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Molecular epidemiology and clinical manifestations of human cryptosporidiosis in Sweden

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SUMMARY

This study describes the epidemiology and symptoms in 271 cryptosporidiosis patients in Stockholm County, Sweden. Species/genotypes were determined by polymerase chain reaction–restriction fragment-length polymorphism (PCR–RFLP) of the *Cryptosporidium* oocyst wall protein (COWP) and 18S rRNA genes. Species were *C. parvum* ($n=111$), *C. hominis* ($n=65$), *C. meleagridis* ($n=11$), *C. felis* ($n=2$), *Cryptosporidium* chipmunk genotype 1 ($n=2$), and a recently described species, *C. viatorum* ($n=2$). Analysis of the Gp60 gene revealed five *C. hominis* allele families (Ia, Ib, Id, Ie, If), and four *C. parvum* allele families (IIa, IIc, IId, IIe). Most *C. parvum* cases (51%) were infected in Sweden, as opposed to *C. hominis* cases (26%). Clinical manifestations differed slightly by species. Diarrhoea lasted longer in *C. parvum* cases compared to *C. hominis* and *C. meleagridis* cases. At follow-up 25–36 months after disease onset, 15% of the patients still reported intermittent diarrhoea. In four outbreaks and 13 family clusters, a single subtype was identified, indicating a common infection source, which emphasizes the value of genotyping for epidemiological investigations.

Key words: Clinical manifestations, cryptosporidiosis, molecular epidemiology.

INTRODUCTION

Cryptosporidium spp. are intestinal protozoan parasites that infect a wide range of hosts including ruminants and humans [1]. The parasites are ubiquitous and several species cause acute gastroenteritis in humans. Cryptosporidiosis is usually a self-limiting disease, but can be life threatening in immunocompromised and malnourished individuals. So far, 25 species, as well as a number of potentially new variants of *Cryptosporidium* have been described [2–7]. Most human cases are caused by *Cryptosporidium parvum*, which also infects some other mammals, notably cattle, and *Cryptosporidium hominis*, which

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primarily infects humans. Infection is acquired by ingestion of oocysts, which are shed in the stool of infected animals or humans.

Cryptosporidium oocysts are resistant to chlorine at concentrations used for water treatment, and waterborne transmission has been frequently reported. Outbreaks have been associated both with drinking water and swimming pools [8–10]. The largest known outbreak occurred in Milwaukee in 1993 and affected more than 400 000 persons [10]. The largest known Swedish outbreak occurred during winter 2010–2011 and affected about 20 000 individuals [11]. In recent years, powerful molecular tools have been developed to subtype *C. hominis* and *C. parvum* [12]. The subtypes of the latter species differ in host specificity, some are zoonotic, some anthroponotic, and some bovine [13].

Although cryptosporidiosis is a notifiable disease in Sweden, only 100–400 cases are reported annually. The true incidence is likely to be higher since most laboratories do not test for *Cryptosporidium* unless requested by the clinician. The aim of the current study was to describe epidemiological features and clinical symptoms in patients with cryptosporidiosis in Stockholm County.

METHODS

Patients

From 1 April 2006 to 31 November 2008, all patients with cryptosporidiosis, living in Stockholm County, Sweden, were included in the study through mandatory notifications of confirmed cases by the parasitological laboratory. A questionnaire was sent out enquiring about travel abroad during the 2 weeks before disease onset, symptoms, and symptoms in household members. A follow-up questionnaire 6–36 months after disease onset provided information about treatment, persisting symptoms and complications. A second follow-up questionnaire was completed after a further 9 months by those individuals who reported persisting symptoms or complications at the first follow-up. The study was approved by the ethics committee of Karolinska Institutet, Stockholm, Sweden.

Microbiological investigations

Three parasitological laboratories in Stockholm participated in the study. Submitted stool specimens were screened for the presence of parasites including *Cryptosporidium* by light microscopy on wet smears after formol-ethyl acetate concentration. Correct identification of *Cryptosporidium* oocysts was confirmed by modified Ziehl–Neelsen staining. This staining was also performed on all specimens where *Cryptosporidium* was specifically requested. In all, 271 specimens contained confirmed *Cryptosporidium* oocysts. One hundred and ninety five (72%) stool specimens, most analysed at the Karolinska University Laboratory, Stockholm, were forwarded to the Swedish Institute for Communicable Disease Control (SMI), Solna for molecular analyses. The remaining 76 (28%) samples were initially preserved in sodium acetate-acetic acid-formalin fixative and therefore not suitable for molecular analyses. Stool specimens were also cultured on selective media for bacterial enteropathogens. Detected *Salmonella* spp., *Shigella* spp., *Yersinia* spp., *Aeromonas* spp., *Plesiomonas* spp., and *Campylobacter* spp. were identified by use of routine diagnostic methods (details can be found in the Swedish Institute for Communicable Disease Control database [14]). Stool specimens from patients with bloody diarrhoea were additionally analysed for enterohaemorrhagic *Escherichia coli* by detection of the verotoxin 1 and/or 2 genes by polymerase chain reaction (PCR), and serological typing, as described previously [15].

Molecular analysis

DNA was extracted directly from stool specimens using the QIAamp DNA mini kit (Qiagen, Germany) according to the manufacturer's recommendations. Disruption of oocysts was performed before extraction using a Mini-BeadBeater (Biospec Products Inc., USA) [16]. On a limited number of samples where no amplicons were obtained, a new extraction was performed on oocysts isolated by a sucrose gradient [16].

The *Cryptosporidium* oocyst wall protein (COWP) and the 18S rRNA genes were examined on all isolates using PCR and subsequent restriction fragment-length polymorphism (RFLP) [17, 18]. Sequencing in both directions using standard techniques was performed on a limited number of isolates, (i) to confirm species, (ii) in case of ambiguous or unusual RFLP profiles, (iii) when amplicons were obtained at one locus only.

For subtype analysis of isolates identified as *C. hominis* or *C. parvum*, a nested PCR protocol was used to amplify the 60 kDa glycoprotein (Gp60) gene

Table 1. *Cryptosporidium spp. distribution* and probable area of origin of the disease in 271 patients with cryptosporidiosis, as related to species*

Species (*n*)	Number of isolates (%)					
	Sweden	Other European countries	Africa	Asia	Latin America	North America
C. parvum (111)	57 (51)	35 (32)	4 (4)	13 (12)	2 (2)	0
C. hominis (65)	17 (26)	12 (18)	14 (22)	13 (20)	8 (12)	1 (2)
C. hominis and *C. parvum* (1)	1	0	0	0	0	0
C. meleagridis (11)	0	0	1 (9)	10 (91)	0	0
C. felis (2)	1	0	0	1	0	0
Cryptosporidium chipmunk genotype I (2)	2	0	0	0	0	0
C. viatorum (2)	0	0	1	0	1	0
All cases† (271)	114 (42)	65 (24)	25 (9)	47 (17)	17 (6)	3 (1)

* *Cryptosporidium* isolates from 195/271 patients were available for molecular analysis.
† Includes 76 cases that were not available for species determination and one isolate that remained negative despite repeated PCR trials.

as described by Chalmers *et al.* [19]. All sequences obtained were compared with published sequences in the GenBank database using BLAST [Basic Local Alignment Search Tool, NCBI (http://www.ncbi.nlm.nih.gov/BLAST)]. Representative nucleotide sequences have been deposited in GenBank under accession numbers JN867334–JN867336.

Statistical methods

Fisher's exact test and χ^2 test were used to evaluate differences between characteristics in patients infected with different *Cryptosporidium* spp. Mann–Whitney *U* test was used to compare ages of patients infected with different *Cryptosporidium* spp. JMP software (SAS Institute, USA.) was used for all statistical calculations.

RESULTS

Microbiological and genotyping results

Cultures for bacterial enteropathogens, performed on faecal samples of 232/271 patients, revealed 13 (6%) individuals with bacterial infections including *Campylobacter* ($n=6$), *Salmonella* ($n=4$), *Shigella* ($n=1$), enterohaemorrhagic *E. coli* ($n=1$), and mixed *Salmonella* and *Shigella* infection ($n=1$). Parasitological examination of all samples detected, in addition to *Cryptosporidium* spp., the following parasites: *Giardia intestinalis* ($n=3$), *Blastocystis hominis* ($n=1$), and non-pathogenic amoebas ($n=6$).

Species determination was successfully accomplished in 194/195 analysed isolates (Table 1). RFLP analysis of amplified products of the COWP and 18S rRNA genes revealed identical results in 185 isolates: *C. hominis* ($n=64$), *C. parvum* ($n=110$) and *C. meleagridis* ($n=11$). Thirteen randomly selected isolates were sequenced at the 18S rRNA locus, all confirming the initial results. One isolate demonstrated a *C. hominis* pattern in the COWP–RFLP but a mixed *C. hominis*/*C. parvum* pattern in the 18S rRNA–RFLP. Sequencing confirmed a mixed infection. Two isolates were only amplified at the COWP locus, where RFLP and subsequent sequencing identified one as *C. hominis* and one as *C. parvum*. In addition, six isolates were either negative, or showed inconclusive RFLP patterns, at the COWP locus. At the 18S rRNA locus two of these isolates were identified as *C. felis* (RFLP and sequencing), two as *Cryptosporidium* chipmunk genotype 1 (sequencing), and two had 100% homology with isolate W14532 (GenBank accession no. HM485434), a recently described species designated *C. viatorum* [7]. One isolate remained negative despite repeated PCR trials (Table 1).

Sixty-three of 65 *C. hominis* isolates were successfully subtyped at the Gp60 locus. Isolates belonged to allele families Ia ($n=5$), Ib ($n=44$), Id ($n=10$), Ie ($n=1$), and If ($n=3$) (Table 2). IbA10G2 was the dominating subtype with 37 cases, and this was the only *C. hominis* subtype identified in domestic cases. One patient who had travelled to China was

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Table 2. *Subtypes of* C. hominis *and* C. parvum *from 171 cases of human cryptosporidiosis in Stockholm County, Sweden*

Species	Allele family	Subtype	No. of infected	Origin of infection (number of cases)
C. hominis			65 (63 subtyped)	
	Ia		5	
		IaA21R4	1	The Philippines
		IaA23R3	2	Eritrea (1), Sweden (1 secondary case)
		IaA26R3	1	Egypt
		IaA27R3	1	Bangladesh
	Ib		44	
		IbA9G3	4	India (1), Ethiopia (1), Somalia (1), Republic of Congo (1)
		IbA10G2	37	Sweden (3 sporadic, 9 outbreak related), Thailand (9), Venezuela (5), Portugal (3), Spain (3), Turkey (2), Brazil (1), France (1), Albania (1)
		IbA12G3	2	Mexico
		IbA19G2	1	China
	1d		10	
		IdA14	2	Afghanistan (1), Thailand (1)
		IdA15	4	Spain (3), Ethiopia (1)
		IdA15G1	1	India
		IdA17	1	Ethiopia
		IdA20	1	South Africa
		IdA24	1	South Africa
	Ie	IeA11G3T3	1	Uganda
	If		3	
		IfA12G2	1	South Africa
		IfA14G1	2	The Gambia
C. parvum			111 (107 subtyped)	
	IIa		69	
		IIaA13G1R2	2	Sweden (1), England (1)
		IIaA15G1R1	1	Syria
		IIaA15G2R1	14	Sweden (5), Costa Rica (2) Portugal (4), Azores (1), England (1), France (1)
		IIaA16G1R1	18	Sweden (7 sporadic, 10 outbreak related), Austria (1)
		IIaA16G3R1	1	Sweden
		IIaA16R1	4	Malta
		IIaA17G1R1	16	Sweden (2 sporadic, 13 outbreak related), Mauritius (1)
		IIaA17G2R1	1	Sweden
		IIaA18G1R1	1	Sweden
		IIaA18G3R1	1	Scotland
		IIaA20G3R1	1	Morocco
		IIaA21R1	9	Sweden (1), Norway (8)
	IIc		11	
		IIcA5G3a	6	Syria (3), Egypt (1), Ethiopia (1), Asia (1)
		IIcA5G3k	5	India (5)
	IId		24	
		IIdA16G1	1	Tunisia
		IIdA17G1	3	Portugal (2), Sweden (1)
		IIdA19G1	2	Sweden (1), Portugal (1)
		IIdA20G1c	1	France
		IIdA20G1e	1	Spain
		IIdA22G1	5	Sweden (2 sporadic, 2 outbreak related), Spain (1)
		IIdA22G1c	9	Sweden (8 sporadic), Spain (1)
		IIdA25G1	1	Sweden
		IIdA26G1	1	Spain
	IIe	IIeA7G1	1	India
	IIo	IIoA16G1	2	Thailand
C. hominis + *C. parvum*	Ia, IIc	IaA23R3, IIcA5G3a	1	Sweden (secondary to IaA23R3 from Eritrea)

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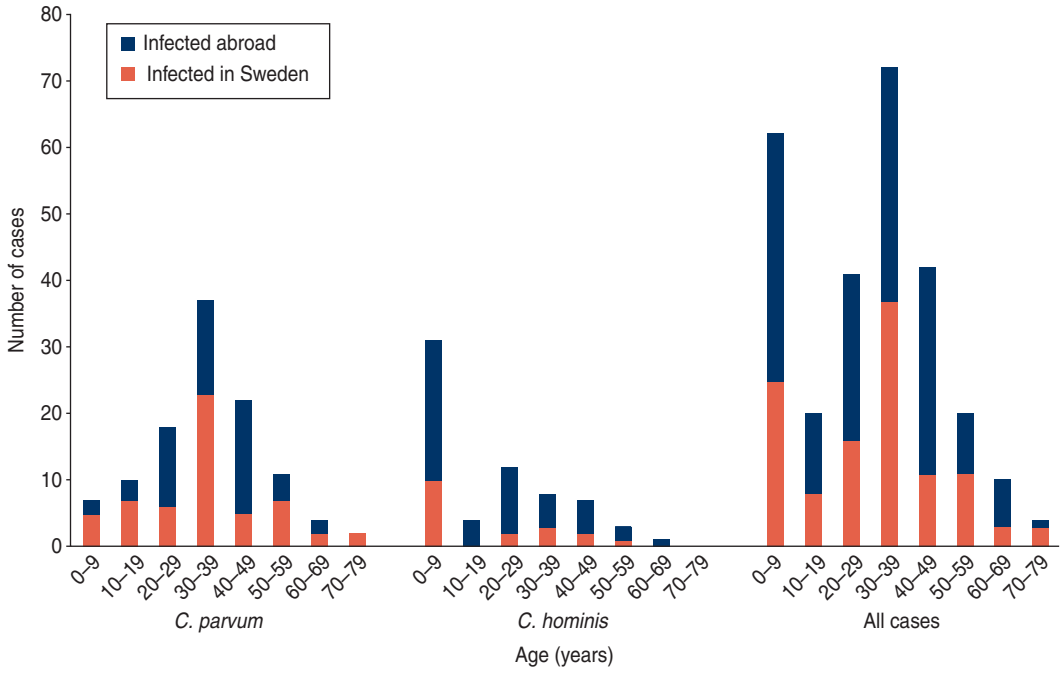

Fig. 1. Age distribution of *Cryptosporidium parvum* and *Cryptosporidium hominis* cases, diagnosed in Stockholm County during the study period, April 2006 to November 2008.

infected with subtype IbA19G2. A IfA12G2 isolate originating from South Africa was identical to a *C. hominis* sequence from a baboon in Kenya (GenBank accession no. JF681172).

Of 111 *C. parvum* isolates, 107 were successfully subtyped and belonged to allele families IIa ($n=69$), IIc ($n=11$), IId ($n=24$), IIe ($n=1$) and novel allele family IIo ($n=2$) (Table 2). All domestic cases were from allele families IIa and IId, with subtypes IIaA16G1R1 and IIaA17G1R1 dominating due to their involvement in confirmed outbreaks. The 11 IIc isolates were of two different subtypes, IIcA5G3a ($n=6$) and novel subtype IIcA5G3k ($n=5$, all from Kerala, India). The novel allele family was matched to IIdA16G1 in the BLAST search but had 69 mutations in the 647-bp post-repetitive sequence compared to the best matching sequence (Gen Bank accession no. FJ917372), including substitutions as well as insertions and deletions. Gp60 analysis of the case with mixed *C. hominis*/*C. parvum* infection identified subtypes IaA23R3 and IIcA5G3a.

Patient characteristics

Patients were either sporadic cases ($n=181$), belonged to outbreaks ($n=60$) or were identified by contact tracing, i.e. follow-up of household contacts to *Cryptosporidium* index cases ($n=30$). There were 126 male and 145 female patients. The age distribution of the patients was bimodal with peaks in children aged <9 years and adults aged 30–39 years (Fig. 1). No differences in age distribution between males and females were found. Median age in patients with *C. hominis* (11 years, range 0–63 years) was lower compared to all cases (32 years, range 1–73 years), *C. parvum* (34 years, range 1–72 years) cases, and *C. meleagridis* (33 years, range 1–73 years) cases ($P<0{\cdot}0001$). The monthly distribution of cases is illustrated in Figure 2. Most cases occurred during August–October, when also four of the five outbreaks were identified, one in 2007 and three in 2008. If outbreaks were excluded, the seasonal pattern was less evident (Fig. 2).

Travel history

Of the 271 patients, 157 (58%) had travelled outside Sweden during the previous 2 weeks (Table 1). Half of the *C. parvum* cases, 57/111 (51%), were probably infected in Sweden as opposed to *C. hominis* cases, that were most likely infected abroad, 48/65 (74%) ($P=0{\cdot}002$). Of 17 *C. hominis* cases infected in Sweden, 14 were most likely secondary to index cases infected abroad. Only seven patients had contacts with farms and farm animals, all of them were infected with *C. parvum* belonging to zoonotic allele families IIa

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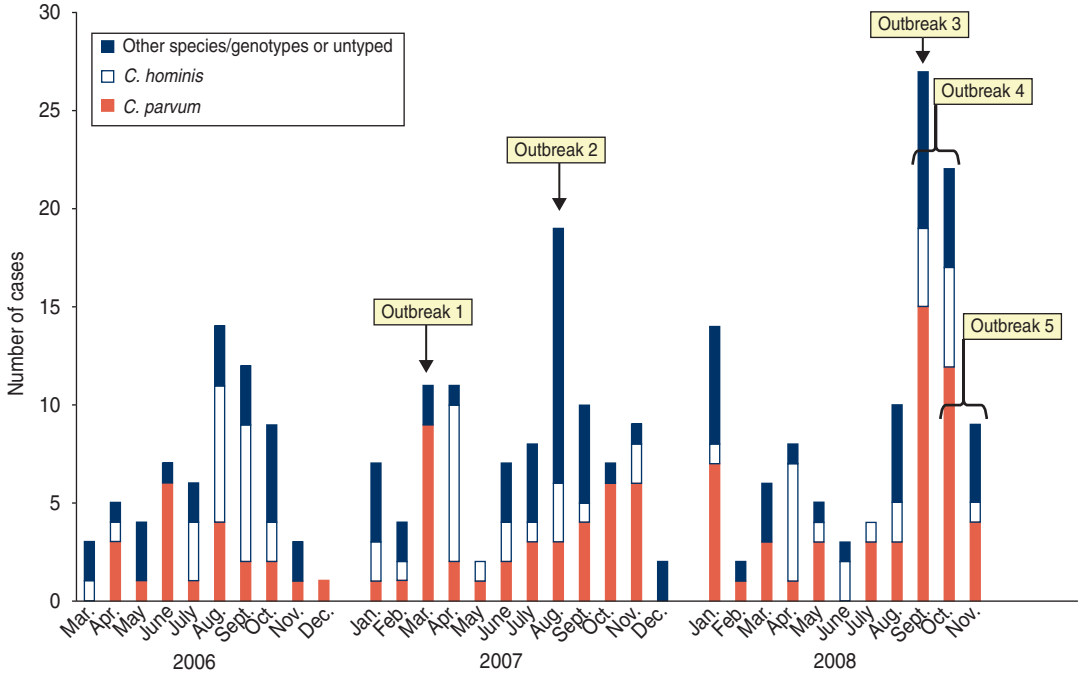

Fig. 2. Monthly distribution of *Cryptosporidium parvum* and *Cryptosporidium hominis* cases diagnosed in Stockholm County during the study period, April 2006 to November 2008. The five outbreaks are described in the text.

and IId. The patient with both *C. parvum* and *C. hominis* was a household contact of a patient who had visited Eritrea and from whom only *C. hominis* could be isolated. All but one of 11 *C. meleagridis* cases were infected in Asia. One of the two patients with *C. felis* was infected during a vacation in India. The other patient had contact with a kitten with diarrhoea 3 weeks before disease onset.

Two unrelated patients, infected in Sweden, carried *Cryptosporidium* chipmunk genotype 1. Two other patients, who had travelled to Kenya and Guatemala, respectively, were infected with *C. viatorum* [7]. These four cases will be described in more detail in a forthcoming publication.

Clinical manifestations

Clinical manifestations in relation to species/genotypes are shown in Table 3. Frequent diarrhoea that lasted for >10 days was more common in patients with *C. parvum* than in patients with *C. hominis* ($P=0{\cdot}003$) or *C. meleagridis* ($P=0{\cdot}005$). Vomiting was more common in *C. meleagridis* patients compared to *C. parvum* patients ($P=0{\cdot}006$) or *C. hominis* patients ($P=0{\cdot}04$). Fever was reported by 50% of patients, but there were no significant differences between species. Forty (15%) patients were hospitalized. There was no correlation between infection with a specific species and hospitalization. Four patients were immunocompromised, three of whom were HIV-positive. *Cryptosporidium* isolates from these patients were not available for genotyping.

Persisting symptoms at different time intervals after disease onset, based on the combined results from the two follow-up questionnaires, are shown in Table 4. The first follow-up questionnaire was completed 6–36 months after disease onset by 196/271 patients (72%). The second follow-up questionnaire was completed after another 9 months by 22/32 patients (69%) who reported persisting symptoms at the first follow-up. The response rates for the various questions at follow-up were 69% and 75%, respectively. After 25–36 months, intermittent diarrhoea and abdominal pain were still reported by 15% and 9% of the patients, respectively. There was no difference in frequency of persisting symptoms between patients infected with *C. parvum* or *C. hominis* (data not shown).

Outbreaks and family clusters

During the study period, five outbreaks and 16 family clusters of cryptosporidiosis, involving 60 and 47 laboratory-confirmed cases, respectively, were identified.

Outbreak 1. At a conference, 20/50 participants became ill with watery diarrhoea. Nine of 17

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Table 3. *Reported symptoms in 251 cryptosporidiosis patients that answered the specific questions of the first questionnaire*. Patients with mixed infections with other enteropathogens are excluded. Data are findings/no. of patients who answered the specific questions* (%)

	Patients infected with (%)			
Symptoms	*C. parvum* ($n=105$)	*C. hominis* ($n=61$)	*C. meleagridis* ($n=9$)	All patients† ($n=251$)
Diarrhoea‡	105/105 (100)	60/61 (98)	9/9 (100)	249/251 (99)
Bowel movements				
<5/day‡	19/90 (21)	15/56 (27)	3/9 (33)	47/217 (22)
5–10/day‡	28/90 (31)	22/56 (39)	5/9 (56)	72/217 (33)
>10/day§	43/90 (48)	19/56 (34)	1/9 (11)	98/217 (45)
Abdominal pain‡	93/105 (89)	46/57 (81)	7/9 (78)	205/237 (86)
Vomiting¶	31/105 (30)	24/59 (41)	7/9 (78)	83/243 (34)
Fever >38 °C‡	50/105 (48)	27/59 (46)	7/9 (78)	117/236 (50)
Duration of symptoms				
1–3 days‖	1/103 (<1)	5/58 (9)	1/9 (11)	8/236 (4)
4–10 days#	19/103 (18)	19/58 (33)	5/9 (56)	64/236 (27)
>10 days$	83/103 (81)	34/58 (59)	3/9 (33)	164/236 (69)

* Response rates for the different questions in the questionnaire varied from 86% to 100%.
† Includes 71 cases where species was not determined and five cases with species/genotypes other than *C. parvum*, *C. hominis*, or *C. meleagridis*.
‡ No significant differences.
§ Significant difference between *C. parvum* and *C. meleagridis* ($P=0{\cdot}03$).
¶ Significant difference between *C. parvum* and *C. meleagridis* ($P=0{\cdot}006$), and *C. hominis* and *C. meleagridis* ($P=0{\cdot}04$).
‖ Significant difference between *C. parvum* and *C. hominis* ($P=0{\cdot}02$).
Significant difference between *C. parvum* and *C. hominis* ($P=0{\cdot}04$), and *C. parvum* and *C. meleagridis* ($P=0{\cdot}02$).
$ Significant difference between *C. parvum* and *C. hominis* ($P=0{\cdot}003$), and *C. parvum* and *C. meleagridis* ($P=0{\cdot}005$).

individuals who supplied faecal specimens were positive for *Cryptosporidium* by microscopy. All nine contained *C. parvum*. Eight samples were successfully subtyped and contained subtype IIaA21R1. Epidemiological data suggested an association with in-house water consumption [20].

Outbreak 2. One adult and 8/14 children with diarrhoea at a day-care centre were positive for *Cryptosporidium*. Two samples were available for molecular analysis and *C. parvum* subtype IIdA22G1 was identified. The infection was probably caught from a diarrhoeal index child by swimming together in a pool [21].

Outbreak 3. Twenty-one cases of diarrhoea occurred among guests and staff at a wedding reception. Sixteen of the cases were positive for *Cryptosporidium* and all 13 isolates that were available for molecular analysis contained *C. parvum* subtype IIaA17G1R1. The suspected vehicle of infection was chopped fresh parsley [22].

Outbreak 4. At a day-care centre, seven children and one household contact were infected with *Cryptosporidium*. Six samples were used for molecular analysis, and *C. hominis* subtype IbA10G2 was identified. The infection was probably acquired from the index child who had fallen ill with diarrhoea after a trip abroad.

Outbreak 5. An increase of sporadic domestic cases of cryptosporidiosis was observed in Stockholm County in autumn 2008. In total, 18 cases were notified. The suspected source of infection, based on a case-control study, was arugula salad. Molecular analysis of 15 samples identified four subtypes of *C. parvum* in this outbreak, IIaA16G1R1 ($n=10$), IIaA15G2R1 ($n=1$), IIdA22G1 ($n=3$), and IId19G1 ($n=1$).

Family clusters. *Cryptosporidium* isolates from 39/47 cases that belonged to 16 different family clusters were analysed. In the 13 clusters where more than one sample was subtyped, isolates from patients within

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Table 4. *Persisting symptoms in 196 cryptosporidiosis patients that answered the follow-up questionnaires. Patients with mixed infections with other enteropathogens were excluded. Data are findings/no. of patients who answered the specific questions after different time intervals*

	No. of patients who reported persisting symptoms after			
Symptoms	6–12 months*	13–24 months*	25–36 months*	>36 months*
Intermittent diarrhoea	6/66	9/76	8/53	1/1
Abdominal pain	1/66	5/76	5/53	
Flatulence	2/66	8/76	8/53	
Myalgia/arthralgia	3/66	4/76	4/53	1/1
Fatigue	4/66	4/76	2/53	1/1

* Time after disease onset.

each cluster were of identical subtypes (Table 5). The transmission route was most likely from child to parent in six of the family clusters, from parent to child in one cluster, and between siblings in one cluster. In the remaining family clusters, the source of infection was unknown.

DISCUSSION

The present study is the first to genetically characterize human *Cryptosporidium* isolates from Swedish patients and to compare the associations of species with clinical manifestations.

The predominance of *C. parvum* is in contrast to findings from many other industrial and developing countries, where *C. hominis* often dominates [23–27]. In Europe, the two species are rather evenly distributed, with *C. parvum* being more prevalent in some reports [28] and *C. hominis* in others [24, 29, 30].

A primarily zoonotic transmission route of domestic *C. parvum* infection was indicated, because all such isolates belonged to zoonotic allele families IIa and IId, whereas infections with subtypes from anthroponotic allele families IIc and IIe were all apparently acquired abroad. However, since the present study was performed in an urban area, where contact with farm animals is minimal, other transmission routes like consumption of contaminated food or water might possibly have been more important. It is also possible that some of the subtypes identified as zoonotic circulate within the human population without intermingling with animal hosts, as has been shown in Scotland [13].

Epidemiological characteristics such as source of infection and transmission routes may explain differences in geographical distribution between *C. hominis* and *C. parvum*. *C. hominis* is transmitted only between humans whereas *C. parvum* infections can result from either zoonotic or anthroponotic transmission. Infections with *C. parvum* have, accordingly, been linked to contact with farms and farm animals, and infections with *C. hominis* with travel abroad and contact with other individuals with diarrhoea [24, 29, 31]. Both species have been associated with drinking-water and swimming-pool outbreaks [8, 10]. Consequently, data from different reports are most likely influenced by the characteristics of the population studied, such as whether people included were living in urban or rural areas, socioeconomic, seasonal and demographic factors and the occurrence of outbreaks during the study period. The predominance of *C. hominis* in developing countries, suggesting primarily anthroponotic transmission, is probably associated with hygiene practices and contaminated drinking water.

Cryptosporidium spp. other than *C. parvum* and *C. hominis* were identified in 8·7% of the cases, the most common being *C. meleagridis* (6%). This figure is high compared to studies from other developed countries, where *C. meleagridis* usually accounts for only about 1% of cases [31, 32]. Most of our patients were infected in Thailand (data not shown), a very popular tourist destination for Swedes, and a country where *C. meleagridis* seems to be more prevalent than *C. parvum*, at least in immunocompromised patients [33–35]. We also report a recently described species, *C. viatorum*, identified in two patients. This species has been identified in ten persons that had travelled to India, Nepal, Pakistan or Bangladesh [7]. Interestingly, our patients had travelled to South America and Africa and had no connection with each other. One patient had a mixed *C. hominis* and

Table 5. *Subtypes of* C. parvum *and* C. hominis *from 39 of 47 individuals belonging to 16 different family clusters*

Cluster	No. cases (no. typed)	Origin of infection	*Cryptosporidium* spp.	Subtype
1	3 (3)	Sweden	*C. hominis*	IbA10G2
2	4 (2)	Sweden	*C. parvum*	IIdA22G1
3	3 (3)*	Eritrea	*C. hominis*	IaA23R3
4	2 (2)	India	*C. parvum*	IIcA5G3k
5	4 (2)	Sweden	*C. hominis*	IbA10G2
6	2 (1)†	Sweden	*C. parvum*	IIaA16G1R1
7	6 (6)	Thailand	*C. hominis*	IbA10G2
8	3 (3)	Portugal	*C. hominis*	IbA10G2
9	2 (2)	Spain (Mallorca)	*C. hominis*	IdA15
10	2 (2)	Sweden	*C. parvum*	IIaA16G1R1
11	5 (5)	Venezuela	*C. hominis*	IbA10G2
12	3 (1)	Spain (Mallorca)	*C. hominis*	IbA10G2
13	2 (2)	Costa Rica	*C. parvum*	IIaA15G2R1
14	2 (1)	Sweden	*C. parvum*	IIaA16G1R1
15	2 (2)	India	*C. parvum*	IIcA5G3k
16	2 (2)	Sweden	*C. parvum*	IIaA16G1R1

* One of the cases was also infected with *C. parvum*.
† One *Cryptosporidium* isolate could not be subtyped.

C. parvum infection, a proportion comparable with data from other reports [24, 26, 32, 36].

The median age of patients differs from reports from both developed and developing countries, where children aged <10 years usually predominate [9, 24, 29–31]. High drinking-water quality and sanitary conditions in Sweden may prevent infections in children. Moreover, three of the five outbreaks involved mainly adults.

The bimodal age distribution is in agreement with studies from France [31], The Netherlands [30], and the USA [37]. Patients infected with *C. hominis* were significantly younger compared to the whole group of patients as well as those infected with *C. parvum*. Many were children aged <10 years. This difference has also been found in other European countries [30, 38] and may reflect child behaviours that favour infections with *C. hominis*, like close person-to-person contacts and frequent swimming in pools. In contrast to *C. parvum* cases, the age distribution of *C. hominis* cases was bimodal. This may reflect transmission between parents and their children.

The predominance of cases in late summer and autumn has also been noted in reports from the USA and other European countries [9, 30, 31, 36–38], except for the UK and Ireland, where a spring peak, mainly due to *C. parvum* infections, was noted [28]. Four of the five outbreaks in the present study occurred in late summer and autumn, which in part may explain the increased number of cases during this season. Seasonal behaviours, e.g. increased outdoor activities, international travel, and swimming pool use in summer and autumn may be another explanation.

Almost 60% of cases reported travel abroad in the 2 weeks prior to disease onset. That travel is a risk factor for cryptosporidiosis among Swedes may, however, be biased by the fact that people are more prone to seek healthcare for diarrhoea acquired abroad and physicians more likely order *Cryptosporidium* investigation in cases with travellers' diarrhoea than in indigenous cases. There was, however, a difference between *C. parvum* cases, where 51% were infected in Sweden and *C. hominis* cases, where 74% acquired their infection abroad. A predominance of *C. hominis* in travel-associated cases has also been observed in previous studies from developed countries [24, 29, 36] and most likely reflects the local incidence of different *Cryptosporidium* spp. in different countries, as well as differences in behaviour and exposure during travel abroad. Moreover, the majority of indigenous *C. hominis* cases in this study probably acquired their infection by person-to-person transmission from an index case infected abroad.

Main clinical symptoms were frequent diarrhoea and abdominal pain, as described previously [8, 24, 25]. More patients were hospitalized and in need of intravenous rehydration compared to 8·9% in a study by Chalmers *et al.* [24]. Moreover, up to 70% of the patients had symptoms for >10 days, and 25–36 months after disease onset 15% of patients still

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reported intermittent diarrhoea and 8% complained of musculoskeletal symptoms. A few reports have shown clinical manifestation differences between *C. hominis* and *C. parvum* [39–41] suggesting a higher pathogenicity of *C. hominis*, especially in HIV-infected persons, and also differences between subtype families [39, 40, 42]. There were, however, only slight differences in the intensity of clinical symptoms by species in patients in the present study, in agreement with reports from the UK and France [24, 31]. The duration of diarrhoea was longer in patients infected with *C. parvum* compared to those infected with *C. hominis*. Persistent symptoms after cryptosporidiosis have been described by others, but especially after infection with *C. hominis* in children [25, 39, 40]. Only four patients in the present study were immunocompromised, which emphasizes that immunocompetent individuals are also susceptible to many *Cryptosporidium* spp. and genotypes.

Subtyping is a vital tool in epidemiological investigations due to the wide range of intra-species diversity. For example, multilocus typing (MLT) of three microsatellites of isolates from a presumed single *C. parvum* outbreak in Sweden, involving two swimming pools, identified different MLTs suggesting two parallel outbreaks [43]. Some of these isolates were later typed at the Gp60 locus, identifying subtypes IIaA16R1 and IIcA5G3a, confirming two separate infection sources (M. Lebbad, unpublished data). In four of the outbreaks as well as in suspected family clusters described in this study, a single subtype was identified, providing evidence of a common infection source. In the fifth outbreak, involving arugula salad as the suspected source, we identified four subtypes from allele families IIa and IId. These allele families are recognized as zoonotic, and contamination due to fertilization with animal faeces during cultivation cannot be ruled out. Subtype clonality of *C. parvum* has been identified in some cattle herds, whereas some herds seem to harbour multiple subtypes [23, 44]. Thus, it is difficult to determine whether one or several infection sources were involved in this particular outbreak.

C. hominis subtype IbA10G2 was predominant, and this subtype has also been identified as the most common *C. hominis* subtype worldwide [28, 30, 45]. Even if ten outbreak-related cases were excluded, 43% of *C. hominis* cases were due to this subtype. In agreement with findings from the UK, having a non-IbA10G2 subtype was associated with recent travel outside Europe [46]. The IbA19G2 subtype, identified in one visitor to China, has so far only been identified in China [47, 48], and could thus currently be geographically isolated.

Domestic *C. parvum* cases in this study all belonged to zoonotic allele families IIa and IId. Except for dairy cattle, little is known about *Cryptosporidium* prevalence, species and subtype distribution and zoonotic potential in Swedish animals. In a previous study we identified *C. parvum* in only 20% of 115 analysed *Cryptosporidium*-positive calf samples from calves aged 1–62 days [49], indicating that the overall zoonotic potential of Swedish dairy cattle is low. However, subtype IIaA16G1R1, frequently identified in domestic cases in this study, was common in calves whereas IIaA17G1R1, which was involved in outbreak 3, was only identified in one calf [49]. We also identified three novel subtypes (IIaA21G1R1, IIdA16G1, IIdA23G1) and two subtypes with post-repetitive variations (IIdA20G1e, IIdA22G1c) in calves [49]. Interestingly, IIdA22G1c was identified in seven human domestic cases and in two travel cases in the present study. IIdA16G1 and IIdA20G1e were identified in one travel case each, whereas IIaA21G1R1 and IIdA23G1 were not identified. This further emphasizes that calves should be taken into consideration as a source of infection. Subtype IIaA15G2R1, which has been highly prevalent in both humans and cattle in previous reports [30, 45] was identified in 14 cases, most of them apparently infected abroad. We have not found this subtype in Swedish dairy cattle [49]. The widespread occurrence of this subtype in humans might suggest that this subtype also circulates in human populations without zoonotic transmission.

Our study has some limitations. First, only patients in Stockholm County, representing about 20% of the Swedish population, were included and data are thus not representative for the whole country. Second, *Cryptosporidium* isolates from only about 70% of cases were available for molecular analysis.

In conclusion, the majority of cryptosporidiosis patients in this study were infected by *C. parvum* followed by *C. hominis* and *C. meleagridis*. Clinical manifestations differed by species. A high diversity of *Cryptosporidium* spp. and subtypes was identified and molecular characterization of isolates was crucial for epidemiological investigations and contact tracing. There is need for increased awareness of cryptosporidiosis among physicians and laboratory personnel to correctly assess the burden of cryptosporidiosis. Stool specimens from individuals with diarrhoea

should routinely be tested for *Cryptosporidium* and notification of confirmed cases to public health agencies should be mandatory in order to improve the prevention of cryptosporidiosis and the understanding of its epidemiology.

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DECLARATION OF INTEREST

None.

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