Available online at www.sciencedirect.com
ScienceDirect
Veterinary Parasitology 149 (2007) 265–270
veterinary parasitology
www.elsevier.com/locate/vetpar

Short communication

Fecal and serological survey of *Neospora caninum* in farm dogs in Costa Rica

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Received 23 May 2007; received in revised form 26 July 2007; accepted 1 August 2007

Abstract

To detect oocysts of *Neospora caninum* in dog feces and to determine the excretion pattern in dogs from specialized dairy farms in Costa Rica, a total of 265 fecal samples from 34 dogs were collected at intervals from February to August 2005. Fecal samples were examined for *N. caninum*-like oocysts microscopically, by DNA detection using the polymerase chain reaction (PCR), and by bioassay. *N. caninum* DNA was detected by PCR in four fecal samples, twice from one dog, but oocysts were not detected microscopically in these dogs. Sera of 31 of 34 dogs were tested for antibodies to *N. caninum* by a competitive-inhibition ELISA (VMRD®). Fifteen (48.4%) of 31 dogs had antibodies to *N. caninum* by ELISA. Seroconversion was not found in 28 dogs that were bled twice, 4 months apart (March and July 2005). Only one dog tested positive to *N. caninum* by both ELISA and PCR. This is the first report of finding *N. caninum* DNA in feces of naturally infected dogs in Costa Rican dairy farms.
Published by Elsevier B.V.

Keywords: *Neospora caninum*; Oocysts; Fecal samples; PCR; Dogs; Costa Rica

1. Introduction

Neospora caninum is an important cause of abortion in dairy cattle worldwide (Dubey, 2003). Dogs, coyotes, and possibly foxes are its definitive hosts (McAllister et al., 1998; Gondim et al., 2004; Wapenaar et al., 2006). Although *N. caninum* is transplacentally transmitted very efficiently in cattle, dogs are considered essential in the life cycle of this parasite (Dubey et al., 2007). However, unlike other coccidian parasites, relatively few oocysts are excreted in canine feces. Furthermore, the detection of *N. caninum* oocysts in feces is problematic because *N. caninum* oocysts morphologically resemble oocysts of three other coccidians (*Hammondia heydorni*, *Hammondia hammondi*, *Toxoplasma gondii*) that might be present in canine feces (Schares et al., 2005). Although molecular methods to detect *N. caninum*-like oocysts have been described (Hill et al., 2001; Slapeta et al., 2002; Sreekumar et al., 2004) the sensitivity is not high because of the low numbers of oocysts in canine feces. A comprehensive survey of *N. caninum* infection in feces of dogs from Germany by Schares et al. (2005) highlighted the difficulties of identification of *N. caninum* oocysts in canine feces. *N. caninum*-like oocysts were found in the feces of 47 of 24,089 fecal samples. Oocysts could be isolated from 29 of these 47 dogs and 28 of the 29 fecal samples were bioassayed in gerbils. Based on seroconversion in bioassayed gerbils,

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0304-4017/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.vetpar.2007.08.007

seven samples were considered to be *N. caninum*; five samples were definitively identified as *N. caninum* based on successful in vitro cultivation. *N. caninum* DNA was detected in four dogs by PCR directly from the dog feces. Among the other isolates, 12 were considered to be *H. heydorni*, two *T. gondii*, and two *H. hammondi*; *T. gondii* and *H. hammondi* are pseudo-parasites in dog feces and result from the ingestion of cat feces by dogs.

Detection of antibodies to *N. caninum* in canine serum does not provide epidemiologically important information because most dogs that excrete *N. caninum* oocysts do not have serum antibodies (McAllister et al., 1998; Dubey et al., 2007).

Previous to the detection of *N. caninum* oocysts in dog feces in Germany (Schares et al., 2005), *N. caninum* oocysts were reported in dogs from Argentina (Basso et al., 2001), United Kingdom (McGarry et al., 2003), Czech Republic (Slapeta et al., 2002), and New Zealand (McInnes et al., 2006). Basso et al. (2001) found a few *N. caninum* oocysts in the feces of a 45-day-old Rottweiler from La Plata, Argentina. Viable *N. caninum* was recovered from the gerbils that were fed these oocysts and the strain was successfully cultured in vitro. Slapeta et al. (2002) found oocysts in a 1-year-old German shepherd from Czech Republic; these oocysts were considered *N. caninum* based on PCR, and bioassay was not reported. McGarry et al. (2003) examined a total of 15 fecal samples from two foxhound kennels in U.K. (10 from one kennel of 80 and 5 from the second kennel of 60 dogs) and found *N. caninum* oocysts in two samples. One of these samples was identified as *N. caninum* based on PCR; a second fecal sample from this dog taken 4 months later revealed a few oocysts that were identified *N. caninum* based on PCR. McInnes et al. (2006) in New Zealand detected *N. caninum* DNA in the feces of a dog 2.5 years after they had isolated viable *N. caninum* from the skin of the dog.

Cattle are important for the economy of Costa Rica. In a cross-sectional study of 94 dairies in Costa Rica, 94.7% of the farms surveyed had *N. caninum* seropositive cows with an overall seroprevalence of 43.3% (Romero et al., 2005). Nothing is known of the epidemiology of *N. caninum* in Costa Rica. There are three reports of fatal neosporosis in Costa Rica, one in a dog (Morales et al., 1995), one in a goat (Dubey et al., 1996), and one in cattle (Pérez et al., 1998). The objective of the present study was to detect oocysts of *N. caninum* in farm dogs from specialized dairy farms with a high seroprevalence of *N. caninum* in Costa Rica.

2. Materials and methods

2.1. Study population

Twelve specialized dairy farms from the northern zones of Alajuela and Heredia were used in this study. On the selected farms, the within-herd seroprevalence of *N. caninum* ranged between 25.0% and 70.5% (Romero et al., 2002). Fecal samples from 34 dogs were analyzed and serum samples were collected from 31 of these dogs.

2.2. Sampling of dogs

In order to detect *N. caninum* oocysts and determine the excretion pattern, procedures were established to collect fresh feces of 34 dogs every 15 days during 7 months from February to August 2005. Feces from seven dogs from three farms were collected as planned, but feces from other dogs were collected inconsistently, depending on the cooperation of the owners. The dogs were isolated for 1 day before the farms were visited. Approximately 10 g of feces were picked up directly from the ground, and transported in a cooler at 4 °C to the Laboratory of Parasitology, School of Veterinary Medicine, Universidad Nacional of Costa Rica (UNA), where they were kept at 4 °C until processed (48 h maximum). A total of 265 feces were collected.

To detect *N. caninum* antibodies, dogs were bled twice, 4 months apart (March and July 2005). The blood was taken from the cephalic vein with a 20-guage needle fitted to a Vacutainer® blood collection tube. The samples were centrifuged at 2500 × *g* for 5 min and the serum stored at −20 °C until processed. Fifty-nine blood samples were collected, 31 in the first sampling and 28 in the second sampling.

2.3. Coprological examination

The direct examination and sporulation of the fecal samples were done in the Laboratory of Parasitology, UNA. Feces (10 g) were mixed with 10 ml of distilled water and 50 ml of sucrose solution (specific gravity 1.28) was added. The mixture was sieved through gauze, and centrifuged in a 50 ml conical tube at 2500 × *g* for 10 min. A few drops were taken from the meniscus and transferred to a slide for microscopic examination to detect *N. caninum*-like oocysts. After microscopic examination 5 ml of the supernatant from the very top of the tube were removed and mixed with 45 ml of distilled water; the mixture was centrifuged at 2500 × *g* for 10 min. After discarding the supernatant,