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Short Communication

Two outbreaks of cryptosporidiosis associated with cattle spring pasture events

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ABSTRACT

Over a period of less than four weeks, 50 human cases of cryptosporidiosis were reported from a relatively small geographical area in Sweden. All cases were associated with visits to cattle spring pasture events at two farms (referred to as Farm A and B). Epidemiological and microbiological evidence show that contact with calves at the farms was the most likely source of *Cryptosporidium* infections. Gp60 sequences from human and calf isolates at Farm A were identical to each other, but differed from those at Farm B where, again, human and calf gp60 sequences were identical, proving that the two outbreaks had no common origin. As a direct consequence of these two outbreaks, and guided by knowledge gained from the outbreak investigations, the Swedish Board of Agriculture and all relevant farmer advisory organizations have updated their hygiene instructions for farm visits.

1. Introduction

Cryptosporidium spp. are protozoan parasites with an environmentally robust oocyst stage, infective to humans and other animals. The parasite is transmitted by the faecal-oral route, usually through oocyst-contaminated water or food or by direct contact with infected persons or animals, and the infective dose can be very low (Chappell et al., 2006; Chappell et al., 1996; Davies and Chalmers, 2009; DuPont et al., 1995). Several species can infect humans, with the zoonotic *Cryptosporidium parvum* and the anthroponotic *Cryptosporidium hominis* accounting for the vast majority of cases worldwide (Ryan et al., 2014). Surveillance of cryptosporidiosis in humans varies in the European countries and cryptosporidiosis is underdiagnosed throughout this region (Caccio and Chalmers, 2016). Cryptosporidiosis in humans has been a notifiable disease in Sweden since 2004. In Sweden, several food- and waterborne, as well as person-to-person related, outbreaks of *Cryptosporidium* have occurred (Insulander et al., 2005; Insulander et al., 2013; Persson et al., 2007). The largest known outbreaks in Sweden (and Europe) to date occurred in Östersund and Skellefteå in 2010 and 2011. Both were caused by contamination of municipal drinking water with *C. hominis,* and 45,500 people were infected, in total (Bjelkmar et al., 2017; Andersson et al., 2014). Also several direct zoonotic outbreaks or transmissions have also been reported (Beser et al., 2015; Silverlas et al., 2012; Kinross et al., 2015). *C. parvum* is the most common species causing cryptosporidiosis acquired in Sweden (Insulander et al., 2013). The vast majority of Swedish cattle herds have *Cryptosporidium* spp. infected individuals (Bjorkman et al., 2015; Silverlas et al., 2009). Calves, as young as one day of age can shed oocysts (Bjorkman et al., 2015). However, the non-zoonotic *C. bovis* is the most common species in Swedish cattle and *C. parvum* is rarely detected from calves older than six weeks of age (Bjorkman et al., 2015; Silverlas et al., 2010). In contrast to *C. parvum*, infection of *C. bovis* is not associated with calf diarrhoea (Silverlas et al., 2009).

Many farms in Sweden invite the public to watch when cattle are let

Abbreviations: VGR, Västra Götaland Region; RMO, Regional Medical Officer; SAF, Sodium acetate, acetic acid, formaline; SSU rRNA, Small subunit ribosomal RNA; RFLP, Restriction Fragment Length Polymorphism; FITC, Fluorescein isothiocyanate

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out to spring pasture. Sometimes visitors may pet calves, and festivities may include distribution and consumption of milk and snacks. These events attract thousands of visitors and are very popular among families with children. In spring 2015, an unusually high number of cryptosporidiosis cases were reported in Västra Götaland Region (VGR), Sweden. Two cattle spring pasture events could be connected to the cases. An outbreak investigation was initiated to determine scope and source, and to prevent further spread.

2. Materials and methods

2.1 Outbreak investigation

On May 4th, 2015, the Regional Medical Officer (RMO) in VGR was informed by the regional office of Healthcare Guide 1177 about diarrhoea in eight children and a teacher of a primary school class. The school class had visited a spring pasture event at Farm A on April 23rd. Local primary health care centers were contacted to determine if there were an unexpected number of diarrhoeal cases. Between April 23rd and May 7th, several other diarrhoeal cases were found among visitors to the event. On May 7th, *Cryptosporidium* was confirmed as causative agent, and the RMO initiated a joint outbreak investigation team with the County Veterinary Officer, Unilabs Clinical Microbiology Laboratory, the National Veterinary Institute, the Public Health Agency of Sweden, the Swedish Board of Agriculture, and the National Water Catastrophe Group (VAKA).

On May 11th the RMO was informed about diarrhoeal cases in another municipality. These cases were associated with a spring pasture event at Farm B, located more than 50 km from Farm A, on May 1st, and a parallel outbreak investigation team, including the same members, was formed. During the investigation, telephone meetings were held in which all of the participants took part, in addition to several sub-issue specific meetings. Local health care centres were encouraged to test symptomatic persons coupled to either of the two farm events. Initial information to the public was communicated via media and social media. Farm A reported approximately 500 visitors and Farm B reported approximately 3000 visitors. Visitors were allowed to pet young calves in pens at both farms. Also, visitors were offered pasteurised milk and buns. Both farms had biosecurity routines for visitors, which included offering visitors individually wrapped sanitising wipes for hand hygiene.

2.2 Case definition and confirmed cases

A suspected case was defined as a person with any of the following gastrointestinal symptoms: vomiting, diarrhoea, abdominal pain, or malaise; who had visited either of the two events on April 23rd (Farm A) or May 1st (Farm B); or had been in close contact with a person who visited these events. A confirmed case was defined as a suspected case with detection of *Cryptosporidium* spp. in stool by microscopy or by PCR.

2.3 Cryptosporidium diagnostics, species identification and subtyping

Human faecal samples were either transported in sodium acetate, acetic acid and formalin (SAF) fixative and examined by microscopy after concentration and modified Ziehl-Neelsen staining or transported in ESwab™ (Copan) tubes and examined by PCR using previously published primers and probe (Verweij et al., 2004) or examined by both methods. For species identification and subtyping nonfixed stool samples or faeces preserved in ESwab™ tubes were used for DNA extraction. DNA was extracted directly from stool specimens using the QIAamp DNA mini kit (Qiagen, Germany) according to the manufacturer's recommendations. In the case of ESwab™ tubes, liquid was centrifuged and the sediment used for extraction with QIAamp DNA mini kit as above. Disruption of oocysts was performed before extraction by beadbeating using a BulletBlender (Techtum, Sweden). Species identification was performed by amplification of the small subunit ribosomal RNA (SSU rRNA) gene followed by restriction fragment length polymorphism analysis (RFLP) (Xiao et al., 2001; Xiao et al., 1999) and subtyping through sequencing the 60-kDa glycoprotein (gp60) gene (Alves et al., 2003).

At each farm, individual rectal samples were taken from seven preweaned calves.

Oocysts were enriched using flotation based on saturated sodium chloride, labelled with a Fluorescein isothiocyanate (FITC) antibody (Crypto/GiardiaCel, Cellabs Pty Ltd., Australia) and examined by microscopy. Disruption of oocysts was performed using a FastPrep24 homogenizer (MP Biomedicals, USA), and DNA was extracted from all remaining material using the QIAamp DNA stool kit (Qiagen, Germany) according to manufacturer recommendations. Species identification and subtyping were performed as with human samples, but without RFLP. Nucleotide sequences have been deposited in GenBank under accession numbers KT895368–9 (human samples), and MF953283–4 (calf samples).

3. Results and discussion

In recent years, many Swedish farmers have opened their farms for the public to watch when cattle are let out to spring pasture. These events are growing in popularity and are also used to inform the public about farming and are a way for local entrepreneurs to promote their products. In 2015, approximately 200,000 persons participated in these events in Sweden (Lantbrukarnas Riksförbund, LRF 2016), with hundreds or even thousands of visitors at each event. In addition to viewing the cows, petting young calves in pens is a highlight, especially for young visitors.

During the period of May 5th to May 27th, 121 faecal samples were analysed from persons associated with either of the two spring pasture events. Of these, 50 (41.3%) were positive for *Cryptosporidium* sp. and considered confirmed cases (Table 1). Twenty-seven of the confirmed cases were females, 23 were male, and 34 cases (68%), were children under the age of 12. Children, in particular, had visited the petting pens. Among visitors to Farm A, there were 38 confirmed cases, ranging from 1 to 41 years of age (median age 5.5 years).

Symptoms persisted for 7.4 (n = 38) days on average. All these cases had diarrhoea, and some also fever, vomiting, rash and abdominal pain. Among visitors to Farm B, there were 12 confirmed cases with ages ranging from 3 to 62 years (median age 32.5 years).

All but one of the confirmed cases had visited one of the two spring pasture events. However, family members of this case had visited one of the spring pasture events, and the fact that this case had a later time of onset of symptoms, indicates that this case may be a secondary infection. To our knowledge, no cases had visited both events.

Both farms had, due to the risk of zoonotic infections, and in accordance with Swedish regulations (Jordbruksverk, 2013), hygiene routines for visitors: sanitising wipes were offered after animal contact. However, *Cryptosporidium* is, due to oocyst formation, very robust and resistant to most commonly used disinfectants, such as chlorine and

Table 1
Human stool specimens (n = 121) analysed with microscopy and/or PCR. Total positive cases (n = 50) is the total of all PCR positives regardless of microscopy result (n = 45) and microscopy positives when PCR was not performed (n = 5).

	PCR positive	PCR negative	PCR not performed	Total
Microscopy positive	32	0	5	37
Microscopy negative	3	16	29	48
Microscopy not performed	10	26	0	36
Total	45	42	34	121