

Cryptosporidium infection in Brazil: implications for veterinary medicine and public health

Infecção por *Cryptosporidium* no Brasil: implicações em medicina veterinária e em saúde pública

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Abstract

The aim of this review paper is to report the results of cryptosporidiosis research in Brazil, mainly its occurrence in animals and implications for veterinary medicine and public health. An increasing number of papers related to *Cryptosporidium* spp. infection in Brazil are available at national and international literature. The main focus described in these papers is the occurrence of *Cryptosporidium* spp. in food, environmental samples, in humans and several animal species, particularly birds, cattle, dogs and cats. Using molecular biology techniques, most *Cryptosporidium* species and genotypes identified in other countries have been described in Brazil. In mammals, there are descriptions of infection by *C. bovis*, *C. canis*, *C. felis*, *C. meleagridis*, *C. parvum*, and the cervine genotype; in birds, the following species and genotypes have been described: *C. baileyi*, *C. galli*, *C. meleagridis*, *C. parvum* and the avian genotypes I, II and III. Several species have been described in humans, such as *C. parvum*, *C. hominis*, and some species adapted to animal hosts such as *C. canis*, *C. felis* and *C. meleagridis*.

Keywords: *Cryptosporidium* spp., animals, humans, Brazil.

Resumo

O objetivo deste trabalho foi relatar, por meio de revisão de literatura, os resultados de pesquisas sobre a criptosporidiose no Brasil, com ênfase em sua ocorrência em animais e suas implicações em medicina veterinária e em saúde pública. Um número crescente de trabalhos sobre a infecção por *Cryptosporidium* spp. no Brasil está disponível na literatura nacional e internacional. Nestes trabalhos, são abordados principalmente aspectos relacionados à ocorrência de *Cryptosporidium* spp. em alimentos, amostras ambientais, no homem e em diversas espécies animais, particularmente em aves, bovinos, cães e gatos. Por meio de técnicas de biologia molecular, a maioria das espécies e alguns genótipos identificados em outros países foram descritos no Brasil. Em mamíferos, houve identificação de *C. bovis*, *C. canis*, *C. felis*, *C. meleagridis*, *C. parvum* e o genótipo cervídeo; em diversas espécies de aves, foi descrita infecção por *C. baileyi*, *C. galli*, *C. meleagridis*, *C. parvum* e pelos genótipos I, II e III de aves. Várias espécies foram descritas no homem, como *C. parvum* e *C. hominis*, além de algumas espécies adaptadas a hospedeiros animais, como *C. canis*, *C. felis* e *C. meleagridis*.

Palavras-chave: *Cryptosporidium* spp., animais, homem, Brasil.

Introduction

Cryptosporidiosis is a parasitic disease that affects amphibians, birds, mammals, fish and reptiles, particularly in the gastrointestinal tract, causing clinical or subclinical infections (XIAO et al., 2004; FAYER, 2008). Although the first description of infection due to *Cryptosporidium* was made by Tyzzer, in 1907, it was only in the 1970s, after the first reports of infection in cattle (PANCIERA et al., 1971) and in humans (MEISEL et al., 1976; NIME et al., 1976),

that cryptosporidiosis aroused the attention of researchers because of its potential as an important cause of zoonotic and anthroponotic infections, and clinical or subclinical illnesses, in humans and animals. Until then, it had been considered to be a supposedly rare and opportunist infection (XIAO et al., 2004).

In Brazil, there has been an increasing amount of research and growing numbers of published papers relating to cryptosporidiosis, particularly in poultry, cattle, dogs, cats and humans, and to a lesser extent, in other species of domestic and wild mammals. Most of the published papers have dealt with occurrences of infection by the genus *Cryptosporidium* by means of microscopy, without species classification (Table 1). Studies aiming to classify

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the species of this protozoon, using molecular techniques, remain scarce albeit growing in number (Table 2).

Articles in review form published in several countries have discussed a wide variety of aspects of cryptosporidiosis in humans and animals, but there is little information from studies developed in Brazil (RAMIREZ et al., 2004; XIAO et al., 2004; JEX et al., 2008; BOWMAN; FORSTER, 2010; RYAN, 2010). The aim of the present study was to report, through a review of the literature, the results from studies on cryptosporidiosis in Brazil, with emphasis on its occurrence in animals and its importance within veterinary medicine and public health.

Etiological Agent

The genus *Cryptosporidium* belongs to the phylum Apicomplexa, class Conoidasida, subclass Coccidiasina, order Eucoccidiorida, suborder Eimeriorina and family Cryptosporidiidae (ADL et al., 2005; FAYER, 2008). However, from molecular phylogenetic data, Carreno et al. (1999), Barta and Thompson (2006) and Kuo et al. (2008) suggested that this genus is genetically more closely related to the class Gregarinia. This theory was based on the existence of extracellular stages in the biological cycle of *Cryptosporidium andersoni*; on its multiplication in cell-free culture medium (HIJJAWI et al., 2002); and on the cross-reactivity of anti-*Cryptosporidium* monoclonal antibodies with sporocysts of the genus *Monocystis*, in the direct immunofluorescence reaction (BULL et al., 1998).

There is still no clear and definitive documentation regarding how many and which *Cryptosporidium* species infect amphibians, birds, mammals, fish and reptiles. Around 19 species have been described by means of morphological, biological and molecular data. Because of the absence of data relating to biological and morphological characteristics, several isolates have been classified as genotypes, without species definition (FAYER, 2010).

Occurrence of *Cryptosporidium* in Samples from the Environment, Food and Domestic and Wild Animals in Brazil

1. Samples from the environment and food

The presence of *Cryptosporidium* in samples from the environment and food is important in public health terms. For this reason, research has been conducted resulting in descriptions of *Cryptosporidium* sp. in water treatment works (NISHI et al., 2009a), samples from sewage and rivers (FRANCO et al., 2001; FARIAS et al., 2002; IACOVSKI et al., 2004; NISHI et al., 2009b; RAZZOLINI et al., 2010), from well water for human consumption and from ditches (GAMBA et al., 2000), from bivalve mollusks and oysters (GUIGUET LEAL et al., 2008) and from vegetables (SILVA et al., 2005).

2. Domestic poultry and wild birds kept in captivity

All the species and some genotypes of *Cryptosporidium* that have been reported in birds in other countries (RYAN, 2010) have been described in Brazil (Table 2). The prevalence of 4.86% (47/966) among birds reared in captivity (NAKAMURA et al.,

2009) is similar to what was described by Ng et al. (2006) in Australia (6.25%; 27/430).

There are reports of infection caused by *Cryptosporidium baileyi* in domestic chickens, quails and ducks (MEIRELES; FIGUEIREDO, 1992; CARDOZO et al., 2005; HUBER et al., 2007), including evaluations of the biological, zootechnical and pathogenetic aspects of infections due to this species, following experimental inoculation in domestic chickens (MEIRELES et al., 1999) and quails (CARDOZO et al., 2005). Two species with zoonotic potential have been described in Brazil: *Cryptosporidium meleagridis* in domestic chickens (HUBER et al., 2007; NAKAMURA et al., 2009) and *Cryptosporidium parvum* in cockatiels (*Nymphicus hollandicus*) (NAKAMURA et al., 2009).

A new genotype of *Cryptosporidium* that is present in ostriches and was described by Santos et al. (2005) and Meireles et al. (2006), and which has been correlated with the presence of prolapse of the cloaca and mortality, was subsequently classified as avian genotype II (NG et al., 2006). Oliveira et al. (2008) analyzed 77 samples of ostrich feces from five properties in the State of Rio de Janeiro, and found that 50 samples (44.4%) were positive for *Cryptosporidium* sp.

Passeriformes and Psittaciformes present chronic infection due to *Cryptosporidium galli*, characterized by intermittent shedding of oocysts, which in most cases is of subclinical nature (ANTUNES et al., 2008; SILVA et al., 2010).

There are also reports of infection by *Cryptosporidium* in broiler chickens (JACOBSEN et al., 2006) and in emus (*Rhea americana*) (LUDWIG; MARQUES, 2008), although without species classification.

3. Domestic mammals

The first report on *Cryptosporidium* infection in cattle, in Brazil, was made by Modolo et al. (1988), among dairy calves in the Botucatu region (SP). The prevalence of *Cryptosporidium* in dairy cattle in Brazil ranges from 0.6 to 72.13% (Table 1), with greatest occurrence in animals up to the age of two months (GARCIA; LIMA, 1994; SOUZA; LOPES, 1995; FEITOSA et al., 2004; LANGONI et al., 2004; CARDOSO et al., 2008).

In several Brazilian States, a correlation has been observed between the presence of clinical signs such as diarrhea and the presence of other etiological agents, with occurrences of infection due to *Cryptosporidium* in fecal samples from dairy cattle (GARCIA; LIMA, 1994; SOUZA; LOPES, 1995; LANGONI et al., 2004; CARDOSO et al., 2008; FEITOSA et al., 2008) or beef cattle (OLIVEIRA FILHO et al., 2007).

The epidemiological and clinical characteristics of most of the reports of infection in cattle suggest that the species described was *C. parvum*. *Cryptosporidium* species have been classified according to the morphological characteristics of the oocysts, as *C. parvum* (CARDOSO et al., 2008), *Cryptosporidium muris* (in cattle, currently classified as *Cryptosporidium andersoni*) (SOUZA; LOPES, 1995; PENA et al., 1997) or *C. andersoni* (CARDOSO et al., 2008). Molecular characterization of *Cryptosporidium* species was only done in two studies, with identification of *C. parvum* (HUBER et al., 2007; THOMAZ et al., 2007) and *Cryptosporidium bovis* (THOMAZ et al., 2007).

Table 1. Prevalence of *Cryptosporidium* spp. in fecal samples of bovines, dogs and cats submitted to different diagnostic techniques without species level identification.

Host	State	Nº of positive samples/Nº of samples (% of positive samples)	Diagnostic technique*	Reference
Bovines	Mato Grosso	30/130 (40.0)	AFS	Oliveira Filho et al. (2007)
	Minas Gerais	85/305 (27.87)	AFS	Garcia and Lima (1993)
	Minas Gerais	51/262 (19.5)	AFS	Garcia and Lima (1994)
	Paraná	6/849 (0.6)	CF-BF	Cardoso et al. (2008)
	Rio de Janeiro	36/59 (72.13)	CF-PC, SMB	Souza and Lopes (1995)
	São Paulo	53/308 (17.3)	CF-BF	Pena et al. (1997)
	São Paulo	79/203 (38.9)	AFS	Langoni et al. (2004)
	São Paulo	56/459 (12.4)	CF-PC, ELISA	Feitosa et al. (2004)
Dogs	Ceará	9/88 (10.3)	AFS	Newman et al. (1993)
	Minas Gerais	5/269 (1.85)	ELISA	Figueiredo et al. (2004)
	Minas Gerais	6/256 (2.3)	SMB	Mundin et al. (2007)
	São Paulo	43/1755 (2.4)	CF-BF	Funada et al. (2007)
	Rio de Janeiro	4/81 (4.9)	CF-BF	Pereira et al. (2010)
	Rio de Janeiro	131/500 (26.2)	SMB	Balassiano et al. (2009)
	São Paulo	43/450 (9.5)	AFS, PCR	Lallo and Bondan (2006)
	São Paulo	10/420 (2.4)	ELISA	Bresciani et al. (2008)
	São Paulo	8/240 (3.1)	AFS	Katagiri and Oliveira-Sequeira (2008)
Cats	Rio de Janeiro	6/48 (12.5)	CF-BF, SMB	Huber et al. (2002)
	São Paulo	27/187 (14.4)	CF-BF	Gennari et al. (1999)
	São Paulo	37/327 (11.3)	CF-BF	Funada et al. (2007)
	São Paulo	2/51 (3.9)	MG, ELISA	Coelho et al. (2009)

*AFS: acid-fast stain; SMB: safranin methylene blue; CF-BF: centrifugal-flotation using sucrose solution/bright field microscopy; CF-PC centrifugal-flotation using sucrose solution/phase contrast microscopy; PCR: polimerase chain reaction; ELISA: enzyme-linked immunosorbent assay; MG: malachite green negative strain.

The presence of *Cryptosporidium* oocysts in fecal samples from domestic buffalos was described by Ribeiro et al. (2000), in asymptomatic animals or in animals with symptoms characterized by diarrhea.

The prevalence of infection due to *Cryptosporidium* in dogs, in Brazil, ranges from 2.3% (MUNDIN et al., 2007) to 26.2% (BALASSIANO et al., 2009), according to epidemiological studies that aimed to detect oocysts or soluble antigens in fecal samples. In cats, the prevalence of *Cryptosporidium* ranges from 3.9% (COELHO et al., 2009) to 14.44% (GENNARI et al., 1999) (Table 1). The two *Cryptosporidium* species most commonly found in dogs and cats (*Cryptosporidium canis* and *Cryptosporidium felis*) were described by Huber et al. (2007) and Thomaz et al. (2007), respectively in dogs and cats (Table 2).

The first report of cryptosporidiosis in goats, in Brazil, was made by Vieira et al. (1997), at the Veterinary Hospital of the Federal University of Minas Gerais, among 22 goats aged one to two weeks that presented diarrhea and mortality. On six properties located in the State of Rio de Janeiro, out of a total of 105 fecal samples from goats (from 56 adult animals and 49 young animals) that were examined using the safranin-methylene blue technique, Bomfim et al. (2005) found five samples (4.8%) that were positive for *Cryptosporidium*, all from young animals.

In horses, cryptosporidiosis is generally of subclinical nature, with low prevalence, as demonstrated by Souza et al. (2009), who found a prevalence of 0.75% (3/396) in fecal samples in the State of Rio de Janeiro, which were examined by means of centrifugation-flotation in Sheather solution and the safranin-methylene blue technique.

Asymptomatic infection due to *Cryptosporidium*, in sheep, was observed by Green et al. (2004), in the State of São Paulo, with a prevalence of 55.5% (102/184) during the rainy season and 17.3% (31/179) during the dry season. In the municipality of Ibimirim (State of Pernambuco), the rate of positive findings in fecal samples examined using the Kinyoun technique was 3.7% (3/81), and two animals presented subclinical infection (TEMBUE et al., 2006). In another study, the rate of positive findings in fecal samples from sheep was 6.7% (31/460), which were found in 38.1% (8/21) of the properties investigated in the Araçatuba region (State of São Paulo). *C. parvum* and the cervid genotype were identified by means of nested PCR for amplification of fragments of the 18S subunit of rRNA gene and of the actin gene, with sequencing of amplified fragments (FÉRES et al. 2009).

The prevalence of infection in pigs in Brazil has been found to be 1.7% (2/114) in the State of Minas Gerais and 1.2% (2/174) to 7.6% (32/423) in the State of São Paulo (NISHI et al., 2000; CALDERARO et al., 2001). Clinical infection in piglets, with the presence of *Cryptosporidium* associated with *Escherichia coli*, rotavirus and picobirnavirus was reported by Alferi et al. (1994), in the State of Paraná.

4. Wild mammals and reptiles

In addition to the possibility of clinical illness, the presence of *Cryptosporidium* in wild animals, particularly mammals, represents a possibility for transmission of zoonotic species to humans, including

Table 2. *Cryptosporidium* species and genotypes identified in humans and animals in Brazil using molecular diagnostic techniques.

	Host	Species or genotype	Gene target	Diagnostic technique	Reference
Mammals	Bovine	<i>C. bovis</i> , <i>C. parvum</i>	18S rRNA	PCR/RFLP, PCR/sequencing	Huber et al. (2007); Thomaz et al. (2007)
	Capybara (<i>Hydrochoerus hydrochaeris</i>)	<i>C. parvum</i>	18S rRNA, gp60	PCR/sequencing	Meireles et al. (2006)
	Cat	<i>C. felis</i>	18S rRNA	PCR/RFLP, PCR/ sequencing	Huber et al. (2007); Thomaz et al. (2007)
	Dog	<i>C. canis</i>	18S rRNA	PCR/RFLP, PCR/ sequencing	Huber et al. (2007); Thomaz et al. (2007)
	Guinea pig	<i>Cryptosporidium</i> sp.	18S rRNA	PCR/RFLP, PCR/ sequencing	Huber et al. (2007)
	Human	<i>C. canis</i> , <i>C. felis</i> , <i>C. hominis</i> , <i>C. meleagridis</i> , <i>C. parvum</i>	18S rRNA, COWP, TRAP-C1, ML1	PCR/RFLP, PCR/ sequencing	Brantley et al. (2003); Gatei et al. (2003); Gonçalves et al. (2006); Bushen et al. (2007); Araújo et al. (2008); Lucca et al. (2009)
	Lamb	Cervine genotype, <i>C. parvum</i>	18S rRNA, actin	PCR/ sequencing	Féres et al. (2009)
Birds	Black vulture (<i>Coragyps atratus</i>)	<i>C. baileyi</i>	18S rRNA, actin	PCR/sequencing	Nakamura et al. (2009)
	Canary (<i>Serinus canaria</i>)	Avian genotype I; <i>C. galli</i>	18S rRNA, actin	PCR/sequencing	Antunes et al. (2008); Nakamura et al. (2009)
	Cockatiel (<i>Nymphicus hollandicus</i>)	<i>C. galli</i> , <i>C. parvum</i> , avian genotype III	18S rRNA, actin	PCR/sequencing	Antunes et al. (2008); Nakamura et al. (2009)
	Domestic chicken (<i>Gallus gallus domesticus</i>)	<i>C. baileyi</i> , <i>C. meleagridis</i>	18S rRNA	PCR/RFLP, PCR/ sequencing	Huber et al. (2007); Nakamura et al. (2009)
	Duck*	<i>C. baileyi</i>	18S rRNA	PCR/RFLP, PCR/ sequencing	Huber et al. (2007)
	Great-billed seedfinch (<i>Oryzoborus maximiliani</i>)	<i>C. galli</i>	18S rRNA	PCR/sequencing	Silva et al. (2010)
	Indian peafowl (<i>Pavo cristatus</i>)	Avian genotype I	18S rRNA, actin	PCR/sequencing	Nakamura et al. (2009)
	Lesser seed-finch (<i>Oryzoborus angolensis</i>)	<i>C. galli</i>	18S rRNA, actin	PCR/sequencing	Antunes et al. (2008); Nakamura et al. (2009)
	Ostrich (<i>Struthio camelus</i>)	Avian genotype II	18S rRNA, actin, hsp70	PCR/sequencing	Meireles et al. (2006); Nakamura et al. (2009)
	Quail*	<i>C. baileyi</i>	18S rRNA	PCR/RFLP, PCR/ sequencing	Huber et al. (2007)
	Rosy-faced Lovebird (<i>Agapornis roseicollis</i>)	Avian genotype III	18S rRNA, actin	PCR/sequencing	Nakamura et al. (2009)
	Rusty-collared seedeater (<i>Sporophila collaris</i>)	<i>C. galli</i>	18S rRNA	PCR/sequencing	Silva et al. (2010)
	Saffron finch (<i>Sicalis flaveola</i>)	<i>C. baileyi</i>	18S rRNA, actin	PCR/sequencing	Nakamura et al. (2009)
	Ultramarine grosbeak (<i>Cyanocompsa brissonii</i>)	<i>C. galli</i>	18S rRNA	PCR/sequencing	Silva et al. (2010)

* Avian species was not reported.

the cervid genotype. However, the importance of wild animals in the epidemiology of human cryptosporidiosis has still not been defined (SMITH et al., 2006; XIAO; FAYER, 2008; FENG, 2010).

Among the wild mammals investigated in Brazil, oocysts of *Cryptosporidium* sp. have been found in manatees (*Trichechus manatus*) (BORGES et al., 2009), in fecal samples from wild

mammals of the Atlantic forest (*Akodon serrensis* and *Oryzomys ratticeps*) in three mountainous areas in southeastern Brazil (DALL'OLIO; FRANCO, 2004) and in rodents (*Akodon montensis*, *Sciurus aestuans* and *Thaptomys nigrita*) in a deforested area in the State of São Paulo (LALLO et al., 2009). Meireles et al. (2007) found a zoonotic subtype of *C. parvum* in fecal samples from

capibaras (*Hydrochoerus hydrochaeris*) that were gathered in the city of Araçatuba (State of São Paulo).

In reptiles, there is only one report of the presence of oocysts of *Cryptosporidium* sp., in rattlesnakes housed at the Venomous Animal Study Center (CEVAP) of UNESP, in Botucatu (KARASAWA et al., 2002).

5. Public health

Infections in humans are commonly caused by *C. hominis*, a species that is almost exclusively found in human beings, or by *C. parvum*, which is the main etiologic agent of zoonotic infections, with many cases described in the literature. However, there are sporadic reports of infections in humans caused by other species or genotypes, including *C. canis*, *C. felis*, *C. meleagridis*, *C. muris*, *Cryptosporidium suis* and the rabbit, cervid, squirrel, horse, monkey and pig genotypes (XIAO, 2010).

In Brazil, some species and genotypes with zoonotic potential, such as *C. canis*, *C. felis*, *C. meleagridis*, *C. parvum* and the cervid genotype, have been identified in animals. In humans, there are reports of infection caused by *C. parvum*, *C. hominis* and species that are adapted to animal hosts, such as *C. canis*, *C. felis* and *C. meleagridis* (Table 2).

Concluding remarks

Although there are significant numbers of studies on cryptosporidiosis in Brazil, the molecular epidemiology of the parasite is still little explored. Viewing oocysts under the microscope is a valuable means for diagnosing and determining the intensity of the infection, and for making a morphometric and morphological evaluation on the oocysts, but it does not enable classification of the parasite species. In the Brazilian literature, because of the high cost of molecular techniques, methods based on microscopy are predominantly used for diagnosing cryptosporidiosis (Table 1).

One factor to be borne in mind is that oocysts and other evolutionary stages of the genus *Cryptosporidium* are very small and occasionally very similar, in their morphological and staining characteristics, to yeasts, fungal spores and other structures present in fecal samples. This may result in false-positive diagnoses from routine diagnostic techniques such as Kinyoun, modified Ziehl Neelsen or safranin-methylene blue, or from oocyst observation without staining, after concentration in saturated sucrose, sodium chloride or zinc sulfate solutions.

In the event that there are few oocysts in the fecal samples, or doubts regarding the diagnosis, it is recommended that the diagnosis should be confirmed through combining the techniques described above or, preferably, through using techniques that are more specific, such as immunofluorescence, staining with 4',6'-diamidino-2-phenylindole (DAPI), differential interference contrast microscopy or the polymerase chain reaction.

Although information on certain animal species remains scarce, the information that is available in Brazil demonstrates that cryptosporidiosis presents clinical and epidemiological characteristics that are similar to those found in other countries, particularly in relation to ruminants, horses, dogs and cats; the species and genotypes found in birds and mammals (including

humans) have already been described in other countries. Table 2 shows the *Cryptosporidium* species and genotypes that have been identified using molecular biology techniques in animals and humans in Brazil.

Because *Cryptosporidium* has multiple hosts and transmission cycles, along with genetic variation between species and between genotypes, it is of fundamental importance to undertake molecular characterization of the Brazilian isolates, whether of environmental, food, animal or human origin, in order to epidemiologically evaluate, prevent and control outbreaks of cryptosporidiosis in humans and animals.

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