Veterinary Parasitology 254 (2018) 1–5

Contents lists available at ScienceDirect

Veterinary Parasitology

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

Research paper

Cryptosporidium species in post-weaned and adult sheep and goats from N.W. Spain: Public and animal health significance

P. Díaz*, E. Navarro, A. Prieto, A. Pérez-Creo, M. Viña, J.M. Díaz-Cao, C.M. López, R. Panadero, G. Fernández, P. Díez-Baños, P. Morrondo

Department of Animal Pathology (INVESAGA Group), Faculty of Veterinary Sciences, Universidade de Santiago de Compostela, 27002, Lugo, Spain

ARTICLE INFO

Keywords:
Cryptosporidium
Sheep
Goats
SSU rRNA PCR-RFLP
GP60 subtypes
NW Spain

ABSTRACT

Application of molecular approaches has led to a significant progress on the knowledge of the epidemiology of *Cryptosporidium* spp. Nevertheless, molecular information on the occurrence of cryptosporidiosis in domestic small ruminants, especially in goats, are limited and restricted to the study of a modest number of isolates, mainly from diarrhoeic neonates. In order to determine the *Cryptosporidium* species present in healthy post-weaned and adult small ruminants from north-western Spain and to analyse a possible age-related distribution of species, faecal specimens were collected in sheep and goat farms without neonatal diarrhoea outbreaks the year before the sampling. *Cryptosporidium* spp. DNA was detected by SSU-rRNA PCR-RFLP, using restriction *enzymes SspI*, *VspI* and *MboII*. *C. parvum* and *C. ubiquitum* isolates were further characterized at the GP60 locus.

Our results reveal that *Cryptosporidium* spp. is widely distributed in small ruminant farms (47.4–50.0%), although its prevalence is low in both hosts (5.9-6.0%). No significant differences in individual prevalence were detected between age groups. *C. xiaoi* and the zoonotic *C. parvum* and *C. ubiquitum* were identified. In sheep, *C. parvum* was the predominant species and its prevalence increased with age, in contrast to *C. xiaoi*; *C. ubiquitum* was an occasional finding in adults. In goats, *C. xiaoi* and *C. ubiquitum* were the most frequent species and slightly more prevalent in adults than in post-weaned kids, in contrast to *C. parvum*. Subtyping analysis of *C. parvum* isolates revealed the presence of IIaA15G2R1 and IIaA14G2R1 in sheep, whereas IIaA13G1R1 and IIdA17G1 were restricted to goats; only the *C. ubiquitum* XIIa subtype 3 was found.

Although the prevalences detected are low, these values are probably underestimated due to, amongst others, the cross-sectional design of the study and the intermittent oocyst-excretion of post-weaned and adult small ruminants. Thus, these animals may play an important role in the appearance of cryptosporidiosis outbreaks in humans and domestic ruminant neonates and therefore should be considered as a potential threat to animal production and human health.

1. Introduction

Cryptosporidium is an apicomplexan protozoan globally recognized as a significant source of gastrointestinal illness for a wide range of vertebrate hosts, including humans (Xiao, 2010). Among animals, cryptosporidial infection is especially important in domestic ruminants since it is commonly associated with diarrhoeal disease in suckling animals (Castro-Hermida et al., 2002; Díaz et al., 2010, 2015), leading to significant economic losses to farmers.

Application of molecular approaches for the diagnosis of *Cryptosporidium* infections has led to a significant progress on the knowledge of the epidemiology of this protozoan, allowing the identification of both "host-specific" and zoonotic *Cryptosporidium* species as well as their transmission routes (Xiao, 2010; Chalmers and Katzer, 2013). Regarding farm animals, most data on the species and prevalence of *Cryptosporidium* infections concern cattle; thus, molecular information on the occurrence of cryptosporidiosis in domestic small ruminants, especially in goats, are limited and restricted to a modest number of isolates. Up to now, ten and six *Cryptosporidium* species have been identified in sheep and goats respectively, including *C. andersoni*, *C. baileyi*, *C. bovis*, *C. canis*, *C. fayeri*, *C. hominis*, *C. parvum*, *C. scrofarum*, *C. suis*, *C. ubiquitum*, *C. xiaoi* as well as several genotypes (Ryan et al., 2005; Karanis et al., 2007; Pritchard et al., 2008; Díaz et al., 2010, 2015; Koinari et al., 2014; Mi et al., 2014; Wang et al., 2014; Squire et al., 2017; Zhang et al., 2018).

The age of the animals is one of the most significant risk factors related to the presence of cryptosporidiosis outbreaks in ruminant farms. Thus, it has been widely demonstrated that suckling calves,

* Corresponding author at: Parasitoloxía e Enfermidades parasitarias, Facultade de Veterinaria, Pabellón I Planta Baja, Campus Universitario s/n, 27002, Lugo, Spain
E-mail address: pablo.diaz@usc.es (P. Díaz).

https://doi.org/10.1016/j.vetpar.2018.02.040
Received 16 January 2018; Received in revised form 19 February 2018; Accepted 24 February 2018

Table 1
Cryptosporidium species and *C. parvum* and *C. ubiquitum* GP60 subtypes identified in post-weaned and adult sheep and goats from farms in north-western Spain.

		Positive (%)	*Cryptosporidium* species				GP60 subtypes (positives)	
			C. parvum	*C. ubiquitum*	*C. xiaoi*	Not identified	*C. parvum*	*C. ubiquitum*
Sheep	Post-weaned	5/84 (6.0%)	3	0	2	0	IIaA15G2R1 (1)	–
	Adult	14/240 (5.8%)	10	1	1	2	IIaA14G2R1 (2) IIaA15G2R1 (5)	XIIa (1)
	Total	19/324 (5.9%)	13	1	3	2		
Goats	Post-weaned	3/63 (4.8%)	1	1	1	0	IIdA17G1 (1)	XIIa (1)
	Adult	11/171 (6.4%)	2	4	4	1	IIaA13G1R1 (1) IIdA17G1 (1)	XIIa (2)
	Total	14/234 (6.0%)	3	5	5	1		
	Total	33/558 (5.9)	16	6	8	3		

lambs and kids are the most susceptible to infection by *Cryptosporidium* spp., developing the clinical disease, whereas cryptosporidial infection in adult animals is usually asymptomatic (Castro-Hermida et al., 2007; Mueller-Doblies et al., 2008; Díaz et al., 2015). Molecular techniques have allowed the recent detection of an age-related variation of Cryptosporidium species in cattle, identifying the zoonotic *C. parvum* as the responsible for most of cryptosporidial infections in pre-weaned calves, especially in clinical animals that are considered the main oocyst shedders and major contributors to environmental contamination (Castro-Hermida et al., 2002; Díaz et al., 2010). Older cattle are mainly infected by more adapted and less pathogenic *Cryptosporidium* species, such as *C. ryanae*, *C. bovis* and *C. andersoni* (Santín and Trout, 2008; Xiao, 2010); thus, these animals are probably not responsible for the infection of calves (Paraud et al., 2014), playing a minor role as a source of zoonotic cryptosporidiosis for humans (Chalmers and Katzer, 2013).

In contrast to cattle, the current available information in domestic small ruminants is not complete since the limited number of molecular investigations carried out in those hosts has not allowed proving the existence of a similar age distribution of *Cryptosporidium* species. In fact, most of those studies are mainly focused on cryptosporidiosis outbreaks in diarrhoeic pre-weaned lambs and/or kids, where *C. parvum* is the main species identified (Quílez et al., 2008a; Díaz et al., 2010, 2015; Mueller-Doblies et al., 2008; Pritchard et al., 2008; Cacciò et al., 2013; Imre et al., 2013); information on *Cryptosporidium* infections in post-weaned an adult domestic small ruminants is very scarce, especially in healthy animals, and molecular species identification is not always performed (Robertson, 2009).

The main aim of this study was to determine the *Cryptosporidium* species present in healthy post-weaned and adult sheep and goats from north-western Spain, and to analyse a possible age-related distribution of species. In addition, the role of those animals as shedders of *Cryptosporidium* species causing zoonotic cryptosporidiosis and neonatal diarrhoea outbreaks in small ruminant farms was also assessed.

2. Material and methods

2.1. Study area and characteristics of studied flocks

The study was performed in Galicia, a region located in the northwest of Spain, covering an area of 29,574 km^2. Most of Galician ovine and caprine flocks are noncommercial meat-producing farms with a traditional and semi-intensive rearing system. In general, facilities are small, overcrowded and poorly ventilated, leading to poor hygienic conditions; in addition, it is a common practice that ruminant livestock share grazing areas and facilities (Díaz et al., 2015). Animals freely graze in pastures for some hours a day, depending on the season and weather conditions, and are housed at night-time. Females are mated naturally and the peak lambing period occurs mainly in winter months. During the breeding season, a large number of lambs and kids born in a short period of time; newborn animals are kept indoors with their dams in a separate pen of the shed, that is usually crowded, for a few days before they are integrated into the adult flock.

2.2. Collection of samples

Faecal specimens from post-weaned (2 month-1 year-old) and adult (> 1 year) sheep (n = 324) and goats (n = 234) were collected individually from the rectum over a 2-year period (February 2013–February 2015). Animals originated from 15 and 12 sheep and goat farms, respectively, in north-western Spain. Four additional mixed farms, with both small ruminant species, were investigated. No neonatal diarrhoea outbreaks were reported in the investigated farms the year before the sampling. Between 1 and 65 samples were collected from each farm (Mean = 16.0; Standard deviation = 12.9). All animals were healthy at the time of sampling; moreover, all samples were normally formed faeces. The number of samples collected considering the age of the animals is summarized in Table 1.

2.3. Molecular methods

Cryptosporidium oocysts were firstly concentrated using a diphasic sedimentation technique. Briefly, 2 g of faeces were diluted in 20 ml of distilled water, filtered through a 40 µm mesh size sieve (Filtra Vibración, Badalona, Spain) and centrifuged at 2000 × *g* for 10 min. The supernatant was removed and the sediment resuspended in 8 ml of distilled water and 2 ml of ethyl-acetate. After centrifugation at 2000 × *g* for 10 min, the resulting top three layers were discarded and the sediment was washed in phosphate buffered saline (PBS) pH 7.2. DNA was directly extracted from 200 mg of sediment using a commercial DNA extraction kit (REALPURE Spin Food-Stool, REAL, Valencia, Spain) according to the manufacturer´s protocol, preceded by three cycles of freezing and thawing. DNA extracts were stored at −20 °C.

Detection of *Cryptosporidium* spp. DNA was performed by nested PCR at the SSU-rRNA gene, using primers previously described (Xiao et al., 2001; Jiang et al., 2005). Afterwards, PCR products were digested with restriction *enzymes SspI*, *VspI* and *MboII* (New England BioLabs, Beverly, MA, USA); species identification was made based on the comparison of band patterns with those reported in the literature (Feng et al., 2007; Xiao and Ryan, 2008). A subset of representative positive isolates, which included at least one of each *Cryptosporidium* species identified, was sequenced for confirming endonuclease restriction results.

All *C. parvum* and *C. ubiquitum* isolates were further characterized at the GP60 locus using previously reported protocols (Alves et al., 2003; Li et al., 2014). Products were sequenced using an ABI 3730xl sequencer (Applied Biosystems, Foster City, California, USA). Sequences were aligned and edited with Chromas Pro (Technelysium, Brisbane, Australia), and consensus sequences were searched against the