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Prevalence of *Neospora caninum* and *Toxoplasma gondii* in sheep and dogs from Guarapuava farms, Paraná State, Brazil

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Abstract

Sheep and dog blood samples were collected from nine farms in the county of Guarapuava, Paraná, Brazil. The indirect fluorescent antibody test (IFAT) was used to detect *Neospora caninum* and *Toxoplasma gondii* antibodies. Herein, serum samples from 305 sheep were evaluated, being 29 (9.5%) and 157 (51.5%) seropositives to *N. caninum* and *T. gondii*, respectively. Seven (29.1%) and five (20.8%) out of 24 dogs were seropositives to *N. caninum* and *T. gondii*, respectively. There were no differences among the sheep serology for *N. caninum* and reproductive problems, management and animal feeding variables, neurological problems and presence of other animals species on the farm ($P \geqslant 0.05$). The simultaneous frequency of antibodies between *N. caninum* and *T. gondii* was 5.2% in the herds. Age, breed, farm size, semi-intensive activity, mineral salt supplementation, water origin, stage of the pregnancy when reproduction problems occurred, neurological problems in lambs, presence of rodents in the food room and pasture cat access were identified as associated factors for the occurrence of toxoplasmosis in sheep ($P < 0.05$). There were no differences among the seropositivity in dogs for *N. caninum* and *T. gondii* and breed, age and sex ($P \geqslant 0.05$). The present work is the first report on serum prevalence of *N. caninum* in sheep from the state of Paraná, Brazil.
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Keywords: *Neospora caninum*; *Toxoplasma gondii*; Ovine; Dogs; Indirect fluorescent antibody test; Risk factors

1. Introduction

Toxoplasma gondii is one of the most important causes of sheep and goat abortions in the United States and Scotland (Dubey and Kirkbride, 1984; Buxton, 1990). Freire et al. (1995), Garcia et al. (1999) and Ogawa et al. (2003) have reported on prevalence of anti-*T. gondii* antibodies in sheep herds from Northern Paraná, Brazil.

Dubey et al. (1988a) isolated and classified the parasite *Neospora caninum*, a protozoan morphologically similar to the *T. gondii* and *Sarcocystis* species (Dubey et al., 1988b). Its life cycle was determined in 1998 (McAllister et al., 1998), and dogs and coyotes (*Canis latrans*) have been described, so far, as definitive hosts (McAllister et al., 1998; Gondim et al., 2004).

Several productive and wild animal species have been identified as intermediate *N. caninum* hosts (Dubey and Lindsay, 1996). Animals can be born seronegatives, and later be infected by the ingestion of water and food

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contaminated by oocysts produced by dogs. Thus, showing the involvement of horizontal transmission in the epidemiological chain of this coccidia (McAllister et al., 1998; Wouda et al., 1999).

Dubey et al. (1990b) reported for the first time the occurrence of *N. caninum* in a newborn lamb of a week old, which died presenting nervous clinical signs. Ultra structural and Immunohistochemical analysis permitted a definitive diagnosis of *N. caninum* tissue cysts in the brain and spinal cord of this lamb. Kobayashi et al. (2001) communicated a case of natural infection by *N. caninum* affecting a pregnant ewe and her two twin fetuses, showing the vertical transmission of the agent in this animal species. Otter et al. (1997) investigated by IFAT 129 pleural fluids from 141 aborted lambs, but they had none success in positive results. Finally, Hässig et al. (2003) reported for the first time abortion associated with *N. caninum* in naturally infected sheep.

There are few studies about *N. caninum* in sheep, and *T. gondii* is one of the most important ethiologic agents in that animals. Focusing on this point, we studied the prevalence of anti-*N. caninum* and anti-*T. gondii* antibodies in sheep sera from Central-Western region of Paraná state, Brazil.

2. Material and methods

2.1. Study area and sampling

The survey was carried out at the Guarapuava county, located in the Central-Western region of Paraná state, Southern Brazil, where is concentrated most part of the sheep raisers in this State. The 305 serum samples were obtained from nine sheep raisers farms. The sample size was determined by the EPI-INFO statistical program 6.04 version (CDC-Atlanta). A prevalence estimated at 50% was adopted, 5% precision at the confidence level of 95% in a population of 1250 sheep. All the dogs present on the nine farms also took part in the study, in a total of 24 animals.

2.2. Questionnaire

An epidemiological questionnaire was developed to collect information on the following management variables: farm size, type of activity carried out (livestock rearing, crop cultivation or mixed), system of livestock raising (intensive, semi-intensive or extensive), breed, animal feeding variables: intensive feeding system (pasture or pasture and hay), type of concentrated ration offered (commercial, produced on the farm or none), mineral supplementation (common salt or mineral salt), water origin (artesian well and spring or dam/reservoir/stream) water tank (presence or absence); and on the following animal health variables: reproductive problems (presence or absence), frequency of the reproductive problems (first offspring, from second to fifth offspring or all), stage of pregnancy when the reproductive problems occurred (first third, second third or last third), neurological problems in lambs (presence or absence); presence of other animals species on the farms (rodents, cats), access of cats (food deposits, pasture).

2.3. Indirect fluorescent antibody test (IFAT)

Three hundred twenty nine serum samples, 305 from sheep and 24 from dogs, were collected from May to June 2001, by jugular vein puncture and stored at −18 °C until be tested. Sheep and dog sera were analyzed by IFAT to detect antibodies against *N. caninum*, according to Conrad et al. (1993), and against *T. gondii*, according to Camargo (1964).

Antigen of *N. caninum* culture-derived tachyzoites of the NC-1 isolate was produced and prepared according to Yamane et al. (1997). Sheep and dog test sera were diluted twofold starting at a dilution of 1:50, incubated on antigen slides for 30 min at 37 °C, and washed in PBS (pH 9.0). Ovine and dog IgG antibodies were detected with anti-sheep IgG-FITC conjugated (whole molecule, Sigma, F7634) and anti-dog IgG-FITC conjugated (whole molecule, Sigma, F7884), respectively. Sera were considered positive if the entire surface of the tachyzoites was fluorescent (Paré et al., 1995).

Cut-off points for *N. caninum* and *T. gondii* in sheep, were considered of 1:50 (Figliuolo et al., 2004) and 1:64 (Garcia et al., 1999; Figliuolo et al., 2004), respectively. These were chosen to avoid unspecific reactions (Atkinson et al., 2000). *T. gondii* and *N. caninum* cut-off points for dog sera were 1:16 and 1:50, respectively. All IFAs included known positive and negative sheep and dogs serum samples as control.

2.4. Statistical analysis

Variables were analyzed by the Chi-square test (χ^2) corrected by Yates and the Fisher Exact Test, using the Epi Info program (CDC, 6.04b version). Association among variables and occurrence of seropositives were estimated from values obtained by the odds ratio (OR), a confidence interval at 95%. We have considered as significant a *P*-value of $\leqslant 0.05$.

3. Results

3.1. Seroprevalence of T. gondii and N. caninum

The prevalence results are presented in Table 1. Population analyses showed a 9.5% (29/305) prevalence of *N. caninum* in ovine and a 29% (7/24) in dogs. This same population showed a 51.4% (157/305) prevalence of *T. gondii* in ovine and 20.8% (5/24) in dogs. Simultaneous antibody frequency between *N. caninum* and *T. gondii* was 5.2% (16/305) for ovine and 20.8% (5/24) for dogs. Seropositive sheep for *N. caninum* were detected in eight out of the nine farms studied, with a prevalence that ranged from 4.7% to 17%. When *T. gondii* was the focus of study,

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