Available online at www.sciencedirect.com
ScienceDirect
Veterinary Parasitology 150 (2007) 291–296
veterinary parasitology
www.elsevier.com/locate/vetpar

Molecular identification of *Cryptosporidium* spp. from fecal samples of felines, canines and bovines in the state of São Paulo, Brazil

Alexandre Thomaz [a], Marcelo V. Meireles [b], Rodrigo M. Soares [a], Hilda F.J. Pena [a], Solange M. Gennari [a,*]

[a] *Departamento de Medicina Veterinária Preventiva e Saúde Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo. Av. Prof. Orlando Marques de Paiva, 87, Cidade Universitária, CEP 05508-270, São Paulo, SP, Brazil*
[b] *Departamento de Clínica, Cirurgia e Reprodução Animal, Universidade Estadual Paulista, Campus de Araçatuba, Araçatuba, Brazil*

Received 13 May 2007; received in revised form 24 September 2007; accepted 24 September 2007

Abstract

The objective of this study was to obtain information of epidemiological nature through genotypic characterization of *Cryptosporidium* isolates from dogs, cats and bovines from the state of São Paulo, Brazil. The extraction of DNA from oocysts was carried out and polymerase chain reaction was accomplished using specific primers to 18S rRNA gene. The amplicons were directed sequenced. Seven cat samples, nine dog samples and nine bovine samples were analysed. From the seven cat samples the genotypic analyses revealed *Cryptosporidium felis* in all. These were the first genotypic characterization of *Cryptosporidium* from domestic felines in Brazil. In nine sequenced samples from dogs, genotypic identities compatible with *Cryptosporidium canis* were revealed in all samples. The genotypic analyses in bovines revealed *Cryptosporidium parvum* in eight samples and *Cryptosporidium bovis* in another sample, the last one being a non-zoonotic species, not related to clinical symptoms and described for the first time in Brazil.

Keywords: *Cryptosporidium*; Genotypic characterization; Cats; Dogs; Bovines

1. Introduction

In 1907, Ernest Edward Tyzzer described a protozoa parasite, which he found in gastric glands of laboratory mice, observed the spore formation (oocysts) and described the fecal-oral transmission of the parasite. Since then species recognized to be infecting bovines, dogs and cats are described: *C. parvum*, *C. andersoni*, *C. bovis* (bovines), *C. canis* (dogs) and *C. felis* (cats) (Dubey et al., 1990; Fayer et al., 2005; Fayer, 2004).

There are few descriptions of infection by *Cryptosporidium* in dogs, most cases involving puppies less than 6-month old. The first evidence of *Cryptosporidium* spp. in this species was registered in 1981 by Tzipori and Campbell who detected antibodies in 16 out of 20 serum samples. The association of the agent with the disease process was described in 1983 when the parasite was detected in a dog carrying distemper (Wilson and Holscher, 1983), demonstrating an immunosuppressed onset and the appearing of parasite disease.

In cats, *Cryptosporidium* was first described in Japan (Iseki, 1979). Ever since, this parasite was described in asymptomatic and symptomatic cats having persistent

* Corresponding author.
E-mail address: sgennari@usp.br (S.M. Gennari).

0304-4017/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetpar.2007.09.023

diarrhea, anorexia and weight loss (Fayer et al., 2006b; Monticello et al., 1987). The potential for zoonotic transmission among both cats and humans was demonstrated accompanying the infection of 6-year old cats, orally inoculated with *Cryptosporidium* oocysts obtained from an immunosuppressed person (Current et al., 1983). The agent was also found in an 8-year old child who was infected with *Cryptosporidium* after being in contact with an infected cat (Egger et al., 1990).

Studies made with human and bovine genotypes of *C. parvum* associated with biological information, proposed a human genotype named *Cryptosporidium hominis* (genotype I) (Morgan-Ryan et al., 2002), and *C. parvum* bovine genotype (genotype II) which is capable of infecting humans and other mammals (Olson et al., 2004).

Thus, two transmission cycles of *Cryptosporidiosis* are recognized, one being anthroponotic, with *C. hominis* circulating among humans and the other being zoonotic, with *C. parvum* circulating among humans and other host species, mainly bovines, however with a possible involvement of other species (Abe and Iseki, 2003; Peng et al., 1997; Widmer et al., 1998). Therefore, the best way to determine a zoonotic transmission is to compare the *Cryptosporidium* isolates from different hosts using molecular methods and genetic characterization of these isolates (Sargent et al., 1998).

The objective of this study was to isolate *Cryptosporidium* from fecal samples of naturally infected cats, dogs and bovines from the state of São Paulo, Brazil and to determine the genetic characterization of these isolates.

2. Materials and methods

2.1. Sample collection and coprodiagnosis

Stool samples from dogs and cats were obtained through the laboratory routine of the Laboratory of Parasitic Diseases of the Faculty of Veterinary Medicine and Zootechny, University of São Paulo (FMVZ-USP). The cat and dog samples were taken from animals from few days up to 10 and 16 years of age.

The bovine fecal samples were obtained from animals from herds located in the municipalities of Pirassununga, Botucatu, Araçatuba, Presidente Epitácio, Morungaba, Piracaia and Piracicaba, in the state of São Paulo, Brazil. The samples were collected from calves with and without diarrhea within 8 days to 6 months of age.

All the stool samples were previously tested for the presence of oocysts of *Cryptosporidium* spp. using the flotation–concentration method in sucrose, with a specific gravity of 1.205 g/cm^3 (Ogassawara and Benassi, 1980). The samples positive in the coprodiagnostic test were stored at 4 °C in 2.5% potassium dichromate. All the samples were collected from January 2004 to July 2006.

2.2. Morphometric analyses of oocysts

The oocysts were measured using a graduated ocular lens and a 40× objective lens (400× magnification) in a light microscope. In average, four oocysts per sample were measured and a final average was made.

2.3. Oocyst purification

The positive samples for *C. parvum*-like oocysts stored in 2.5% potassium dichromate were submitted again to the flotation–concentration method using disposable material and the floated material used in the diagnosis was washed from the slide and the glass coverslip using 1 ml of TE (Tris–EDTA pH 8.0), in a disposable Petri dish, placed in a 1.5 ml microtube, and later submitted to DNA extraction.

2.4. DNA extraction from oocysts

The purified oocysts were cleared by centrifugation at 12,000 × *g* for 5 min, and then ressuspended in 200 μl TE–SDS (Tris–HCl 10 mM, EDTA 1mM, SDS 1%). After that, the oocysts suspension was submitted to five freeze and thaw cycles in liquid nitrogen and dry-bath at 65 °C, respectively and then proteinase K was added to a concentration 10 μg/ml. The suspension was incubated at 56 °C for 1 h. After incubation, the DNA was extracted using a mixture of phenol–chlorophorm–isoamil-alcohol (25:24:1) and ethanol precipitated as described elsewhere (Sambrook et al., 1989).

2.5. Nested-PCR

The nested-PCR was directed to the 18S rRNA gene (Xiao et al., 1999; Xiao et al., 2000). The primary PCR cycling conditions used were 94 °C for 3 min, followed by 35 cycles of 94 °C for 45 s, 55 °C for 45 s and 72 °C for 60 s. The PCR was finished with final extension of 72 °C for 7 min. The primers (P1: 5′-CCC ATT TCC TTC GAA ACA GGA-3′ and P2: 5′-TTC TAG AGC TAA TAC ATG CG-3′), dNTPs and $MgCl_2$ were used at a final concentration of 0.3 μM, 200 μM and 2.0 mM, respectively. Platinum *Taq* DNA polymerase (Invitrogen, Carlsbad, CA) was used at a final concentration of