Cryptosporidium infections in Denmark, 2010-2014

Christen Rune Stensvold1, Steen Ethelberg2, Louise Hansen2, Sumrin Sahar1, Marianne Voldstedlund1, Michael Kemp3, Gitte Nyvang Hartmeyer2, Erik Otte4, Anne Line Engsbro5, Henrik Vedel Nielsen1 & Kåre Mølbak2

ABSTRACT

INTRODUCTION: The incidence of cryptosporidiosis in Denmark is unknown. Here, we present the number of cases detected in the 2010-2014 period along with data on species and subtypes.

METHODS: Complete national data retrieved from the Danish Microbiology Database and Statens Serum Institut (SSI) comprised test results on cryptosporidia detected by microscopy or polymerase chain reaction (PCR) between 1 January 2010 and 30 April 2014. Samples that tested positive at the SSI were submitted to species and subtype analysis by conventional PCR and sequencing of ribosomal and gp60 genes, respectively.

RESULTS: A total of 689 Cryptosporidium-positive stool samples were submitted by 387 patients. Limiting case episodes to two months (60 days), a total of 388 case episodes representing 387 patients were identified. Cryptosporidiosis was most common among infants and toddlers. Moreover, a peak in incidence was observed among younger adults aged 23-24 years. In 43 Cryptosporidium-positive faecal samples, identification was performed to species and subtype level. Cryptosporidium parvum was found in 34 samples, C. hominis in eight, and C. meleagridis in one sample. C. parvum subtypes Ila15G2R1 (n = 10) and Ila16G3R1 (n = 5) were predominating.

CONCLUSION: Cryptosporidia are a significant cause of diarrhoea in Denmark. Outbreaks may not be detected due to continued use of diagnostic tests of limited sensitivity and due to lack of surveillance. With molecular methods now being introduced in many Danish laboratories, we propose establishing national surveillance of cryptosporidiosis.

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Cryptosporidiosis is an infection caused by single-celled parasites of the Cryptosporidium genus. Infected patients may present with watery diarrhoea lasting for up to weeks [1, 2]. Even in otherwise healthy individuals, pronounced symptoms may arise. The parasites are primarily transmitted through contaminated drinking or recreational water; however, infection may occasionally arise through contaminated food or contact to animals or infected individuals [3, 4].

1-10 oocysts (spores) are sufficient to establish infection. The cryptosporidia invade and multiply in the gut epithelium, which is usually accompanied by development of symptoms; oocysts are shed in the faeces throughout this period. The longevity of oocyst shedding varies. Oocysts are environmentally resilient and may survive for days even in chlorinated water; meanwhile, ultraviolet (UV) irradiation and boiling of water is efficient in terms of limiting waterborne transmission.

Cryptosporidiosis is self-limiting within 1-3 weeks in otherwise healthy individuals, whereas immunosuppressed patients may develop chronic diarrhoea with prolonged oocyst shedding. An effective treatment regimen remains to be identified, although some effect of nitazoxanide, a nitrothiazolyl-salicylamide derivative, has been reported [5, 6].

On a global scale, cryptosporidia are a frequent cause of outbreaks. In Milwaukee, USA, an outbreak in 1993 caused by contaminated drinking water affected more than 400,000 people [7]. In 2010, approximately 27,000 people became infected in Östersund, also through contaminated drinking water [8], and waterborne outbreaks are quite common in Sweden [4, 8-11]. In Sub-Saharan Africa and other regions, cryptosporidiosis is one of the most common and serious causes of diarrhoea-related morbidity and mortality in infants and toddlers [12].

The first known outbreak in Denmark was recorded at Hvidovre Hospital in 1989 [13], and in 2005 an outbreak was described that affected employees in a large Danish company due to oocyst-contaminated raw carrots served in the company’s canteen [3]. Since then, minor outbreaks have been described related to the handling of calves, a significant reservoir of Cryptosporidium parvum infection. C. parvum is possibly the species most commonly associated with cryptosporidiosis in humans; another common species is primate-adapted C. hominis. Zoonotic transmission involving other species, including C. meleagridis, C. canis, and C. felis, is seen occasionally [9, 14]. C. parvum infection is associated with more severe symptoms than cases of cryptosporidiosis caused by C. hominis [9]. Multiple subtypes and subtype variants have been described for several species, and molecular typing is critical to successful outbreak investigations.

Contrary to the situation in our neighbouring countries, no national surveillance of cryptosporidiosis exists in Denmark. Hence, the incidence of the infection is unknown, and no guidelines as to which patients should be
suspected of and tested for cryptosporidiosis are available. The aim of this article is to increase awareness of cryptosporidia as a cause of diarrhoea. Based on currently available national data, we summarised the number of cases detected between January 2010 and April 2014, and generated preliminary data that indicate which species account for cryptosporidiosis detected in Denmark. By introducing sensitive diagnostic methods and by increasing the focus on cryptosporidia as a potential cause of diarrhoea, a more complete and accurate picture of the incidence and transmission patterns will be revealed.

METHODS
Data from the Danish Microbiology Database and molecular typing
Data were retrieved from the Danish Microbiology Database (MiBa) [15, 16], which receives laboratory test results from all microbiology departments in Denmark. Data comprised test results on cryptosporidia detected by microscopy or polymerase chain reaction (PCR) between 1 January 2010 and 30 April 2014. As the national reference laboratory for parasitology at Statens Serum Institut (SSI) did not comply with the national standard protocol for transferring electronic microbiological reports to MiBa during the first two years of the study, data from this laboratory were extracted directly from the SSI database and merged with the MiBa data set to obtain complete nationwide data.

Samples identified as positive by the SSI were furthermore submitted to species and subtype analysis by conventional PCR and sequencing of ribosomal (SSU rRNA) and gp60 genes, respectively, as assays for gp60-based subtyping are now available for the three most common species, *C. parvum*, *C. hominis* and *C. meleagris* [17]. Data were standardised and analysed using Stata v. 13. Results are indicated as number of cases, a case-episode being defined as an individual with one or more positive stool samples, excluding multiple positive test results obtained within a 60-day period.

Trial registration: not relevant.

RESULTS
Incidence and distribution
From January 2010 to April 2014, a total of 689 *Cryptosporidium*-positive stool samples had been submitted by 387 patients, several patients testing positive more than once. Limiting case-episodes to two months (60 days), only one patient had more than one episode. Hence, a total of 388 case-episodes representing 387 patients were identified, including 210 episodes in females and 178 episodes in males. Analysing the patient age distribution (Figure 1), it appears that cases of cryptosporidiosis were most common among infants and toddlers. Moreover, a peak in incidence was observed among younger adults aged 23-24 years. In older adults and seniors, only few positive samples were seen.

Among children less than five years old, boys were more prone to developing infection than girls, whereas in adolescents and younger adults, more cases of cryptosporidiosis were found among females than males (Figure 2). In terms of seasonal variation, there was a general trend towards identifying more cases in late...
summer and autumn, which is in agreement with observations from other countries [18].

GP60 data
For 43 Cryptosporidium-positive faecal samples analysed from 2010 to 2014, parasites were identified to species and subtype level. *C. parvum* was found in 34 samples, *C. hominis* in eight, and *C. meleagridis* was identified in a sample from a patient with a recent history of kidney transplantation. With regard to *C. parvum*, we observed a relatively high number of two subtypes that are common in calves in Scandinavia, IIaA15G2R1 (n = 10) and IIaA16G3R1 (n = 5). Some of the affected individuals were veterinarians who had been handling calves prior to developing the infection.

**DISCUSSION**

**Diagnostic considerations**
Traditional parasitology uses microscopy of Ziehl-Neelsen (ZN)-stained smears of faecal concentrates to detect cryptosporidia or commercial kits based on antigen detection. In Denmark, DNA-based methods are being introduced in several routine clinical microbiology laboratories for detection of parasites such as *Cryptosporidium* spp. and *Giardia intestinalis*. A study including 889 prospective and random faecal samples identified a total of 16 cases of cryptosporidiosis by real-time PCR, while no cases were detected by microscopy of ZN-stained faecal smears [19]. At SSI, a genus-specific real-time PCR is employed to enable the detection of species other than *C. hominis* and *C. parvum*. Even when real-time PCR is used, one of two samples from an infected patient may test negative, and it is recommended to test at least two samples to reduce the risk of overlooking cases.

In Denmark, no national guidelines are available regarding the referral of samples for *Cryptosporidium* testing. In Halland, Sweden, PCR has been implemented in the routine screening of patients with diarrhea regardless of type of diarrhea and region of exposure (domestic or travel-related). Interestingly, Halland has the highest incidence of cryptosporidiosis seen throughout Sweden, 17/100,000 (2013; [20]). Meanwhile, in Denmark, testing for cryptosporidiosis is primarily performed in cases of travel-associated or persisting diarrhea, or in cases of diarrhea in immunocompromised individuals. Otherwise healthy individuals with acute diarrhea and no history of travelling are therefore not tested for cryptosporidiosis on a routine basis.

Preliminary research data from the SSI indicate that the incidence of cryptosporidiosis in patients with acute diarrhea acquired in Denmark is comparable to the incidence among patients suspected of cryptosporidiosis, including patients with chronic and/or travel-associated diarrhea. Based on observations from Halland and the SSI, ongoing studies will address whether testing for *Cryptosporidium* should be implemented consistently in the routine screening of stool samples from patients with diarrhea acquired in Denmark.

The samples that were subtyped had been collected over a period of five years. Therefore, the probability of an unrecognised outbreak of, e.g., IIaA15G2R1 is small, though still possible.

Six out of eight cases of *C. hominis* infection were registered in the winter season. In Sweden, the vast majority of *C. hominis* cases are imported [9].

The typing data may indicate that mostly sporadic cases or cases related to exposure to calves are detected in Denmark. A few cases of travel-associated *C. hominis* are seen, and cases in which the disease is particularly debilitating (as in infants and toddlers) are likely to be
identified, which again may explain the relatively higher incidence among infants and toddlers. However, since cryptosporidiosis is probably under-diagnosed, these data should be interpreted with caution.

Moving towards surveillance

For several reasons, cryptosporidiosis is likely under-diagnosed in Denmark. Most often cryptosporidiosis is a self-limiting disease with only few patients seeking medical assistance. Furthermore, there is often a low index of suspicion of cryptosporidiosis and specific examination of faeces for Cryptosporidium species is rarely requested. Finally, some methods currently in use for the detection of cryptosporidia in patient samples have only limited sensitivity. As more sensitive methods are being introduced in diagnostic laboratories, we propose establishing national surveillance of cryptosporidiosis.

CONCLUSION

In Denmark as well as in other countries, cryptosporidiosis constitute an important pathogen causing diarrhoeal disease in otherwise healthy children and adults. Outbreaks are frequently seen in our neighbouring countries and are also likely to occur in Denmark although probably less frequently. However, outbreaks, if they exist, would likely not be easily recognised because of lack of awareness, lack of national guidelines on testing and because of the continued use of diagnostic methods with limited sensitivity. As more sensitive methods are being introduced in diagnostic laboratories, we propose establishing national surveillance of cryptosporidiosis.

CORRESPONDENCE: Christen Rune Stensvold, Infektionsepidemiologi, Statens Serum Institut, Artillerivej 5, 2300 Copenhagen S, Denmark. E-mail: run@ssi.dk

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LITERATURE


Folkmunskmyndigheten.se/Infektionsepidemiologi, Statens Serum Institut, Artillerivej 5, 2300 Copenhagen S, Denmark.
E-mail: run@ssi.dk

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