minas Gerais.

Acknowledgements

References

Aguiar, D.M., Chiebao, D.P., Rodrigues, A.A.R., Cavalcante, G.T., Labruna, M.B., Gennari, S.M., 2004. Prevalência de anticorpos anti-*Neospora caninum* em ovinos do município de Monte Negro, RO, Amazônia Ocidental Brasileira. Arq. Inst. Biol. 7, 616–618.

Anderson, M.L., Adrianarivo, A.G., Conrad, P.A., 2000. Neosporosis in cattle. Anim. Reprod. Sci. 60-61, 417–431.

Andreotti, R., Matos, M.F.C., Gonçalves, K.N., Oshiro, L.M., Lima-Junior, M.S.C., Paiva, F., Leite, F.L., 2009. Comparison of indirect ELISA based on recombinant protein NcSRS2 and IFAT for detection of *Neospora caninum* antibodies in sheep. Rev. Bras. Parasitol. Vet. 18, 20–24.

ANUALPEC, 2008. Anuário da Pecuária Brasileira, 1st ed. Instituto FNP, São Paulo, 332 p.

Bennett, S., Woods, T., Liyanage, W.M., Smith, D.L., 1991. A simplified general method for cluster-sample surveys of health in developing countries. Rapp. Trimest. Sanit. Mond. 44, 97–106.

Carneiro, A.C.A.V., Carneiro, M., Gouveia, A.M.G., Vilas-Boas, L.S., Vitor, R.W.A., 2009. Seroprevalence and risk factors of sheep toxoplasmosis in Minas Gerais, Brazil. Revue Méd. Vét. 160, 527–531.

Dubey, J.P., 2003. Review of *Neospora caninum* and neosporosis in animals. Korean J. Parasitol. 41, 1–16.

Faria, E.B., Cavalcanti, E.F.T.S.F., Medeiros, E.S., Pinheiro-Júnior, J.W., Azevedo, S.S., Athayde, A.C.R., Mota, R.A., 2010. Risk factors associated with *Neospora caninum* seropositivity in sheep from the state of Alagoas, in the northeast region of Brazil. J. Parasitol. 96, 197–199.

Figliuolo, L.P.C., Kansai, N., Ragozo, A.M., Paula, V.S., Dias, R.A., Souza, S.L.P., Gennari, S.M., 2004. Prevalence of anti-*Toxoplasma gondii* and anti-*Neospora caninum* antibodies in ovine from São Paulo State, Brazil. Vet. Parasitol. 123, 161–166.

Georgieva, D., Prelezov, P.N., Koinarski, V.T.S., 2006. *Neospora caninum* and neosporosis in animals—a review. Bulgarian J. Vet. Med. 9, 1–26.

Guimarães, A.S., Gouveia, A.M.G., Abreu, C.P., Haddad, J.P.A., Leite, R.C., Carmo, F.B., 2009. Características zoossanitárias da ovinocultura em Minas Gerais. Rev. Vet. Zootec. em MG 102, 33–39.

IBGE Censo agropecuário, 1999–2009. Minas Gerais. Avaiable from www.ibge.gov.br (accessed 09.09.2011).

Langoni, H., Greca Júnior, H., Guimarães, F.F., Ullmann, L.S., Gaio, F.C., Uehara, R.S., Rosa, E.P., Amorim, R.M., Silva, R.C., 2011. Serological profile of *Toxoplasma gondii* and *Neospora caninum* infection in commercial sheep from São Paulo State, Brazil. Vet. Parasitol. 177, 50–54.

Machado, Kikuti, G.P., Langoni, M., Paes, H., 2011. Seroprevalence and risk factors associated with neosporosis in sheep and dogs from farms. Vet. Parasitol. 182, 356–358.

Magalhães, H.H., Gouveia, A.M.G., 1985. Diagnóstico da situação da caprinocultura em algumas microrregiões dos Estados de Minas Gerais e Rio de Janeiro. Rev. Cabra Bodes. 1, 5–7.

Munhoz, K.F., Luca Neto, M., Santos, S.M.A., Garcia, J.L., Guimarães Junior, J.S., Vidotto, O., Headley, S.A., Yamamura, M.H., 2010. Occurrence of anti-*Neospora caninum* antibodies in sheep from farms located in northern Paraná, Brazil. Semina: Ciên. Agr. 31, 1031–1040.

Paré, J., Hietala, S.K., Thurmond, M.C., 1995. Interpretation of an indirect fluorescent antibody test for diagnosis of *Neospora* sp. infection in cattle. J. Vet. Diagn. Invest. 7, 273–275.

Pinheiro, R.R., Gouveia, A.M.G., Alves, F.S.F., Haddad, J.P.A., 2000. Aspectos epidemiológicos da caprinocultura cearense. Arq. Bras. Med. Vet. Zootec. 52, 534–543.

Romanelli, P.R., Freire, R.L., Vidotto, O., Marana, E.R.M., Ogawa, L., De Paula, V.S.O., Garcia, J.L., Navarro, I.T., 2007. Prevalence of *Neospora caninum* and *Toxoplasma gondii* in sheep and dogs from Guarapuava farms, Paraná State, Brazil. Res. Vet. Sci. 82, 202–207.

Rossi, G.F., Cabral, D.D., Ribeiro, D.P., Pajuaba, A.C.A.M., Corrêa, R.R., Moreira, R.Q., Mineo, T.W.P., Mineo, J.R., Silva, D.A.O., 2011. Evaluation of *Toxoplasma gondii* and *Neospora caninum* infections in sheep from Uberlândia, Minas Gerais State, Brazil, by different serological methods. Vet. Parasitol. 175, 252–259.

Salaberry, S.R.S., Okuda, L.H., Nassar, A.F.C., Castro, J.R., Ribeiro, A.M.C.L., 2010. Prevalence of *Neospora caninum* antibodies in sheep flocks of Uberlândia country, MG. Rev. Bras. Parasitol. Vet. 19, 148–151.

Soares, H.S., Ahid, S.M.M., Bezerra, A.C.D.S., Pena, H.F.J., Dias, R.A., Gennari, S.M., 2009. Prevalence of anti-*Toxoplasma gondii* and anti-*Neospora caninum* antibodies in sheep from Mossoró, Rio Grande do Norte, Brazil. Vet. Parasitol. 160, 211–214.

Ueno, T.E.H., Gonçalves, V.S.P., Heinemann, M.B., Dilli, T.L.B., Akimoto, B.M., Souza, S.L.P., Gennari, S.M., Soares, R.M., 2009. Prevalence of *Toxoplasma gondii* and *Neospora caninum* infections in sheep from Federal District, central region of Brazil. Trop. Anim. Health Prod. 41, 547–552.