Veterinary Parasitology 177 (2011) 378–382

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Short Communication

# Molecular characterization of *Cryptosporidium* spp. in pre-weaned dairy calves in the Czech Republic: Absence of *C. ryanae* and management-associated distribution of *C. andersoni*, *C. bovis* and *C. parvum* subtypes

M. Kváč[a,b], N. Hromadová[b], D. Květoňová[a], M. Rost[c], B. Sak[a,*]

[a] *Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, v.v.i., Branišovská 31, České Budějovice 370 05, Czech Republic*
[b] *Faculty of agriculture, University of South Bohemia in České Budějovice, České Budějovice, Czech Republic*
[c] *Faculty of Economics, University of South Bohemia in České Budějovice, České Budějovice, Czech Republic*

ARTICLE INFO

*Article history:*
Received 22 June 2010
Received in revised form
22 November 2010
Accepted 29 November 2010

*Keywords:*
Calves
*Cryptosporidium andersoni*
*C. bovis*
*C. parvum*
GP60
SSU

ABSTRACT

A total of 750 faecal samples of dairy calves at up to 2 months of age kept in various housing systems were screened for *Cryptosporidium* spp. infection using the aniline-carbol-methyl violet staining method. DNA was extracted from *Cryptosporidium* positive samples and from 150 randomly selected microscopically negative samples. Nested PCR was performed to amplify the partial SSU rRNA gene of *Cryptosporidium* that was subsequently digested by SspI, VspI and MboII restriction enzymes to determine the present *Cryptosporidium* species and genotype. In addition, the samples characterized as *Cryptosporidium parvum* were subsequently analyzed at the GP60 gene to determine the distribution of zoonotic subtypes. Sequence analyses and RFLP identified *C. parvum* in 137, *Cryptosporidium andersoni* in 21 and *Cryptosporidium bovis* in 3 samples. Neither mixed infections nor *Cryptosporidium ryanae* was detected. Sequencing of the GP60 gene from *C. parvum*-positive samples revealed all five subtypes of family IIa (A15G2R1, A16G1R1, A22G1R1, A18G1R1, and A15G1R1). The obvious management-associated distribution of *Cryptosporidium* spp. was demonstrated. Direct contact with adult animals was found to be a risky factor for *C. andersoni* and *C. bovis* infection. IIaA15G2R1 and IIaA16G1R1 were detected as major subtypes, whereas only the IIaA16G1R1 subtype was found in animals kept in boxes. Three of the five detected subtypes were previously associated with human cryptosporidiosis, and moreover, the IIaA15G1R1 subtype, previously reported in humans only, was detected in calves for the first time.


## 1. Introduction

Previous studies demonstrated that cattle could be infected with 10 different *Cryptosporidium* species or genotypes at least; nevertheless, *Cryptosporidium parvum*, *Cryptosporidium andersoni*, *Cryptosporidium bovis*, and *Cryptosporidium ryanae* were commonly detected in cattle worldwide. All of them were found in most age categories, especially *C. andersoni* (Kváč et al., 2006; Fayer et al., 2008); *C. parvum* is dominant in pre-weaned calves (<2 months) while *C. bovis* and *C. ryanae* are considered to be predominantly infectious to post-weaned calves (2–11 months) (Santín and Trout, 2007).

Economic losses due to cryptosporidiosis of neonatal calves depend on the *Cryptosporidium* species/genotypes causing the infection. While *C. parvum* infection causes diarrhoea, dehydration, growth retardation and losses to the dairy industry in terms of increased labour and veteri-

* Corresponding author. Tel.: +420 38775419; fax: +420 385310388.
*E-mail address:* casio@paru.cas.cz (B. Sak).

0304-4017/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetpar.2010.11.048

nary costs associated with calf morbidity and, occasionally, mortality (Olson et al., 2004), calves infected with other *Cryptosporidium* species or genotypes exhibit no overt clinical signs (Kváč and Vítovec, 2003; Fayer et al., 2008).

Although previous studies demonstrated that some animals were important zoonotic reservoirs of *Cryptosporidium* in humans (Xiao and Fayer, 2008) and cattle could be infected with 6 out of 15 human pathogenic *Cryptosporidium* species (Kváč et al., 2009), only *C. parvum* is important for the public health in relation to cattle management. At least 11 different *C. parvum* families (IIa-IIl) with many subtypes have been described on the basis of sequence analysis of a 60-kDa glycoprotein (GP60) from humans and other mammals including cattle (Xiao, 2010).

The purpose of this study was to document the prevalence of *Cryptosporidium* spp. infections, to evaluate their occurrence in relation to a housing system in calves from dairy herds in the Czech Republic using molecular tools based on the genus-specific amplification of a gene of a small ribosomal subunit and to assess the relation between *Cryptosporidium* infection and diarrhoea. We also assessed the distribution and diversity of potentially zoonotic subtypes of *C. parvum* by sequence analyses of a gene encoding a 60-kDa glycoprotein.

## 2. Materials and methods

Research was performed on 24 dairy farms in the Czech Republic in 2008 and 2009; since the housing system was changed on one farm during our investigations (from cowshed to box housing), the total number of monitored sites was 25 (Table 1). No other ruminants were kept on the studied farms. The distances among farms were in the range of 30–200 km and all calves were born on the farms where samples were collected. The housing technologies used were as follows: INDIVIDUAL BOX – calves were kept in individual boxes with deep straw bedding and were separated from dams immediately after calving; COWSHED – calves spent the first 3–5 days after calving in close contact with dams and then they were group-housed on straw bedding that was replaced every second week. The contact with dams or other adult cattle was minimised, but possible, especially when the calves were kept in the same stable. Faecal samples were collected directly from the rectum of calves between 20 and 60 days of age. Each sample was individually placed into a plastic container without fixation, stored in the dark at 4 °C and analyzed within 24 h using the aniline-carbol-methyl violet staining method (Miláček and Vítovec, 1985). Faecal consistency was noted at the time of sampling.

Genomic DNA from faecal specimens was isolated from all positive samples and from 150 randomly selected microscopically negative samples as described previously (Sak et al., 2008). A fragment of the SSU rRNA gene of *Cryptosporidium* isolates was amplified by nested PCR according to Alves et al. (2003) and analyzed by restriction fragment length polymorphism (RFLP) according to Xiao et al. (2001) and Feng et al. (2007). The nested PCR protocol by Feng et al. (2007) was used for the amplification of the GP60 gene in the case of *C. parvum* detection. DNA isolated from the faecal sample containing *Cryptosporidium meleagridis* oocysts was used as a positive control. The nucleotide sequences obtained in this study were aligned with reference sequences retrieved from the GenBank using the ClustalX 2.012 programme (ftp://ftp-igbmc.ustrasbg.fr/pub/ClustalX/).

Fisher's Exact Factorial Test for count data was used for statistical analysis to evaluate the independence of rows and columns in a contingency table with fixed marginals in the R programming environment (2007).

## 3. Results

A total of 750 faecal samples were examined, of them 159 were microscopically positive for *Cryptosporidium* spp. oocysts. All microscopically positive faecal specimens were also PCR positive. Moreover, two out of 150 randomly selected microscopically negative samples were PCR positive and identified as *C. bovis*. Three *Cryptosporidium* species, namely *C. parvum*, *C. andersoni* and *C. bovis*, were identified using restriction analyses, accounting for 87.2%, 12.2% and 0.6%, respectively (Table 1). Results of a subsequent analysis of partial sequences of the SSU rRNA gene demonstrated 100% correspondence to RFLP results and showed 100% homology with the GenBank-listed species as follows: 137 *C. parvum* (GenBank accession number: AF093490), 21 *C. andersoni* (AB089285) and 3 *C. bovis* (AY120911). No mixed infection was detected by either microscopy or PCR-RFLP.

*C. parvum* was detected as a dominant species infecting pre-weaned dairy calves in this present study on 70% of the monitored farms with prevalence up to 53.3%. While the prevalence of *C. parvum* on farms with individual box housing ranged from 6.7 to 53.3% (75% of farms), its range in cowshed housing was from 13.3 to 33.3% (56% of farms). *C. bovis* was detected only in three cases on two farms with cowshed housing. Whereas *C. andersoni* was observed more frequently in calves kept in the stable, *C. parvum* infection was detected more often on farms with individual box housing ($p < 0.001$) (Table 1). A housing system was changed on "OL" farm during our investigations. The change of technology led to a 2.5-fold increase in *C. parvum* infections in calves (Table 1).

*Cryptosporidium* spp. oocysts were detected more frequently ($p < 0.001$) in calves with diarrhoea (35%) than in animals with non-diarrhoeal faeces (15%). Among diarrhoeal *Cryptosporidium* positive samples, *C. parvum* was identified in 95% and *C. andersoni* in 5% of cases (Table 1). *C. bovis* was not detected in any diarrhoeal sample. While *C. parvum* oocysts were detected in 37% of diarrhoeal samples collected from calves kept in the box housing system (range of 0–75%), diarrhoea associated with cryptosporidiosis was detected only in 29% of calves kept in the cowshed (range of 0–56%) (Table 1). Nevertheless, the statistical analysis did not reveal any significant differences between the monitored housing systems and occurrence of diarrhoea associated with cryptosporidiosis, mainly in *C. parvum* infection.

Altogether, 5 different subtypes of *C. parvum* family IIa were found in calves in this study (Table 1). Whereas the IIaA15G2R1 subtype was dominant in calves kept in individual boxes, the IIaA16G1R1 subtype was identified only