Available online at www.sciencedirect.com
ScienceDirect
Veterinary Parasitology 147 (2007) 166–170
veterinary parasitology
www.elsevier.com/locate/vetpar

Short communication

Natural infection with zoonotic subtype of *Cryptosporidium parvum* in Capybara (*Hydrochoerus hydrochaeris*) from Brazil

Marcelo Vasconcelos Meireles [a,*], Rodrigo Martins Soares [b], Fábio Bonello [c], Solange Maria Gennari [b]

[a] *Departamento de Clínica, Cirurgia e Reprodução Animal, Curso de Medicina Veterinária, UNESP, Rua Clóvis Pestana, 793, CEP 16050-680, Bairro Dona Amélia, Araçatuba, SP, Brazil*
[b] *Departamento de Medicina Veterinária Preventiva e Saúde Animal, FMVZ, USP, São Paulo, SP, Brazil*
[c] *Departamento de Patologia Veterinária, Faculdade de Ciências Agrárias e Veterinárias, Fundação Educacional de Andradina, Andradina, São Paulo, SP, Brazil*

Received 12 January 2007; received in revised form 26 March 2007; accepted 26 March 2007

Abstract

A total of 145 capybara (*Hydrochoerus hydrochaeris*) fecal samples from the state of São Paulo, Brazil, were screened for *Cryptosporidium* spp. oocysts using the malachite green method. Eight samples (5.52%) showed positive results and were further submitted to nested PCR reaction for amplification of fragments of 18S rRNA gene and 60-kDa glycoprotein gene for determination of species, alleles and subtypes of *Cryptosporidium*. Sequencing of the PCR products of the 18S rRNA gene fragments and 60-kDa glycoprotein gene fragments showed that for both genes all *Cryptosporidium* isolates from capybara were respectively 100% genetically similar to a bovine isolate of *C. parvum* and to *C. parvum* subtype IIaA15G2R1. To the best of our knowledge this is the first report of *Cryptosporidium* infection in this rodent. The finding of zoonotic *C. parvum* infection in a semi-aquatic mammal that inhabits anthroponotic habitats raises the concern that human water supplies may be contaminated with zoonotic *Cryptosporidium* oocysts from wildlife.

Keywords: *Cryptosporidium*; *Hydrochoerus hydrochaeris*; 60-kDa glycoprotein gene; 18S rRNA gene; Natural infection

1. Introduction

Capybara (*Hydrochoerus hydrochaeris*) is the largest living rodent and is widespread in Central and South America, where it lives in a great variety of habitats like forests, seasonally flooded savannas and mangroves (Mones and Ojasti, 1986; Moreira and MacDonald, 1997). This rodent has a strong affinity for water that is used for mating and to avoid predators (Escobar and González-Jiménez, 1976; MacDonald, 1981; Schaller, 1983).

In some regions of Brazil, the extinction of large predators, the process of habitat deforestation and abundant food supply represented by corn, sugar cane and rice crops, near anthropogenic habitats, may result in dramatic increase of capybara population (Ferraz et al., 2003; Verdade and Ferraz, 2006), raising problems related to public health (Labruna et al., 2004) and crop damage (Ferraz et al., 2003).

Cryptosporidium consists of many species and genotypes capable of infecting a wide range of mammals' species, including bovines, humans and wildlife. Humans are infected primarily by *Cryptosporidium parvum* and *Cryptosporidium hominis* whereas bovine

* Corresponding author. Tel.: +55 18 3636 3271; fax: +55 18 3622 4542.
E-mail address: marcelo@fmva.unesp.br (M.V. Meireles).

0304-4017/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetpar.2007.03.034

cryptosporidiosis is mainly caused by *C. parvum* (Ramirez et al., 2004).

Although *C. parvum* is the species most commonly found in zoonotic infections in humans, other *Cryptosporidium* species and genotypes have been implicated as zoonotic agents, like *C. canis*, *C. felis*, *C. meleagridis*, *C. muris*, cervine genotype and pig genotype I (Xiao et al., 2004a).

There are hundred species of wildlife mammals that can be infected with *Cryptosporidium* sp., including zoonotic species and genotypes like *C. parvum*, *Cryptosporidium canis* and cervine genotype (Fayer et al., 2000; Ramirez et al., 2004; Appelbee et al., 2005) but the role of wildlife as reservoir of zoonotic *Cryptosporidium* species and their transmission to humans or to domestic animals remains uncertain.

The identification of *Cryptosporidium* species and genotypes are commonly accomplished by the analyses of a broad range of genes such as 18S rRNA (Xiao et al., 1999), hsp-70 gene (Sulaiman et al., 2000), actin gene (Sulaiman et al., 2002) and COWP gene (Pedraza-Díaz et al., 2001).

The 60-kDa glycoprotein gene (GP60) is highly polymorphic (Strong et al., 2000) and has been recently used for *C. hominis* and *C. parvum* subgenotyping. Such analyses have shown nine *C. parvum* subtype families (alleles), two zoonotic (IIa and IId) and seven anthroponotic (IIb, IIc, IIe, IIf, IIg, IIh and IIi). Within each subtype family there are several subgenotypes based primarily on the number of trinucleotide repeats coding for the amino acid serine (Abe et al., 2006; Akiyoshi et al., 2006; Alves et al., 2006; Peng et al., 2003a; Sulaiman et al., 2005; Trotz-Williams et al., 2006; Xiao et al., 2007).

In the present study *Cryptosporidium* isolates from capybaras from the state of São Paulo, Brazil, were characterized by means of nested PCR and sequencing of amplified fragments of 18S rRNA gene and GP60 gene, which allows the identification of species and discrimination between subtypes of *C. parvum* (Xiao et al., 2001; Peng et al., 2003b).

2. Material and methods

2.1. Fecal samples

Fecal samples (145) from capybaras were collected from the ground in wetlands and in margins of rivers at São Paulo state, Brazil, from August 2004 to April 2005. In order to avoid the collection of more than one sample from the same animal, fecal samples were taken only from fresh deposited feces and each collection area was visited just one time. They were placed in plastic bags containing 5% potassium dichromate, preserved at 4 °C and initially screened for *Cryptosporidium* spp. oocysts by purification in Sheather sugar solution and visualization using malachite green method (Elliot et al., 1999).

2.2. Genomic DNA extraction

Oocysts walls from positive samples were disrupted as previously described (Xiao et al., 2004b; Coupe et al., 2005) with some modifications. Briefly, the pellet containing purified oocysts were suspended in 200 μl of lysis buffer containing 12.5% chelex-100 (Bio-rad, Hercules, California), 1% polyvinylpirrolidone-K-90 (PVP) (USB, Cleveland, Ohio), 10 mM Tris and 10 mM EDTA plus 10 μl of 10% sodium dodecyl sulfate. Samples were incubated at 99 °C for 20 min at 900 rpm following the addition of 30 μl of PVP-TE (10% (w/v) PVP in Tris–EDTA buffer), 66.6 μl of 1 M KOH and 18.6 μl of 1 M dithiothreitol and incubation at 65 °C for 20 min at 900 rpm. The solution was then neutralized with 8.6 μl of 25% hydrochloric acid. DNA extraction was performed using guanidine thiocyanate and silica (Sigma, St. Louis, MO) (McLauchlin et al., 2000).

2.3. Nested PCR reaction

A nested PCR protocol targeting a highly conserved locus (18S rRNA gene) that enables the identification of all known *Cryptosporidium* spp. was used for species and genotypes differentiation (Xiao et al., 2001). For subgenotyping a nested PCR protocol targeting conserved sequences of GP60 gene was used (Peng et al., 2003b). Although these primers were designed based on conserved sequences among many *C. parvum* allele families we cannot assure that they allow the amplification of GP60 fragments of all *C. parvum* allele families and subtypes described to date.

Ultra-pure autoclaved water was used as negative control. DNA of a bovine *C. parvum* previously identified as type B 18S rRNA (GenBank EF175936) and as subtype IIaA15G2R1 (GenBank EF175937) was used as positive control.

2.4. DNA sequence analysis

Sequence accuracy was confirmed by sequencing secondary PCR products from at least two reactions in both directions on an ABI377 Automated Sequencer using an ABI Big Dye™ Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). The primers

Table 1
Sequence identities[a] (*p* distance) among capybara isolates and *C. parvum* allele families subtypes at GP60 gene

GenBank accession	Allele families	1	2	3	4	5	6	7	8	9	10
EF175939	Capybara isolates	100									
DQ192503	IIa	100	100								
AY166805	IIb	85.6	85.6	100							
AY738195	IIc	67.8	67.8	67.8	100						
AY738194	IId	71.2	71.2	76.4	62.9	100					
AY382675	IIe	75.5	75.5	77.0	68.1	72.4	100				
AY738188	IIf	66.6	66.6	64.7	64.1	60.7	67.8	100			
AY873780	IIg	96.9	96.9	85.0	68.1	71.2	73.9	67.5	100		
AY873781	IIh	68.1	68.1	68.7	96.0	63.5	69.6	64.4	68.1	100	
AY873782	IIi	69.6	69.6	68.4	67.5	62.6	70.2	74.2	70.6	66.9	100

[a] Sequence identities calculated corresponding to residues 140 to 574 of *C. parvum* GP60 gene, complete sequence, GenBank AF164489.

used for cycle sequencing were the same as those used in the nested PCR reactions.

The nucleotide sequences obtained in this study were aligned with reference *Cryptosporidium* sequences using the Clustal X software (Thompson et al., 1997) and the degree of similarity among sequences was determined using BIOEDIT (Hall, 1999) for identification of *Cryptosporidium* species, allele families and subtypes.

2.5. Nucleotide sequence accession number

The nucleotide sequences of capybara isolates have been deposited in GenBank under the accession numbers EF175938 and EF175939 for 18S rRNA gene and GP60 gene, respectively.

3. Results

Of the 145 fecal samples examined by microscopy, eight (5.52%) were positive for *Cryptosporidium* sp. oocysts. Nested PCR resulted in amplification of ~850 bp 18S rRNA gene and ~550 bp GP60 gene fragments in all samples positive by microscopy.

Sequencing of the PCR products of the 18S rRNA gene fragments and GP60 gene fragments showed that for both genes all capybara isolates were respectively 100% genetically similar to a bovine isolate of *C. parvum* type A 18S rRNA (Le Blancq et al., 1997) (GenBank AF015772) and to *C. parvum* subtype IIaA15G2R1 (GenBank DQ192503).

Comparisons of sequence identities (*p* distance) among capybara isolates and GP60 *C. parvum* allele families are shown in Table 1. Data showing sequence identities among capybara isolates and other *C. parvum* 18S rRNA types were included in Table 2. Sequence identities were calculated using the software MEGA version 3.0 (Kumar et al., 2004).

Table 2
Sequence identities[a] (*p* distance) among capybara isolates and different types of *C. parvum* 18S rRNA gene

GenBank accession	Types	1	2	3	4	5
EF175938	Capybara isolates	100				
AF015772	Type A	100	100			
AF093490	Type A	100	100	100		
EF175936	Type B	99.9	99.9	99.9	100	
AF308600	Type B	99.9	99.9	99.9	99.9	100

[a] Sequence identities calculated corresponding to residues 239 to 974 of *C. parvum* 18S rRNA gene, complete sequence, GenBank AF093490.

4. Discussion

There are few studies related to *Cryptosporidium* infection in wildlife. Many wildlife mammals may be infected and become reservoirs of *C. parvum*, like deers, raccoon dog, mountain gorilla, nutria, wild horse and alpaca (Appelbee et al., 2005). The analysis of 18S rRNA gene sequence revealed 100% genetic similarity between capybara isolates and *C. parvum* type A 18S rRNA, which is the most common found in bovines.

The analysis of GP60 gene fragments showed that all capybara isolates belong to zoonotic subtype IIaA15G2R1, also with 100% genetic similarity. To date only *C. parvum* allele families IIa and IId are considered zoonotic. The former are the most common found in both humans and bovines and is widespread in many countries (Abe et al., 2006; Alves et al., 2006; Sulaiman et al., 2005; Trotz-Williams et al., 2006; Xiao et al., 2007).

The majority of samples available were from adults, being associated with low levels of oocysts excretion. The shedding of low number of oocysts by a small proportion of adult animals was also observed by Sturdee et al. (2003), and probably contributed to the small prevalence of cryptosporidiosis observed in this study.

Although there were other native species and domestic animals, mainly bovines, in the overlapping areas with some of the capybara samples areas, this study was not designed to determine the prevalence of cryptosporidiosis in animals other than capybaras.

There are no any data about experimental infection with *Cryptosporidium* sp. in capybaras. In Brazil this species is protected by government laws that prevents its utilization in most experimental studies. More extensive studies should be accomplished to determine if capybaras are prone to develop clinical signs of *Cryptosporidium* infection, and if this species are able to shed large numbers of oocysts and contribute to environmental load.

To the best of our knowledge this is the first report of *Cryptosporidium* infection in capybaras. The finding of zoonotic *C. parvum* infection in a semi-aquatic mammal that inhabits anthroponotic habitats raises the concern that human water supplies may be contaminated with zoonotic *Cryptosporidium* oocysts from wildlife.

Acknowledgments

We thank Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq for the following grants: post doctoral scholarship to M.V.M. and a scholarship for productivity in research to S.M.G.

References

Abe, N., Matsubayashi, M., Kimata, I., Iseki, M., 2006. Subgenotype analysis of *Cryptosporidium parvum* isolates from humans and animals in Japan using the 60-kDa glycoprotein gene sequences. Parasitol. Res. 99, 303–305.

Akiyoshi, D.E., Tumwine, J.K., Bakeera-Kitaka, S., Tzipori, S., 2006. Subtype analysis of *Cryptosporidium* isolates from children in Uganda. J. Parasitol. 92, 1097–1100.

Alves, M., Xiao, L., Antunes, F., Matos, O., 2006. Distribution of *Cryptosporidium* subtypes in humans and domestic and wild ruminants in Portugal. Parasitol. Res. 99, 287–392.

Appelbee, A.J., Thompson, R.C.A., Olson, M.E., 2005. *Giardia* and *Cryptosporidium* in mammalian wildlife—current status and future needs. Trends Parasitol. 21, 370–376.

Coupe, S., Sarfati, C., Hamane, S., Derouin, F., 2005. Detection of *Cryptosporidium* and identification to the species level by nested PCR and restriction fragment length polymorphism. J. Clin. Microbiol. 43, 1017–1023.

Elliot, A., Morgan, U.M., Thompson, R.C.A., 1999. Improved staining method for detecting *Cryptosporidium* oocysts in stools using malachite green. J. Gen. Appl. Microbiol. 45, 139–142.

Escobar, A., González-Jiménez, E., 1976. Estudio de la competencia alimenticia de los herbívoros mayores del llano inundable con referencial especial al chiqüire (*Hydrochoerus hydrochaeris*). Agronomía Trop. 26, 215–227.

Fayer, R., Morgan, U.M., Upton, S.J., 2000. Epidemiology of *Cryptosporidium*: transmission, detection and identification. Int. J. Parasitol. 30, 1305–1322.

Ferraz, K.P.M.B., Lechevalier, M.A., Couto, H.T.Z., Verdade, L.M., 2003. Damage caused by capybaras on a corn field. Sci. Agric. 60, 191–194.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. 41, 95–98.

Kumar, S., Tamura, K., Nei, M., 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief. Bioinform. 5, 150–163.

Labruna, M.B., Whitworth, T., Horta, M.C., Bouyer, D.H., McBride, J.W., Pinter, A., Popov, V., Gennari, S.M., Walker, D.H., 2004. *Rickettsia* species infecting *Amblyomma cooperi* ticks from an area in the state of São Paulo, Brazil, where Brazilian spotted fever is endemic. J. Clin. Microbiol. 42, 90–98.

Le Blancq, S., Khramtsov, N.V., Zamani, F., Upton, S.J., Wu, T.W., 1997. Ribosomal RNA gene organization in *Cryptosporidium parvum*. Mol. Biochem. Parasitol. 90, 463–478.

MacDonald, D.W., 1981. Dwindling resources and the social behavior of capybaras (*Hydrocoerus Hydrocaeris*) (Mammalia). J. Zool. 194, 371–392.

McLauchlin, J., Amar, C., Pedraza-Diaz, S., Nichols, G.L., 2000. Molecular epidemiological analysis of *Cryptosporidium* spp. in the United Kingdom: results of genotyping *Cryptosporidium* spp. in 1,705 fecal samples from humans and 105 fecal samples from livestock animals. J. Clin. Microbiol. 38, 3984–3990.

Mones, A., Ojasti, J., 1986. *Hydrochoerus hydrocaeris*. Mammalian species. Am. Soc. Mammal. 264, 1–7.

Moreira, J.R., MacDonald, D.A., 1997. Técnicas de manejo de capivaras e outros grandes roedores na Amazônia. In: Valladares-Padua, C., Bodmer, R.E., Cullen, Jr., L. (Eds.), Manejo e Conservação de Vida Silvestre no Brazil. Sociedade Civil Mamirauá, Belém, Brazil, pp. 186–213.

Pedraza-Díaz, S., Amar, C., Nichols, G.L., Mclauchlin, J., 2001. Nested polymerase chain reaction for amplification of the *Cryptosporidium* oocyst wall protein gene. Emerg. Infect. Dis. 7, 49–56.

Peng, M.M., Meshnick, S.R., Cunliffe, N.A., Thindwa, B.D.M., Hart, C.A., Broadhead, R.L., Xiao, L., 2003a. Molecular epidemiology of cryptosporidiosis in children in Malawi. J. Eukaryot. Microbiol. 50, 557–559.

Peng, M.M., Wilson, M.L., Holland, R.E., Meshnick, S.R., Lal, A.L., Xiao, L., 2003b. Genetic diversity of *Cryptosporidium* sp. in cattle in Michigan: implications for understanding the transmission dynamics. Parasitol. Res. 90, 175–180.

Ramirez, N.E., Ward, L.A., Sreevatsan, S., 2004. A review of the biology and epidemiology of cryptosporidiosis in humans and animals. Microbes Infect. 6, 773–785.

Schaller, G.S., 1983. Mammal and their biomass in a Brazilian ranch. Arq. Zool. 31, 1–36.

Strong, W.B., Gut, J., Nelson, R., 2000. Cloning and sequence analysis of a highly polymorphic *Cryptosporidium parvum* gene encoding a 60-Kilodalton glycoprotein and characterization of its 15- and

45-Kilodalton zoite surface antigen products. Infect. Immun. 68, 4117–4134.

Sturdee, A.P., Bodley-Tickell, A.T., Archer, A., Chalmers, R.M., 2003. Long-term study of *Cryptosporidium* prevalence on a lowland farm in the United Kingdom. Vet. Parasitol. 116, 97–113.

Sulaiman, I.M., Hira, P.R., Zhou, L., Al-Ali, F.M., Al-Shelahi, F.A., Shweiki, H.M., Iqbal, J., Khalid, N.B., Xiao, L., 2005. Unique endemicity of cryptosporidiosis in children in Kuwait. J. Clin. Microbiol. 43, 2805–2809.

Sulaiman, I.M., Lal, A.A., Xiao, L., 2002. Molecular phylogeny and evolutionary relationships of *Cryptosporidium* parasites at the actin locus. J. Parasitol. 88, 388–394.

Sulaiman, I.M., Morgan, U.M., Thompson, R.C.A., Lal, A.A., Xiao, L., 2000. Phylogenetic relationships of *Cryptosporidium* parasites based on the 70-kilodalton heat shock protein (HSP70) gene. Appl. Environ. Microbiol. 66, 2385–2391.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality tools. Nucleic Acids Res. 24, 4876–4882.

Trotz-Williams, L.A., Martin, D.S., Gatei, W., Cama, V., Peregrine, A.S., Martin, S.W., Nydam, D.V., Jamieson, F., Xiao, L., 2006. Genotype and subtype analyses of *Cryptosporidium* isolates from dairy calves and humans in Ontario. Parasitol. Res. 99, 346–352.

Verdade, L.M., Ferraz, K.M.P.M.B., 2006. Capybaras in an anthropogenic habitat in southeastern Brazil. Braz. J. Biol. 66, 371–378.

Xiao, L., Bern, C., Limor, J., Sulaiman, I., Roberts, J., Checkley, W., Cabrera, L., Gilman, R.H., Lal, A.A., 2001. Identification of 5 types of *Cryptosporidium* parasites in children in Lima, Peru. J. Infect. Dis. 183, 492–497.

Xiao, L., Escalante, L., Yang, C., Sulaiman, I., Escalante, A.A., Montali, R.J., Fayer, R., Lal, A.A., 1999. Phylogenetic analysis of *Cryptosporidium* parasites based on the small-subunit rRNA gene locus. Appl. Environ. Microbiol. 65, 1578–1583.

Xiao, L., Fayer, R., Ryan, U., Upton, S.J., 2004a. *Cryptosporidium* taxonomy: recent advances and implications for public health. Clin. Microbiol. Rev. 17, 72–97.

Xiao, L., Ryan, U.M., Graczik, T.K., Limor, J., Li, L., Kombert, M., Junge, R., Sulaiman, I.M., Zhou, L., Arrowood, M.J., Koudela, B., Modry, D., Lal, A.A., 2004b. Genetic diversity of *Cryptosporidium* spp. in captive reptiles. Appl. Environ. Microbiol. 70, 891–899.

Xiao, L., Zhou, L., Santin, M., Yang, W., Fayer, R., 2007. Distribution of *Cryptosporidium* subtypes in calves in eastern United States. Parasitol. Res. 100, 701–706.