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Neospora caninum seropositivity and reproductive risk factors in dogs

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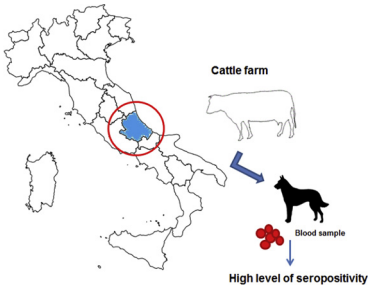
HIGHLIGHTS

- The seropositivity for Neospora caninum in breeding and farm dogs was determine.
- Seropositivity was performed by indirect immunofluorescent antibody test (IFAT).
- Dogs living in the cattle farms showed a higher seropositivity for *N. caninum*.
- The potential risk of horizontal transmission of *N. caninum* between dogs and cattles was confirmed.

GRAPHICAL ABSTRACT



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ABSTRACT

Despite the importance of *Neospora caninum* in veterinary medicine, knowledge of distribution of neosporosis in dog populations in some countries is still poor. The aims of the present study were to determine the occurrence of anti-*N. caninum* antibodies in one-hundred dogs living in cattle farms or dog breedings in central Italy and to evaluate the risk factors associated with seropositivity. The incidence of reproductive system disorders (e.g. infertility after first pregnancy) was also evaluated. Serum from breeding and farm dogs was tested to an indirect immunofluorescent antibody test (IFAT) to assess the occurrence of seropositivity. Management and individual data were collected and analysed both by linear and logistic multiple-regression models to find reliable predictors of seroprevalence and anti-*N. caninum* antibody level. The seropositivity for *N. caninum* was 32%. Dogs reared for breeding and presence of cattle on the farm were associated with seropositivity for *N. caninum*. Dogs living in the cattle farms showed a higher seropositivity for *N. caninum* (46%) compared with those living in dogs breeding (18%) ($P < 0.05$). The high presence of seropositive dogs in cattle farms of the study region demonstrates the potential risk of horizontal transmission of *N. caninum* between dogs and cattle, regardless the occurrence of reproductive system disorders or with infectious bovine tissues contact. Although the Neospora seropositivity in dog breedings may appear relatively low if compared with that found in dogs living with livestock, this infection, apparently underestimated, should be considered as a potential serious problem in canine medicine.

1. Introduction

The apicomplexan protozoan *Neospora caninum* infects domestic and wild animals (e.g. ruminants and dogs), causing a number of

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clinical pictures. The domestic dog (i.e. *Canis lupus familiaris*) and other canids are definitive hosts of *N. caninum*. In certain epidemiological settings the domestic dog acts as a potential source of transmission to cattle due to the shedding of resistant oocysts in the environment (McAllister et al., 1998; Gondim et al., 2004). Infection in dogs can occur by both horizontal and vertical transmission. The parasite can be transplacentary transmitted, during pregnancy from an infected dam to the fetus, or postnatally through the ingestion of food or water contaminated with mature oocysts or of tissues containing tachyzoites or tissue cysts (Lindsay et al., 1999; Dubey et al., 2007; Cavalcante et al., 2012). Indeed, adult dogs become infected ingesting carcasses, fetuses and the placental tissues of intermediate hosts, such as livestock. In natural conditions, bitches that delivered pups congenitally infected with *N. caninum* remained clinically normal (Dubey et al., 2007), but they may transmit the parasite to their offspring in successive generations (Barber and Trees, 1998; Crookshanks et al., 2007). Canine neosporosis is characterized by various neuromuscular signs including ataxia, ascending paralysis and other general nervous clinical signs (Dubey and Lindsay, 1996). Also myocardial, pulmonary, dermatological and reproductive disorders could be present (Rasmussen and Jensen, 1996; Barber and Trees, 1998; Lyndsay et al., 1999; Dubey et al., 2007, 2011).

N. caninum is an important cause of abortion in cattle (Dubey and Lindsay, 1996; Björkman et al., 2010) and it is widely distributed among bovine herds worldwide, where animals acquire the infection by ingesting food or water contaminated by the mature oocysts shed by dogs living in the same environment (Dubey and Schares, 2011).

Despite canine hosts play a major role in the epidemiology of neosporosis, studies on the occurrence of the infection are still incipient in several areas. Previous studies carried out on the distribution of *N. caninum* in dogs in countries from central and eastern Europe, e.g. Sweden, Austria, Czech Republic, Serbia and Romania, showed a prevalence between 2.1 and 32.7% (Wanha et al., 2005; Václavek et al., 2007; Gavrea et al., 2012; Kuruca et al., 2013). Prevalence from 10.9 to 36.4% were recorded in some areas of Italy, in different typologies of dogs, i.e. animals living in rural habitat, kenneled, pet and farm dogs (Capelli et al., 2004; Ferroglio et al., 2007; Paradies et al., 2007). There are areas where the occurrence of the pathogen is still unknown, e.g. central regions of the Apennine territories of Italy. Given the merit in enhancing the fragmentary knowledge of neosporosis in the country, the present sero-epidemiological survey aimed at evaluating the occurrence of anti-*N. caninum* antibodies in farm and breeding dogs living in an area with an anecdotic history of neosporosis. Additionally, risk factors and frequency of reproductive problems were analysed in relation to the seropositivity of examined dogs.

2. Materials and methods

2.1. Animals

From January to June 2013, a blood sample was collected from one hundred dogs living in cattle farms (n. 50 – Group 1) and kept in breeding facilities (n. 50 – Group 2) located in different municipalities of Abruzzo region, central Italy. The sampled population was selected based on main convenience, e.g. availability of breeders to collaborate to the study and history of clinical signs that were compatible with canine neosporosis on each site. Each dog was identified and a complete clinical examination was carried out. The owner was interviewed to obtain the following information: age, sex, breed, reproductive status, feeding habits, pregnancy status, history of reproductive disorders.

2.2. Serological testing and procedures

An indirect immunofluorescent antibody test (FULLER Laboratories, California, USA) consisting of *N. caninum* tachyzoites fixed on a slide, anti-canine IgG FITC conjugate and *N. caninum*-positive and -negative controls was used to detect the presence of antibodies to *N. caninum* in the serum samples, with a positive titer of 1:50 considered positive (Dubey et al., 1988). After collection, blood was centrifuged (3000 rpm for 10 min) and separated sera were stored at −20 °C until examination. All sera were tested in duplicate in a final dilution of 1:50 and 10 μl from each sample were distributed in slides in different wells. After distribution in wells the slides were incubated for 30 min at 37 °C in a humid chamber. After three rinsing with PBS, one drop of ITC anti-dog conjugate was added to each well. Samples were incubated at 37 °C for 30 min, two drops of mounting medium were added and samples were read at UV light at 400X magnification under a fluorescence microscope. The sensitivity and specificity values for the test, were 98 and 99%, respectively (Packham et al., 1998).

2.3. Statistical analysis

The difference of seropositivity for *N. caninum* according to the epidemiological data were evaluated by the Chi-square test (χ^2) or Fisher's exact test when appropriate, separately in breeding and farm dogs. The epidemiological data (predictors) common to all the dogs, i.e. provenance (farm/breeding), age (>or < 3 years), sex, and breed (mongrel/pure breed) were offered to binary logistic models to evaluate possible risk factors for *N. caninum*-seropositivity. Breed was introduced in the models as interaction with the provenance. The differences were considered significant with $P < 0.05$. The software used was SPSS for windows, version 13.0.

3. Results

Overall, thirty-two dogs (32%) of the total study population were positive for anti-*N. caninum* antibodies, twenty-tree (46%) and nine (18%) in Groups 1 and 2 respectively.

3.1. Group 1

Out of the 50 dogs belonging to Group 1, 23 scored positive for anti-*N. caninum* antibodies. Seropositive dogs from Group 1 belonged to fourteen of the twenty-three livestock farms examined. Most of them, i.e. 48 of 50, were clinically normal, without past or present clinical signs compatible with neosporosis. Two seropositive animals had a history of reproductive disorders, i.e. infertility after first pregnancy. The positive percentage was significantly higher in dogs that had a full-term pregnancy. Of the 13 animals which were pure breeds, 10 (77%) were seropositive for *N. caninum*, thus indicating that dogs of pure breeds were more likely infected by *N. caninum* in cattle farms than mongrel dogs (Table 1). Of the 42 farm dogs with feeding habits at risk of neosporosis, i.e. ingestion of bovine fetuses and/or placentas, 18 (42.9%) were seropositive. However, no statistical differences were found between the dogs fed with commercial/homemade food (8/50) and dogs with a previous history of contact with bovine tissues. The other variables were not statistically correlated with seropositivity ($P > 0.05$).

3.2. Group 2

Out of the 50 dogs belonging to Group 2, nine (18%) were positive for anti-*N. caninum* antibodies. None of the nine seropositive animals, which belonged to four different breeds, showed past or