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ORIGINAL ARTICLE

NATURAL INFECTION BY *Cryptosporidium* SPP. IN PRODUCTION ANIMALS: FIRST DESCRIPTION OF SUBTYPE IIaA15G2R1 IN GOAT KIDS AND PIGLETS IN BRAZIL

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ABSTRACT

Cryptosporidiosis is a severe enteric disease, with varied clinical manifestations. In young animals the infection is more common and may be more severe. In this study the polymerase chain reaction (PCR) was used to detect *Cryptosporidium* parasites in goat kids, calves, lambs, piglets and colts sharing the same environment. Fecal samples were collected directly from the rectum of 192 goat kids, 184 calves, 44 lambs, 47 piglets and 26 colts aged up to twelve months, males and females, of different breeds, from the Brazilian states of Goiás, Mato Grosso do Sul, Minas Gerais and São Paulo. PCR was used for amplifying a fragment of 18S rRNA gene and the gene encoding the surface glycoprotein GP60. Positive PCR amplification was observed in 16.7% (32/192) goat kids, 6.5% (12/184) calves and 2.1% (1/47) piglets. Based on the sequencing of 18S rRNA PCR products, all samples from goat kids were identified as *C. parvum*. Among calves, *C. parvum* was identified in 41.7% (5/12), *C. andersoni* in 16.7% (2/12), *C. ryanae* in 16.7% (2/12) and *C. bovis* in 25% (3/12) of the animals. All GP60 sequences were classified as genotype IIaA15G2R1 and were found in goat kids, calves and piglets sharing the same environment. This is the first description of the molecular identification and genotyping of *Cryptosporidium* in goat kids and piglets in Brazil. We conclude that *Cryptosporidium* species and *C. parvum* GP60 subtypes that infect livestock in Brazil, may act as sources of zoonotic infection for other animals and humans.

KEY WORDS: Cryptosporidiosis; *Cryptosporidium parvum*; *Cryptosporidium ryanae*; subgenotypes; zoonosis.

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RESUMO

Infecção natural por *Cryptosporidium* spp. em animais de produção: primeira descrição do subtipo IIaA15G2R1 em cabritos e leitões no Brasil

A criptosporidiose é uma doença entérica grave, com manifestação clínica variada e eventual mortalidade, especialmente em animais jovens. Este estudo objetivou realizar a detecção molecular e a subtipagem de *Cryptosporidium* spp. em cabritos, bezerros, cordeiros, leitões e potros que compartilhem o mesmo ambiente. Amostras fecais foram coletadas diretamente do reto de 192 cabritos, 184 bezerros, 44 cordeiros, 47 leitões e 26 potros com até 12 meses de idade, machos e fêmeas, de raças diferentes, provenientes dos estados de Goiás, Mato Grosso do Sul, Minas Gerais e São Paulo. A reação em cadeia da polimerase (PCR) foi realizada para a amplificação dos fragmentos do gene da subunidade 18S rRNA e do gene da glicoproteína GP60. Amplificação positiva para *Cryptosporidium* foi identificada em 16,7% (32/192) dos cabritos, 6,5% (12/184) dos bezerros e 2,1% (1/47) dos leitões. O sequenciamento dos produtos obtidos com o gene 18S rRNA identificou todas as amostras de cabritos como *C. parvum*. Entre os bezerros, as espécies identificadas foram *C. parvum* em 41,7% (12/05), *C. andersoni* em 16,7% (12/02), *C. ryanae* em 16,7% (12/02) e *C. bovis* em 25% (3/12). Para todas as amostras analisadas com o gene GP60, *C. parvum* subtipo IIaA15G2R1 foi encontrado em cabritos, bezerros e leitões que compartilhavam o mesmo ambiente. Esta é a primeira descrição da identificação molecular e subtipagem de *Cryptosporidium* em cabritos e leitões no Brasil. Concluímos que espécies de *Cryptosporidium* envolvidas nas infecções entéricas de animais de fazenda no Brasil podem causar infecções zoonóticas em outros animais, incluindo os seres humanos.

DESCRITORES: Criptosporidiose; *Cryptosporidium parvum; Cryptosporidium ryanae*; subgenótipos; zoonose.

INTRODUCTION

Cryptosporidiosis is an enteric disease of great public health importance, especially for children (Cama et al., 2008; Xiao et al., 2007; Xiao and Feng, 2008) and immunosuppressed individuals (Trotz-Williams et al., 2006; Araújo et al., 2008).

Cryptosporidium parasites have been found in several livestock species such as goats (Quílez et al., 2008; Sanz Ceballos et al., 2009; Pavlović et al., 2010), cattle (Coklin et al., 2009; Fayer et al., 2009; Paul et al., 2009), sheep (Cosendey et al., 2008; Fiuza et al., 2010; Robertson et al., 2010), pigs (Fiuza et al., 2009; Smith et al., 2010) and horses (Chalmers et al., 2005; Toscan et al., 2010).

The genotyping of *Cryptosporidium* infecting animals and humans sharing the same environment is important from a public health point of view, considering the risk of zoonotic transmission of *C. parvum* (Monis & Thompson, 2003; Smith et al., 2007; Bajer, 2008; Tzipori & Widmer, 2008; Wielinga et al., 2008; Xiao & Feng, 2008).

The objective of this study was to use PCR to detect and genotype *Cryptosporidium* oocysts for the feces of multiple livestock species sharing the same environment and assess the transmission potential among different host species.

MATERIAL AND METHODS

Fecal samples were collected directly from the rectum of 192 goat kids, 184 calves, 44 lambs, 47 piglets and 26 colts of different breeds, males and females less than one year of age, from different farms. Some of these animals shared the same farm environment. Two farms were located in the state of Goiás and one farm in the state of Mato Grosso do Sul, one in Minas Gerais and another in São Paulo. The farms were selected for raising animals of different species in the same area.

The extraction of *Cryptosporidium* spp. genomic DNA was performed using a protocol described previously (Silva et al., 2010). For identification of *Cryptosporidium* species, a nested polymerase chain reaction (nPCR) was performed for amplification of the 18S subunit of the ribosomal RNA gene (18SrRNA) (Xiao et al., 2001) following sequencing of amplified fragments sequence in both directions. Samples identified as *C. parvum* were subjected to subtyping using nPCR for amplification of the glycoprotein GP60 gene fragments (Peng et al., 2003). Ultrapure water was used as negative control and DNA of *C. galli* and *C. parvum* were used as positive control for 18SrRNA and GP60 PCR, respectively.

Nested PCR products were purified by using the QIAquick® Gel Extraction Kit (Qiagen) and were sequenced with the ABI Big Dye™ Terminator Cycle Sequencing Kit (Applied Biosystems). Consensus sequences were determined using CodonCode Aligner v. 2.0.4® software (CodonCode Corporation). Sequences were aligned with ClustalW (Thompson et al., 1997) and alignments visualized with BioEdit (Hall, 1999). GenBank sequences were used as reference. Oocysts were not investigated previously in the samples prior to DNA extraction.

These variables were analyzed using the chi-square test ($\chi 2$) or the Fisher's exact test (Zar, 1999), using the SAS program (SAS, 1999), with a significance level of 5%.

This study was approved by the Animal Welfare and Ethics Committee of the School of Agrarian and Veterinary Sciences of UNESP at Jaboticabal, São Paulo State, Brazil, protocol no. 005589-09.

RESULTS

Nested PCR detected *Cryptosporidium* DNA in fecal samples of 16.7% (32/192) of goat kids, 6.5% (12/184) of calves and 2.1% (1/47) of piglets. The association between host species and prevalence was significant (Chi-square=14.2, 2 d.f., $p<0.001$) indicating that cryptosporidiosis was more prevalent in ruminants than in pigs. Of the 45 *Cryptosporidium* positive samples, 38 were classified as *C. parvum* based on the 18S rRNA sequence,

whereas three samples from calves were identified as *C. bovis*, two as *C. ryanae* and two as *C. andersoni*. The *Cryptosporidium* species and GP60 genotypes identified are shown in the Table. GP60 sequences reported in this study were deposited in GenBank under accession numbers KM085969-KM085977.

Table . *Cryptosporidium* species and GP60 genotypes by municipality.

Municipality (State)	Animal species	No. sampled	Species and subtype (No. positive)
Andradina São Paulo	Goat kids	8	*C. parvum* IIaA15G2R1(4)
	Calves	21	*
	Piglets	25	*C. parvum* IIaA15G2R1 (1)
	Colts	1	*
Castilho São Paulo	Goat kids	62	*C. parvum* IIaA15G2R1 (5) *C. bovis* (3)
	Calves	24	*C. parvum* IIaA15G2R1 (2) *C. ryanae* (2)
	Piglets	6	*
	Colts	2	*
Cassilândia Mato Grosso do Sul	Goat kids	19	*C. parvum* IIaA15G2R1 (1)
	Calves	5	*
	Lambs	13	*
Ilha Solteira São Paulo	Goat kids	33	*C. parvum* IIaA15G2R1 (8)
	Calves	39	*
	Lambs	7	*
	Piglets	12	*
	Colts	2	*
Itarumã Goiás	Goat kids	31	*C. parvum* IIaA15G2R1 (9)
	Calves	70	*C. parvum* IIaA15G2R1 (3)
	Lambs	7	*
	Piglets	4	*
	Colts	16	*
Iturama Minas Gerais	Goat kids	16	*C. parvum* IIaA15G2R1 (2)
Três Lagoas Mato Grosso do Sul	Goat kids	23	*C. parvum* IIaA15G2R1 (3)
	Calves	25	*C. andersoni* (2)
	Lambs	17	*
	Colts	5	*

* PCR negative

DISCUSSION

This work is the first molecular identification of *C. parvum* GP60 genotypes in goat kids and piglets in Brazil. *C. parvum* is known to infect livestock, but is less commonly found in pigs (Xiao, 2010). The detection of a single *C. parvum* GP60 genotype in different host species indicates that *C. parvum* is transmitted among host species. However, multiple loci would have to be genotyped to confirm this interpretation. In contrast to our study, Kváč et al. (2011) detected five GP60 subtypes of *C. parvum* in calves, and a predominance of the IIaA15G2R1 and IIaA16G1R1 alleles. Contrasting results were also reported by Drumo et al. (2012). Based on the analysis of multi-locus genotypes, these authors concluded that *C. parvum* infecting goats belonged to a different subpopulation than *C. parvum* isolates from other livestock species.

As in our work, other investigators have reported the occurrence of *C. andersoni, C. bovis* and *C. ryanae* in cattle in Brazil (Meireles et al., 2011). Results similar to ours were obtained by Sevá et al. (2010), who did not observe the occurrence of *Cryptosporidium* in stool samples of equine and ovine.

With respect to cryptosporidosis in pigs, *C. parvum* has been reported as one of the main causative agents of diarrhea in piglets (Calderaro et al., 2001; Guselle et al., 2003; Suárez-Luengas et al., 2007). In addition, *C. parvum* was the predominant species among the calves in the present study, corroborating the findings of Becher et al. (2004) in Australia, Björkman & Mattsson (2006) in Switzerland, Geurden et al. (2006) in Zambia, Coklin et al. (2007) in Canada, and Keshavarz et al. (2009) in Iran. Thus, ruminants have been considered a potential source of *C. parvum* infection for humans (Pirestani et al., 2008; Fayer et al., 2009; Xiao, 2010).

Similarly to the present results, *C. parvum* GP60 subtype IIaA15G2R1 has been frequently detected in cattle (Peng et al., 2003; Díaz et al., 2010), goats (Castro-Hermida et al., 2005; Pavlović et al., 2010), sheep (Santín et al., 2007; Fiuza et al., 2010) and humans all over the world (Abe et al., 2006; Trotz-Williams et al., 2006).

Further studies are needed to assess the impact of infections caused by different species and subtypes of *Cryptosporidium* in production animals raised in Brazil, especially those who share the same environment and can be considered reservoirs of zoonotic subtypes of this protozoan for other animals, including man. Genotyping of additional markers would reveal the extent to which *C. parvum* is transmitted among livestock species and may reveal subpopulations which were not detected in this survey.

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