Veterinary Parasitology 191 (2013) 128–131

Contents lists available at SciVerse ScienceDirect

Veterinary Parasitology

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

Short communication

A rare *Cryptosporidium parvum* genotype associated with infection of lambs and zoonotic transmission in Italy

Simone M. Cacciò[a,*], Anna Rosa Sannella[a], Valeria Mariano[b], Silvia Valentini[c], Franco Berti[d], Fabio Tosini[a], Edoardo Pozio[a]

[a] *Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy*
[b] *Istituto Zooprofilattico Sperimentale del Lazio e della Toscana, Sezione di Grosseto, Italy*
[c] *Sezione Microbiologia, Ospedale della Misericordia di Grosseto, Italy*
[d] *Azienda USL 9, Grosseto, Italy*

ARTICLE INFO

Article history:
Received 30 May 2012
Received in revised form 25 July 2012
Accepted 11 August 2012

Keywords:
Cryptosporidium parvum
Sheep
Outbreak
Zoonotic transmission
Molecular subtyping

ABSTRACT

An outbreak of cryptosporidiosis occurred in a mixed sheep/cattle farm of Central Italy in October 2011. A total of 450 ovines (250 sheep and 200 lambs) and 140 bovines (130 cows and 10 calves) were housed in two separated units, at the time of the outbreak. About half of the lambs had diarrhea due to *Cryptosporidium* sp. with a mortality rate of 80%; calves were not infected. Genomic DNA was extracted from an archived slide and from fecal specimens, and the parasite was identified as *Cryptosporidium parvum* by PCR and sequence analysis at the CpA135 gene. Genotyping at the GP60 gene showed the presence of a very rare genotype, IIaA20G2R1. Shortly after the outbreak was identified, the son of the farm's owner, aged 18 months, experienced an acute gastroenteritis and was hospitalized due to recurrent episodes of diarrhea, fever, vomiting and lack of appetite. The feces tested negative for bacteria and viruses, whereas cryptosporidiosis was diagnosed by microscopy and an immunochromatographic test. Molecular typing identified the *C. parvum* genotype IIaA20G2R1 in the feces of the child. This is the first case of transmission of cryptosporidiosis in Italy involving lambs as source of oocysts infectious to humans.

1. Introduction

Cryptosporidiosis is a common enteric infection of mammals, including domestic and wild animals, and humans (Tzipori and Widmer, 2008). The infection is transmitted through the fecal-oral route, and the extreme robustness of the oocysts, the infective stage of *Cryptosporidium* parasites, accounts for the importance of indirect transmission through ingestion of contaminated water and food (Smith et al., 2006). In humans, cryptosporidiosis is mainly due to *Cryptosporidium hominis*, a species with a predominant anthroponotic cycle, and to *Cryptosporidium parvum*, a zoonotic species with a significant impact on young ruminants (Cacciò et al., 2005; Xiao, 2010). *C. hominis* is the species responsible for the majority of human cases in the United States, Sub-Saharan Africa, and Asia, while *C. parvum* accounts for more human cases in Europe and particularly in the United Kingdom (Chako et al., 2010).

Among livestock, many studies have focused on the bovine host, particularly dairy calves, and the zoonotic role played by these animals is well established (Xiao, 2010). In comparison, less is known about cryptosporidiosis in goats and sheep and discordant opinions exist regarding the zoonotic potential of *Cryptosporidium* sp. oocysts shed by these animals (Robertson, 2009). Epidemiologic investigations have demonstrated the role of sheep in human cryptosporidiosis more than 20 years ago

* Corresponding author. Tel.: +39 06 49902310; fax: +39 06 49903561.
E-mail address: simone.caccio@iss.it (S.M. Cacciò).

0304-4017/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.vetpar.2012.08.010

(Casemore, 1989), and this has been further supported by molecular studies, particularly in the UK (reviewed by Chalmers and Giles, 2010).

It is well established that sheep can be infected with different *Cryptosporidium* species, including *C. parvum*, *Cryptosporidium bovis*, *Cryptosporidium xiaoi*, and *Cryptosporidium ubiquitum*, whereas *C. hominis*, *Cryptosporidium suis*, *Cryptosporidium andersoni*, *Cryptosporidium fayeri* and the pig genotype II have been reported in a very low number of animals (Robertson, 2009; Xiao, 2010). Of these species, only *C. parvum* is of major concern from the perspective of public health, whereas *C. ubiquitum* has been associated only with sporadic cases in defined geographic areas (Fayer et al., 2010). The prevalence of *C. parvum* in sheep is very high in flocks in the UK (Smith et al., 2010), but it is reported to be very low in surveys conducted in Belgium, Norway, Australia, Brazil, China and the USA, where *C. ubiquitum* and *C. xiaoi* predominate (Ryan et al., 2005; Santín et al., 2007; Geurden et al., 2008; Robertson et al., 2010; Wang et al., 2010; Fiuza et al., 2011).

In this report, we have used molecular techniques to investigate a case of cryptosporidiosis in a child and to show that oocysts shed by lambs were the source of infection.

2. Materials and methods

2.1. Source of isolates

On November 5, 2011, a slide was prepared from a fecal sample taken directly from the rectum of a lamb brought to the Istituto Zooprofilattico Sperimentale (IZS) of Grosseto (Tuscany region, Central Italy) for post-mortem examination. The presence of *Cryptosporidium* spp. was investigated microscopically after staining with Kynioun (TCS Biosciences Ltd.), and by an ELISA test (*Cryptosporidium parvum* Elisa Kit, EuroClone).

On November 29, 2011, a fecal sample from the son of the farm's owner, aged 18 months, was collected at the "Ospedale della Misericordia di Grosseto" in Tuscany. On December 2, 2011, fecal samples were collected on farm from 21 adult sheep, 6 lambs and 6 calves. Fat and debris were removed from fecal samples by extraction with ether, followed by centrifugation and washing of the sediment with phosphate buffered saline (PBS). The presence of *Cryptosporidium* sp. oocysts on the concentrated fecal samples was assessed by immunofluorescence using a commercial kit (Merifluor, Meridian Biosciences).

2.2. DNA isolation and molecular characterization

DNA was extracted directly from the concentrated animal and human feces using a commercial kit (QiAmp Stool DNA mini kit, Qiagen). DNA was also extracted from the slide by scraping the surface and washing it with TE buffer (Tris 10 mM, EDTA 1 mM pH = 8.0). Three cycles of freezing in liquid nitrogen and thawing at 80 °C in a thermal block were performed to disrupt the oocysts recovered from the slide. DNA was then extracted using a commercial kit (QiAmp DNA mini kit, Qiagen, Milan, Italy).

PCR amplification of fragments of the CpA135 and of the GP60 genes was performed as described previously (Tosini et al., 2010; Strong et al., 2000) on a Veriti 96 thermocycler (Applied Biosystems). PCR products were purified using spin columns and sequenced on both strands. Sequences were assembled using SeqMan version 7.1 (DNAStar). BLAST search against the GenBank database was used to identify *C. parvum* GP60 genotypes. The novel GP60 sequence determined in this work is available in the GenBank database under the accession number JX110617.

3. Results and discussion

In October 2011, an outbreak of cryptosporidiosis occurred in a farm located in the province of Grosseto (Tuscany, Central Italy), that housed 450 ovines (250 sheep and 200 lambs) and 140 bovines (130 cows and 10 calves). The infection occurred only in lambs, and about 50% of the animals were affected, with a mortality rate close to 80%. The lambs presented with severe, yellowish liquid diarrhea, loss of weight, and depression.

On November 5, 2011, a lamb was brought to the IZS to carry out post-mortem examination and identify a potential cause of death. The animal presented pulmonary edema, liver congestion and catarrhal enteritis. Samples from liver, gut, lung, kidney and an intracardiac cloth were tested for pathogenic bacteria; *Clostridium perfringens* was isolated from the liver and the kidney, whereas *Escherichia coli* was isolated from the gut. The possible eziopathogenic role of these bacteria was investigated but further analysis revealed that enteropathogenic (ETEC), enterotoxic (EPEC) or enterohemorrhagic (EHEC) *E. coli*, as well as *C. perfringens* toxins were absent. The gut also tested negative for Coronavirus and Rotavirus. On the other hand, *Cryptosporidium* sp. oocysts were detected microscopically after Kynioun staining, and cryptosporidiosis was further confirmed by an ELISA test. On December 2, 2011, fecal samples were collected from 21 adult sheep, 6 lambs and 6 calves and tested for the presence of *Cryptosporidium* sp. oocysts. Only one sample from a lamb tested positive by immunofluorescence.

The son of the farm's owner, a child aged 18 months, experienced an acute enteritis in concomitance with the outbreak of cryptosporidiosis in farmed lambs. The main symptom was non-bloody diarrhea, without fever and vomiting, that lasted for at least four weeks. Laboratory tests ruled out common bacterial species (*Salmonella*, *Campylobacter* and *Shigella*) as the cause of diarrhea, and the child was prescribed with a rehydration therapy and probiotics. However, his conditions worsened and he was hospitalized on November 10, 2011, because of episodes of diarrhea, vomiting, dehydration and lack of appetite. Laboratory tests ruled out Rotavirus and Adenovirus as a cause of diarrhea, whereas both microscopy and immunochromatography revealed the presence of *Cryptosporidium* sp. The child received a parenteral rehydration therapy for 18 h and during the hospitalization he had no further episodes of vomiting or diarrhea, and no fever. He started to eat and drink normally and was discharged on November 13, 2011. Notably, a fecal sample collected from the child during a control visit on November 29, 2011, tested positive for *Cryptosporidium* by immunofluorescence, indicating an incomplete clearance of the infection.