Veterinary Parasitology 219 (2016) 53–56

Contents lists available at ScienceDirect

Veterinary Parasitology

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

Short communication

Cryptosporidium parvum: From foal to veterinary students

R. Galuppi*, S. Piva, C. Castagnetti, G. Sarli, E. Iacono, M.L. Fioravanti, M. Caffara

Department of Veterinary Medical Sciences, Alma Mater Studiorum Bologna University, Via Tolara di Sopra 50, 40064 Ozzano Emilia, BO, Italy

ARTICLE INFO

Article history:
Received 5 January 2016
Received in revised form 27 January 2016
Accepted 1 February 2016

Keywords:
Cryptosporidium parvum
Foals
Veterinary students
Zoonosis

ABSTRACT

This paper describes the transmission of a zoonotic subtype of *Cryptosporidium parvum* between two foals hospitalized in an Equine Perinatology Unit (EPU) linked to an outbreak of cryptosporidiosis in veterinary students. Fecal specimens of 36 mares (105 samples) and 28 foals (122 samples) were subjected to Ziehl–Neelsen staining, nested PCR of 18S rDNA. Two foals tested positive for *Cryptosporidium*; PCR restriction fragment length polymorphism (PCR-RFLP) analysis and subtyping by nested PCR of the 60 kDa glycoprotein (gp60) gene revealed *C. parvum* subtype IIdA23G1. The introduction of *Cryptosporidium* into the EPU is suspected to be in a foal showing no initial clinical signs that tested positive for *C. parvum* during an asymptomatic phase. A second foal, hospitalized afterwards for perinatal asphyxia syndrome complicated with failure of passive transfer and sepsis, showed severe watery diarrhea after 4 days of hospitalization and was positive for the same subtype. During this period, six students attending the EPU complained of abdominal pain and diarrhea and were positive for the same subtype of *C. parvum*. To the authors' knowledge, this is the first description of this subtype in foals and the first report of evidence of zoonotic transmission of cryptosporidiosis from foals to human.

Cryptosporidium spp. are ubiquitous protozoan parasites of vertebrates, but until the 1970s the genus was not known to have veterinary or medical significance. First described in mice by Tyzzer (1907), Panciera et al. (1971) described cryptosporidiosis in calves, and currently bovines are the most important animal group known to be infected by *Cryptosporidium*, and a focus of intensive study.

Only in the mid-1970s were *Cryptosporidium* spp. recognized as human pathogens (Nime et al., 1976) with sporadic reports until the 1980s, when the number of cases increased among immuno compromised patients, particularly those with HIV/AIDS (Current et al., 1983). Human infections with *Cryptosporidium* spp. gained further public interest after a massive outbreak of acute watery diarrhea among residents of Milwaukee in 1993 (MacKenzie et al., 1995). Currently, waterborne transmission is the most frequent source of infection for humans. In the following years, several reports demonstrated its worldwide distribution together with its zoonotic potential (Mosier and Oberst, 2000).

Six *Cryptosporidium* species have been reported in humans: *Cryptosporidium hominis*, *C. parvum*, *Cryptosporidium meleagridis*, *Cryptosporidium felis* and *Cryptosporidium ubiquitum* (Li et al., 2014), as well as sporadic reports of *Cryptosporidium muris*, *Cryptosporidium suis* and *Cryptosporidium* cervine genotype (Xiao and Ryan, 2008). *C. parvum* is responsible for most zoonotic infections (Xiao, 2010) and cattle have often been implicated as a source of zoonotic cryptosporidiosis (Anderson et al., 1982; Current et al., 1983; Pohjola et al., 1986; Levine et al., 1988; Reif et al., 1989; Konkle et al., 1997; Mahdi and Ali., 2002; Preiser et al., 2003; Kiang et al., 2006; Gait et al., 2008; Izadi et al., 2014; Webb and Tubach, 2014; Kinross et al., 2015).

In contrast to bovine and human cryptosporidiosis, relatively little is known of the infections, prevalence, clinical manifestation or economic significance of *Cryptosporidium* among equines. In particular, the role of *Cryptosporidium* affecting horses as a source of zoonotic transmission has not been completely elucidated. Majewska et al. (1999) suggested the possibility of transmission, but did not show any direct association between human and equine cryptosporidiosis. The present paper describes the transmission of a zoonotic subtype of *C. parvum* between foals hospitalized in the Equine Perinatology Unit (EPU) "Stefano Belluzzi" (Department of Veterinary Medical Sciences, *Alma Mater Studiorum* University of Bologna—DIMEVET) linked to an outbreak of cryptosporidiosis in veterinary students.

As part of ongoing research on horse cryptosporidiosis at DIMEVET, during foaling seasons 2013 (January–July) fecal specimens were routinely collected directly from the rectum from all the 36 mares (105 fecal samples) and 28 foals (122 fecal samples) hospitalized in the EPU: in foals born at the EPU, the first sample was collected 4 days after birth and then every 4 days until

* Corresponding author. Fax: +39 0512097039.
E-mail address: roberta.galuppi@unibo.it (R. Galuppi).

http://dx.doi.org/10.1016/j.vetpar.2016.02.001
0304-4017/

discharge from the EPU; in pregnant mares, post-partum mares and foals hospitalized after birth, samples were collected upon admission and then every 4 days until discharge from the EPU. Additional stool specimens were collected in cases of diarrhea. The EPU is a university facility attended by people with different qualifications and duties, such as teaching and support staff and veterinary students. All are trained in practices relating to the isolation of diseased animals before beginning their activities at EPU. Students attending the EPU also received a pamphlet about cryptosporidiosis together with instructions to report any episode of diarrhea occurring and, in such cases, to bring in a sample of their feces.

Stool specimens were homogenized in deionized water, filtered through gauze and centrifuged at 900 × *g* for 30 min. A subsample of the sediments were streaked onto a slide, stained with modified Ziehl–Neelsen (ZN) method (Henriksen and Pohlenz, 1981) and observed under a light microscope (400× and 1000× magnification) and another subsample was frozen at −20 °C for molecular analysis. DNA was extracted by QIAamp DNA Stool Mini Kit (Qiagen, Valencia, CA) following the manufacturer's instructions. Nested PCR of the 18S rDNA, PCR-RFLP analysis, and subtyping by nested PCR of the 60 kDa glycoprotein (gp60) gene were performed as previously reported by Caffara et al. (2013).

All stool specimens collected from 36 mares were negative for *Cryptosporidium* while two from 28 foals (7.1%) were positive in ZN or PCR tests. The first positive sample was from an asymptomatic orphan foal (foal A) admitted to the EPU on May 20th 2013: after 5 days one fecal sample was PCR positive without symptoms. Diarrhea appeared 8 days thereafter and continued for a short period, with feces negative for *Cryptosporidium* in both ZN and PCR. During the subsequent period of hospitalization, which lasted 29 days, only one additional sample (June 14), was positive both in ZN and PCR.

The second subject (foal B), a 55 kg, 19-h-old filly, was born in a breeding farm to an 18-year-old multiparous Standardbred mare during the night and was referred at EPU on May 27th, because of weakness after a red bag delivery and was bottle-fed with colostrum. After clinical examinations and laboratory analysis evaluation, the final diagnosis was perinatal asphyxia syndrome (PAS) complicated with failure of passive transfer (FPT), septicemia due to *Streptococcus equi* subsp. *zooepidemicus* and multiple non-displaced fractures from 5th to 7th right ribs, probably due to neonatal resuscitation at the breeding farm. After 4 days of hospitalization, foal B was able to stand and to suckle mare's milk, but she was returned to total parenteral nutrition due to a severe watery diarrhea. Immediately, mare and filly were subjected together to semi-isolation condition within their box inside the Unit, which included dedicated tools for treatments and grooming, physical barriers, and footwear washing and disposable clothes for veterinarians and students. A fecal specimen collected directly from the rectum of foal B was positive for *Cryptosporidium* sp. in both ZN and PCR.

Foal B was treated with supportive therapy, together with one liter of equine plasma, for another 14 days. The other 4 fecal samples collected during this period were positive for *Cryptosporidium* sp. Foal B was euthanized on June 14th (on day 18th of hospitalization) on the owner's instructions, due to fever (39.2 °C), severe abdominal pain and displacement of the rib fractures.

During the necropsy blood suffusions on right lung, corresponding to the fracture of the 5th–7th ribs, and severe necrotizing ileitis were observed. Histologically the ileum was characterized by lesions of variable severity. In the most affected areas the intestinal mucosa was completely lost and replaced by abundant necrotic debris intermixed with scattered degenerated neutrophils; in the less affected areas villi were severely shortened and blunted and the luminal side of enterocytes was lined by a moderate number of 4–6 μm in diameter, amphophilic to basophilic protozoa, referable to *Cryptosporidium* (Fig. 1).

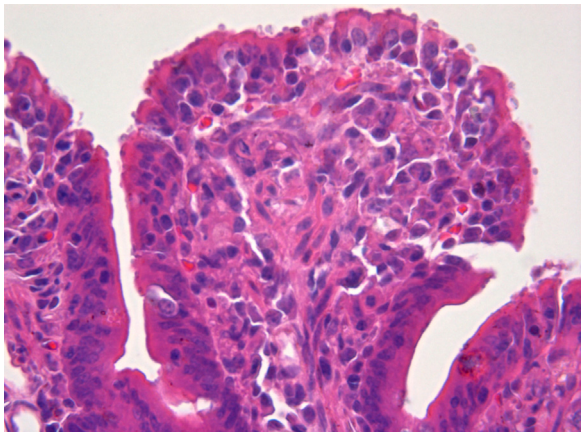

Fig. 1. Foal B. Ileum. The luminal side of enterocytes is lined by a moderate number of amphophilic to basophilic protozoa (cryptosporidia) 4–6 μm in diameter. Hematoxylin-eosin, 63×.

On June 10, a veterinary student attending the EPU reported watery diarrhea, abdominal pain and malaise and delivered to the lab a sample of his stool. Similar complaints were made by two other students the next day, two more students on June 14th and a last student on June 18th, and all brought in stool samples. The last student reported diarrhea since two days before June 18th, and abdominal pain since the preceding 10 days. Along with diarrhea, the students reported headache, nausea, fever, anorexia, weakness/fatigue and, in one case, also a fainting. Almost all students recovered in a week, except one who showed symptoms for over 16 days. All students' fecal samples were positive for *Cryptosporidium* sp. in both ZN and PCR. The Head of the Department and the local Health Authority of Occupational Medicine were informed and students were referred to medical specialists of infectious diseases for possible supportive care. PCR-RFLP analysis of the 18S rDNA gene allowed identification of *C. parvum* in all the positive samples from foals and humans. Subtyping at the gp60 locus (~800 bp) revealed that all the isolates were identical to each other and belonged to the *C. parvum* subtype family IId (IIdA23G1).

Nucleotide sequence data reported in this paper are available in GenBank databases under the accession numbers KR349095–KR349102.

Cryptosporidiosis is one of several causes of diarrhea in foals, together with infectious and noninfectious diseases such as foal heat diarrhea (Frederick et al., 2009). As observed in the foaling season 2012 (Galuppi et al., 2015), *Cryptosporidium* spp. circulates in foals more than might be expected. During the foaling season 2013, an implementation of security measures and rising awareness of the staff with regard to the need of a strict application of cleaning and disinfection procedures and to use basic equipment dedicated to individual box, was carried out in the EPU and this may explain the lower prevalence of infection detected during this survey compared to foaling season 2012 (37.8%). However the presence of non-isolated asymptomatic eliminators is a risk factor for hospitalized critically ill foals that could develop serious clinical signs. In this survey the introduction of *Cryptosporidium* in the EPU could be due to foal A, which initially showed no clinical signs, except for a few days of diarrhea during which 2 stool samples collected were negative. Only two fecal samples out of 12 collected during the asymptomatic phase tested positive for *C. parvum*. Foal B, hospitalized afterwards, was positive for the same *Cryptosporidium* subtype and we hypothesize it became infected by contamination of the EPU environment or equipment. In foal B, the hypoxic-ischemic damages of the intestinal mucosa due to red