Long-term *Cryptosporidium* typing reveals the aetiology and species-specific epidemiology of human cryptosporidiosis in England and Wales, 2000 to 2003

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To improve understanding of the aetiology and epidemiology of human cryptosporidiosis, over 8,000 *Cryptosporidium* isolates were submitted for typing to the species level over a four year period. The majority were either *Cryptosporidium parvum* (45.9%) or *Cryptosporidium hominis* (49.2%). Dual infection occurred in 40 (0.5%) cases and six other known *Cryptosporidium* species or genotypes were found in 67 (0.9%) cases. These were *Cryptosporidium meleagridis*, *Cryptosporidium felis*, *Cryptosporidium canis*, and the *Cryptosporidium* cervine, horse and skunk genotypes. The remaining 3.5% were not typable. Epidemiology differed between infecting species. *C. parvum* cases were younger, although *C. hominis* was more prevalent in infants under one year and in females aged 15 to 44 years. Spring peaks in cases reported to national surveillance were due to *C. parvum*, while *C. hominis* was more prevalent during the late summer and early autumn as well as in patients reporting recent foreign travel. Temporal and geographical differences were observed and a decline in *C. parvum* cases persisted from 2001. Typing of isolates allowed outbreaks to be more clearly delineated, and demonstrated anthroponotic spread of *C. parvum* as well as *C. hominis*. Our findings suggest that national surveillance for *Cryptosporidium* should be conducted at the species level.

Introduction

The aetiological agent of the diarrhoeal disease cryptosporidiosis in humans has been traditionally ascribed to the protozoan parasite *Cryptosporidium parvum*, on the basis of microscopical identification of oocysts or detection of oocyst wall antigens in faeces, and the assumption that all oocysts detected were monospecific [1]. However, *C. parvum* variants, recognised initially phenotypically (designated human or H and cattle or C types) and latterly through genomic studies, segregate into two genotypes (1 and 2), of which genotype 1 is host-adapted for humans [2]. This is now assigned species status and called *Cryptosporidium hominis* and genotype 2 remains *C. parvum* [3]. Although these are the most commonly found species in human cryptosporidiosis worldwide, the distribution varies temporally and geographically [1]. Six other *Cryptosporidium* species have also been found in this host (*Cryptosporidium meleagridis*, *Cryptosporidium felis*, *Cryptosporidium canis*, *Cryptosporidium suis*, *Cryptosporidium muris* and *Cryptosporidium andersoni*), as have *C. hominis* monkey genotype, *C. parvum* mouse genotype and the *Cryptosporidium* cervine, chipmunk genotype I, skunk, horse and rabbit genotypes [4, 5, 6]. The site-specific occurrence and pathogenicity of these unusual *Cryptosporidium* species/genotypes in humans appears to depend on a combination of endemnicity, exposure and parasite-related factors rather than host immune status [7].

Discrimination between *Cryptosporidium* species/genotypes is not possible by methods traditionally applied in routine diagnostic laboratories and national cryptosporidiosis surveillance is usually undertaken and reported without account of the aetiology. One exception is Scotland, where reference laboratory typing results have been incorporated in national surveillance since 2004 [8]. In England and Wales, the parasite is routinely identified at the genus level only [9] and surveillance data show that in the ten years between 1998 and 2007, the number of laboratory confirmed cases reported annually ranged from 3,010 to 5,863 [10]. More cases are reported in one to two year old children and cases are unevenly distributed over time, with peaks in the spring and autumn [11].

Although data have been published on the species identification and occurrence of *Cryptosporidium* spp. in human isolates, numbers studied are often small and / or from selected patient groups, and are rarely representative of community cases routinely seeking medical assistance [1]. The distribution of *C. parvum* and *C. hominis* cases mainly in England between 1998 and 1999, has been shown to vary geographically and temporally [12]. *C. parvum* was detected in 56.1%, *C. hominis* in 41.7%, and the remaining 2.2% comprised *C. meleagridis*, *C. felis*, *C. andersoni*, *C. canis*, *C. suis* and the *Cryptosporidium* cervine type, and samples containing both *C. parvum* and *C. hominis* [13]. While these studies contributed to knowledge of the epidemiology and transmission of *Cryptosporidium* species, national surveillance remained at the genus-level.

In order to improve our understanding of the aetiology and epidemiology of human cryptosporidiosis, and investigate changes over time, an on-going, representative, national collection of *Cryptosporidium* oocysts was established for the whole of England and Wales in January 2000. Here we describe the establishment, baseline aetiology and epidemiological analysis of the national

collection for the first four years (2000 to 2003), and assess the value of *Cryptosporidium* typing for epidemiological and surveillance purposes.

Methods

Between January 2000 and December 2003, faecal samples in which *Cryptosporidium* was detected during routine diagnosis of diarrhoeal disease in publicly funded laboratories through out England and Wales were referred to the Cryptosporidium Reference Unit (CRU) in Swansea for typing to the species level. Briefly, oocysts were separated from faecal debris by salt flotation, and disrupted by boiling, and DNA was extracted by a spin column technique (QIAamp DNA Mini Kit, Qiagen Ltd.) as described previously [14]. The *Cryptosporidium* oocyst wall protein (COWP) gene was investigated for all isolates using polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) [15] and isolates where no amplicons were obtained were further tested by PCR-RFLP analysis of the small sub-unit (SSU) rRNA gene [16]. If amplicons were still not obtained, the stool was examined by microscopy following modified Ziehl-Neelsen staining of fixed smears [17] or immunofluorescence staining (Crypto-Cel, TCS Water Sciences) according to the manufacturer's instructions. PCR products with equivocal or unusual RFLP profiles were purified (Qiaquick, Qiagen Ltd), sequenced in both directions (GeneService Ltd) and edited, consensus sequences compared with published sequences in the GenBank database using the National Institutes of Health National Centre for Biotechnology Information basic local alignment search tool (http://www.ncbi.nlm.nih.gov/BLAST). Sequences were verified and >99.5% similarity, in the region targeted by the PCR, to a published sequence was considered homologous.

Patient demographics (locality, date of birth or age, and sex), clinical details, specimen collection date, history of recent foreign travel and whether the case was considered to be part of a family or household cluster or a general outbreak, was collected from the diagnostic laboratory on a form submitted with each sample and outbreaks verified with the investigating authority. For specimens where the collection date was missing, a proxy date was calculated by deducting from the date of receipt at the CRU the modal time delay for this interval (five days). Cases were geographically located using the Government Office Region of the submitting laboratory. Countries visited by patients reporting recent foreign travel were grouped according to the health advice provided in Health Information for Overseas Travel [18].

To confirm that the submitted samples were representative, the dataset was compared by specimen date, patient age and sex distribution with primary diagnostic laboratory surveillance reports to the Health Protection Agency, using the Mantel-Haenszel version of the chi-squared test and Mann-Whitney two sample test for sex and age distribution respectively.

Differences in demographic data and patient history of first time, confirmed cases of *C. parvum* and *C. hominis* in the whole dataset were compared by univariate logistic regression analysis and age distribution investigated using the Mann-Whitney two-sample test. Patients infected with *C. parvum* were designated as "cases" while patients infected with *C. hominis* were designated as "controls". Further analyses were undertaken separately for each infecting species. Patients co-infected with both species were excluded from these analyses. All statistical analyses were undertaken using EpiInfo (Version 6, Centers for Disease Control and Prevention, Atlanta, GA) and STATA 7 (Stata Corporation, College Station, TX).

Results

Specimen submission

During the four year period from 1 January 2000 to 31 December 2003, a total of 8,075 faecal specimens were received from 133 primary diagnostic laboratories throughout England and Wales, representing 44.3% of the 18,235 *Cryptosporidium* cases reported to national surveillance over the same time period. The monthly distribution of submitted isolates reflected the number of cases reported to national surveillance (Figure 1).

The specimen collection date was available for 7,732 of the 8,075 (95.8%) specimens, and the time delay to date of receipt by the CRU ranged from 1 to 311 days (mean = 6 days, mode = 5 days, median = 5 days). The age of the patient was known for 8,003 (99.1%) specimens. The youngest patient was two months old and the oldest 98 years (mean = 16 years, mode = 1 year, median = 9 years). This was not significantly different from the cases reported to national surveillance (Mann-Whitney two-sample test =-0.031, df=1, p=0.9752).

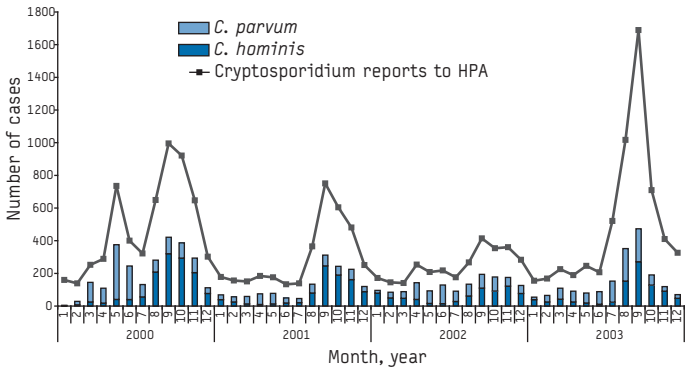
Of the 8,075 specimens received by the CRU, 3,965 (49.1%) specimens were from males, 4,072 (50.4%) were from females and for just 38 (0.5%) the sex of the patient was not known. This was not significantly different from the ratio of male to female cases (1:1.02) reported to national surveillance ($\lambda 2$=0.06, P>0.05, df=1). Foreign travel was indicated on the submission form for 1,049 (13.0%) specimens in the CRU collection compared with 3% of cases reported to national surveillance.

Microbiological and genotyping results

Cryptosporidium was confirmed by microscopy or PCR in 7,829 (97.0%) specimens. Of the remaining 246 (3.0%) specimens, seven were identified by microscopy as Cyclospora cayetanensis, 44 were insufficient in volume for confirmation and the remaining 195 contained yeast cells, mushroom spores, pollen grains or unidentified staining artefacts.

FIGURE 1

Monthly total numbers of cases of *Cryptosporidium* in humans in England and Wales, 2000 to 2003, comparing laboratory surveillance reports and *C. parvum* and *C. hominis* cases identified in the sub-set submitted for typing



Source: Health Protection Agency and United Kingdom Cryptosporidium Reference Unit data

Of the 7,829 confirmed isolates, 7,560 (96.6%) were typable by PCR-RFLP. The positivity rate for COWP PCR-RFLP was 88% on initial test, rising to 92% when a repeat test was included. The overall positivity rate rose to 96.6% following testing of COWP negative samples by SSU rDNA PCR-RFLP. The remaining 3.4% of specimens were confirmed by microscopy, but were not amplified or showed equivocal results (e.g. bands too faint to assign to species/genotypes or multiple bands present) by the PCR methods described here. A total of 141 repeat specimens were received from 70 patients. None of these sequential samples demonstrated a change in the *Cryptosporidium* species from that detected in the initial specimen.

Of the 7,758 first specimens from each patient, 3,817 (49.2%) were *C. hominis*, 3,564 (45.9%) were *C. parvum*, 40 (0.5%) were dual infections with *C. parvum* and *C. hominis*, other *Cryptosporidium* species/genotypes were identified in 67 (0.9%) and 270 (3.5%) were not typable. The unusual species/genotypes were *C. meleagridis* (n=56), *C. felis* (n=4), *Cryptosporidium* cervine genotype (n=4), *C. canis* (n=1), *Cryptosporidium* horse genotype (n=1) and *Cryptosporidium* skunk genotype (n=1). The finding of the horse and skunk genotypes has been described by Robinson et al., [6] and the epidemiology of cases other than *C. parvum* and *C. hominis* is being prepared for publication elsewhere.

Demographics

The patient demographics for *C. parvum* and *C. hominis* are compared in Table 1. The mean age of *C. parvum* cases (15 years, range 0 to 92 years, median 8 years, mode 1 year) was lower than that of *C. hominis* cases (17 years, range 0 to 97 years, median 9 years, mode 1 year) (Mann-Whitney two-sample test=9.69, df=1, p=0.002). Both species were linked to young age (0 to 9 years). There was an excess of *C. parvum* in 10 to 19 year olds, whereas *C. hominis* was common in adults, particularly those between 30 and 39 years of age. More detailed examination of the age-related data (Figure 2) showed that *C. hominis* was also more prevalent than

Table 1

Comparison of demographics and history of 7,381 cases with *Cryptosporidium parvum* and *Cryptosporidium hominis* in England and Wales over four years from 2000 to 2003: analysis using case-control methodology

Variable		*C. parvum* "cases" n=3,564	*C. hominis* "controls" n=3,817	Odds Ratio (95% CI)	p
10 year age group*	0 to 9 years	1,917 (53.8%)	1,946 (51.0%)	1.00	-
	10 to 19 years	579 (16.2%)	494 (12.9%)	**1.19 (1.04 to 1.36)**	**0.012**
	20 to 29 years	362 (10.2%)	352 (9.2%)	1.04 (0.89 to 1.22)	0.597
	30 to 39 years	354 (9.9%)	577 (15.1%)	**0.62 (0.54 to 0.72)**	**0.000**
	40 to 49 years	172 (4.8%)	182 (4.8%)	0.96 (0.77 to 1.19)	0.709
	50 to 59 years	88 (2.5%)	123 (3.2%)	**0.73 (0.55 to 0.96)**	**0.026**
	60 + years	68 (1.9%)	107 (2.8%)	**0.65 (0.47 to 0.88)**	**0.006**
Sex**	Female	1,819 (51.0%)	1,931 (50.6%)	1.00	-
	Male	1,733 (48.6%)	1,866 (48.9%)	0.98 (0.90 to 1.08)	0.761
Immuno-compromise reported	Yes	21 (0.6%)	29 (0.8%)	1.00	-
	No	3,543 (99.4%)	3,788 (99.2%)	0.77 (0.44 to 1.36)	0.373
Foreign travel reported	Yes	304 (8.5%)	621 (16.3%)	1.00	-
	No	3,260 (91.5%)	3,196 (83.7%)	**0.48 (0.41-0.56)**	**0.000**
Outbreak	Yes	276 (7.7%)	226 (5.9%)	1.00	
	No	3,288 (92.3%)	3,591 (94%)	**1.33 (1.11 to 1.61)**	**0.002**
Household cluster	Yes	223 (6.3%)	331 (8.7%)	1.00	-
	No	3,341 (93.7%)	3,486 (91.3%)	**0.70 (0.59-0.84)**	**0.000**
Government Office Region***	West Midlands	286 (8.0%)	253 (6.6%)	1.00	-
	London	37 (1.0%)	155 (4.1%)	**0.21 (0.14 to 0.32)**	**0.000**
	North West	1,044 (29.3%)	911 (23.9%)	1.01 (0.83 to 1.23)	0.889
	South East	191 (5.4%)	435 (11.4%)	**0.39 (0.30 to 0.50)**	**0.000**
	Yorkshire and The Humber	234 (6.6%)	333 (8.7%)	**0.62 (0.49 to 0.79)**	**0.000**
	North East	68 (1.9%)	96 (2.5%)	**0.63 (0.43 to 0.91)**	**0.009**
	East	293(8.2%)	454 (11.9%)	**0.57 (0.45 to 0.72)**	**0.000**
	East Midlands	236 (6.6%)	339 (8.9%)	**0.62 (0.48 to 0.79)**	**0.000**
	South West	621 (17.4%)	456 (11.9%)	1.20 (0.97 to 1.49)	0.079
	Wales	548 (15.3%)	358 (9.4%)	**1.35 (1.09 to 1.69)**	**0.006**

Source: United Kingdom Cryptosporidium Reference Unit data
p: significant values are indicated in bold
* Age not known for 60 cases
** Sex not known for 32 cases
*** Apart from baseline, ranked by decreasing population density per hectare

C. parvum in infants under one year of age. Although *C. parvum* and *C. hominis* cases overall did not differ with regard to sex, this was affected by age with more *C. parvum* in young boys and more *C. hominis*, especially in females, in the 30 and 39 years age group (Figure 2). There was no difference in the distribution of these *Cryptosporidium* species in immunocompetent and immunocompromised patients.

More patients with *C. parvum* belonged to recognised outbreaks but fewer belonged to family or household clusters where *C. hominis* was more common.

Travel history

C. parvum cases were less likely to have reported travel outside the United Kingdom (UK) prior to illness than *C. hominis* cases. The locations visited were Europe (207 *C. parvum*; 378 *C. hominis*), Indian subcontinent (18 *C. parvum*; 42 *C. hominis*), North Africa and the Middle East (18 *C. parvum*; 42 *C. hominis*), sub-Saharan and southern Africa (13 *C. parvum* and 27 *C. hominis*), the Caribbean (6 *C. parvum* and 18 *C. hominis*), South East Asia and Far East (4 *C. parvum* and 2 *C. hominis*), North America, Australia and New Zealand (5 *C. parvum* and 7 *C. hominis*), Central America (3 *C. parvum* and 8 *C. hominis*), South America (2 *C. parvum* and 6 *C. hominis*), mixed locations or country not stated (29 *C. parvum* and 36 *C. hominis*).

Geographical distribution

Regional differences were observed when compared with the West Midlands which had similar numbers of *C. parvum* and *C. hominis* cases. Government Office Regions on the eastern side of the country (i.e. London, South East, Yorkshire and the Humber, North East, East of England and the East Midlands) were more likely to have increased numbers of *C. hominis* while Wales, on the western side, had more *C. parvum* cases. The proportion of *C. parvum* and *C. hominis* cases in the North West and the South West were similar.

Seasonality

The annual proportion of cases of *C. hominis* (49.2% in 2000, 57.5% in 2001, 46.0% in 2002 and 45.1% in 2003) and *C. parvum* (47.1% in 2000, 35.7% in 2001, 49.3% in 2002 and 49.9% in 2003) was approximately equal each year, with the exception of 2001 when there was a much lower proportion of *C. parvum* cases, particularly in the spring. Because of this change over time, and the epidemiological differences highlighted here between *C. parvum* and *C. hominis*, the following data are presented annually and separately for each infecting *Cryptosporidium* species.

All ages were affected by the spring decline in *C. parvum* cases in 2001 (Figure 3), and the spring peak was only partially restored in 2002 and 2003 (Figure 1). During each of the four years most isolates were received during September, this peak being mainly composed of *C. hominis* and to a lesser extent *C. parvum* (Figure 1).

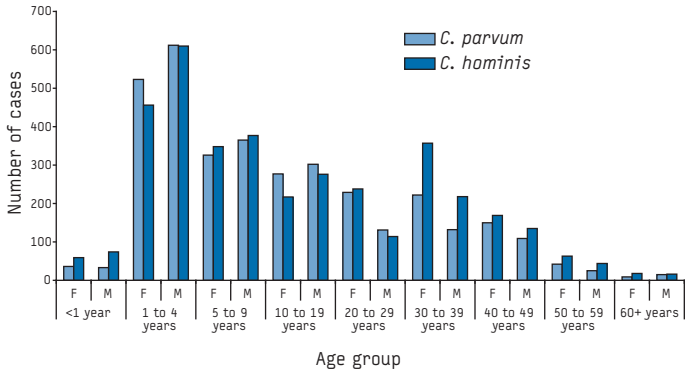
The spring peak in *C. parvum* was almost exclusively composed of indigenous cases, whereas the late summer / autumn *C. hominis* peak included patients who had reported foreign travel (Figure 4). In 2003 there was a substantial peak in *C. parvum*, probably linked to an outbreak originating among holiday makers in Majorca (Table 2). The younger ages particularly were affected by the unusual autumnal peak in *C. parvum* in 2003 (Figure 4).

Cryptosporidiosis outbreaks

Specimens were received from 508 cases linked to 29 locally or nationally recognised outbreaks of cryptosporidiosis during the four year period (Table 2). Outbreaks were caused by *C. hominis*

FIGURE 2

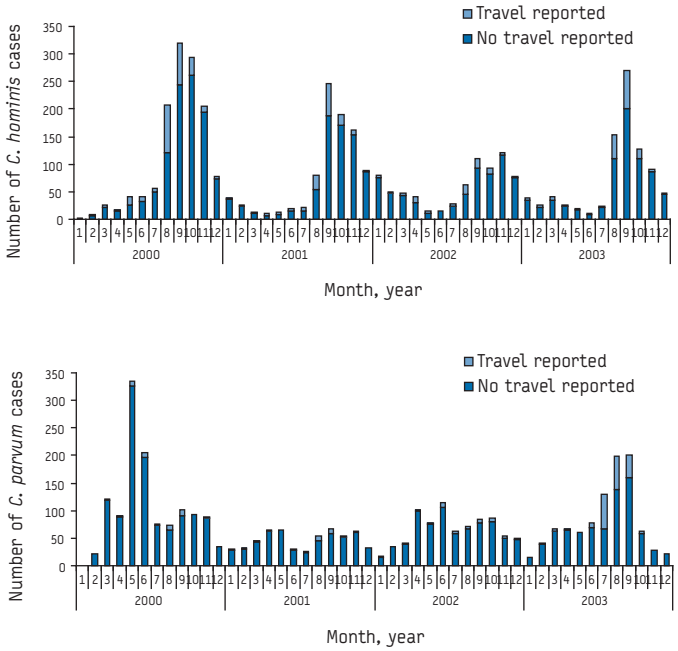
Age and sex distribution of *Cryptosporidium parvum* and *Cryptosporidium hominis* cases in England and Wales over four years from 2000 to 2003 (n= 7,381)



Source: United Kingdom Cryptosporidium Reference Unit data
F: Female; M: Male

FIGURE 4

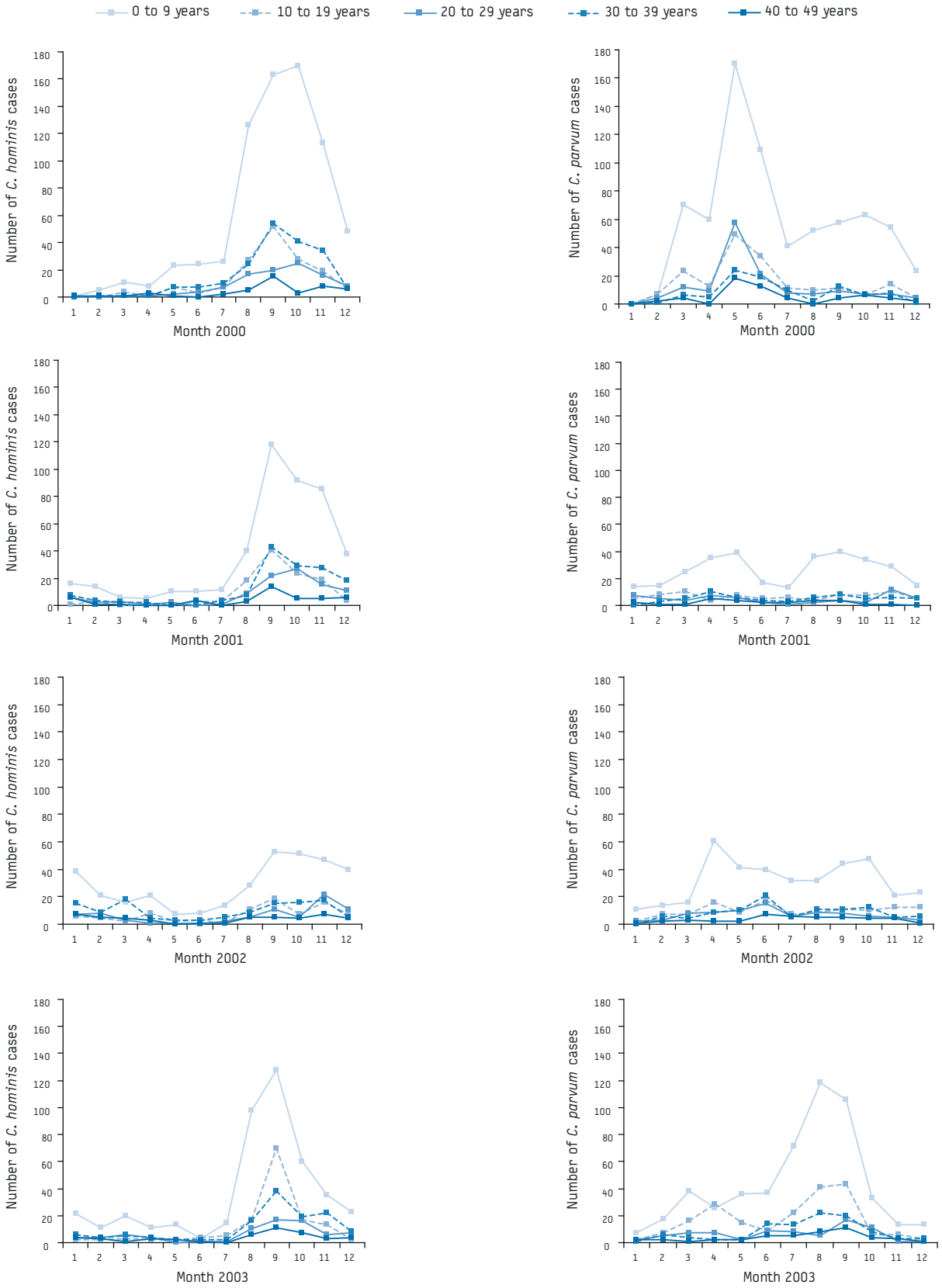
Distribution of *Cryptosporidium hominis* and *Cryptosporidium parvum* in cases reporting travel and not reporting travel outside the United Kingdom over four years from 2000 to 2003



Source: United Kingdom Cryptosporidium Reference Unit data

FIGURE 3

Age distribution of *Cryptosporidium parvum* and *Cryptosporidium hominis* cases in England and Wales, by month, in 2000, 2001, 2002 and 2003 (n=7,381)



Source: United Kingdom Cryptosporidium Reference Unit data

Table 2

***Cryptosporidium* species identified in outbreaks of cryptosporidiosis in England and Wales, from January 2000 to December 2003**

Year	Month	HPA outbreak database number	Government Office Region	Type of supply; source or contact	Cases ill (laboratory confirmed)	Isolates submitted for typing	*C. parvum*	*C. hominis*	Other	References
Drinking water										
2000	March	00/219	North West	Public supply; Spring	58 (58)	48	47	0	1 NT	[19, 20]
2000	May	00/413	North West	Public supply; surface water	207 (207)	134	119	14	1 NT	[21]
2000	May to June	00/440	South West	Private supply at a farm holiday centre	8 (3)	3	3	0	0	[19]
2002	May	~	Wales	Private supply at a child minder's premises on a farm	4 (4)	4	4	0	0	[22]
2002	November	02/1547	South East	Public supply	21 (21)	18	0	18	0	[23]
2002	November to December	02/1701	South East	Public supply; surface and borehole	31 (31)	28	0	28	0	[23]
2002	March	02/018	North West	Private supply; well at a college	50* (1)	1	1	0	0	[23, 24]
Swimming pools										
2000	May to June	00/406	Yorkshire and The Humber	Public pool	41 (41)	34	34	0	0	[19, 22]
2000	July to August	~	London	Club pool	9 (8)	7	1	6	0	Unpublished data
2000	July to August	00/723	London	Public pool	5 (5)	1	0	1	0	[19]
2000	September	00/656	London	Public pool	10 (10)	8	0	8	0	[19]
2000	September	00/870	South West	Public pool	12 (7)	1	1	0	0	[19]
2000	October to November	00/972	South West	Club pool	5 (5)	5	1	4	0	[19]
2001	June	01/347	South East	Outdoor school pool	152* (10)	5	0	5	0	[19, 24]
2001	October to November	01/528	South West	Club pool	3 (3)	3	0	3	0	[24]
2002	September to February	02/1877	South East	Public pool	20 (20)	5	1	4	0	Unpublished data
2003	January to April	03/220	Yorkshire and The Humber	Public pool	66 (48)	21	0	21	0	[23]
2003	August to September	03/409	South East	Public pool	17 (17)	2	0	2	0	[25]
Other water										
2001	August	01/440	South West	Contact with a stream at a beach	14 (6)	5	3	2	0	[24]
2003	August	03/411	West Midlands	Fountain in public park	122 (35)	32	0	31	1 *C. meleagridis*	[25]
2003	September	03/401	South West	Interactive water feature at an animal attraction centre	63 (27)	31	29	2	0	[25, 26]
Farm										
2003	March	03/167	East of England	Open farm, general public	7 (7)	2	2	0	0	[22]
2003	March	03/197	Wales	Open farm, school visit	17 (6)	6	6	0	0	[22]
2003	April	~	Wales	Residential farm centre, school visit	36 (12)	10	10	0	0	Unpublished data
Institutions										
2000	October	00/806	London	Day care nursery	13 (13)	13	0	13	0	Unpublished data
2001	September	01/442	South East	Day care nursery	30 (10)	8	0	8	0	Unpublished data
2002	November	02/1794	Yorkshire and The Humber	Day care nursery	47 (12)	9	0	8	1 NT	Unpublished data
International										
2000	Summer	~	Majorca	Hotel pool	>250	48	0	48	0	[27]
2003	July	~	Majorca	Hotel pool	179 (75)	16	14	0	2 NT	[28]

Source: United Kingdom Cryptosporidium Reference Unit, Health Protection Agency (HPA) and National Public Health Service for Wales data
~ Not recorded in the HPA outbreak database
* Concurrent community outbreaks of Norovirus may account for a proportion of cases.
NT = not typable

(13 outbreaks), *C. parvum* (10 outbreaks) and both species were detected in six outbreaks. Public drinking water supplies were associated with two outbreaks caused by *C. hominis*, one caused by *C. parvum* and one outbreak where both species were detected. Three outbreaks were linked to private water supplies and all three were caused by *C. parvum*.

Although more swimming pool-associated outbreaks in England and Wales were caused by *C. hominis* (n=6) than *C. parvum* (n=2), the largest indigenous outbreak linked to a swimming pool was caused by *C. parvum*. Both species were detected in three outbreaks linked to swimming pools. All swimming pool-associated cryptosporidiosis outbreaks except one were at indoor pools, the most common type in the UK. Furthermore, two international outbreaks were investigated, both linked to hotel pools in Majorca, one was caused by *C. hominis* in 2000 and the other by *C. parvum* in 2003, which may have influenced the subsequent increase in *C. parvum* in the autumn that year (Figure 1).

Water features were associated with two outbreaks, one linked to a fountain in a public park caused by *C. hominis* and the other associated with an interactive water feature at an adventure park featuring a petting zoo caused by both *C. parvum* and *C. hominis*. Three outbreaks linked to open or residential farms were caused by *C. parvum*. One outbreak linked to environmental contact was caused by *C. parvum* and *C. hominis*. Three outbreaks at day care nurseries were caused by *C. hominis*.

Discussion

In this paper, long term, *Cryptosporidium* species-specific epidemiological analysis is described for the first time at a national level, demonstrating that aetiological identification of a large proportion of cryptosporidiosis cases is possible, and furthermore, enhances the surveillance data provided by routine genus-level reporting. The epidemiology of *C. parvum* and *C. hominis* differs, and there is evidence for distinct sources and transmission routes. *C. parvum* infections occur all year round but mainly in the spring, although the spring peaks have declined since 2001. Outbreaks caused by *C. parvum* were linked to farm visits, environmental contact, drinking and recreational water. *C. hominis* cases occur mainly in the summer and autumn, in infants under one year of age and in adult females between 30 and 39 years, and in people who travelled abroad. *C. hominis* outbreaks were linked to day care nurseries, drinking and recreational waters. Some of these risks were identified in a case-control study of sporadic cases undertaken in 2001 which found contact with farmed animals as the significant risk factor for *C. parvum* and travel abroad, contact with another person with diarrhoea and changing young children's nappies to be significant risk factors for *C. hominis* [29]. Thus the epidemiology supports human transmission of *C. hominis*, and both zoonotic and anthroponotic transmission of *C. parvum*. Although household clusters of cases were more commonly caused by *C. hominis*, *C. parvum* was also involved. Human transmission of *C. parvum* was also demonstrated in outbreaks linked to indoor swimming pools, indicating a human source of contamination and infection with this species.

Multi-locus genotyping of a subset of *C. parvum* isolates from this collection, analysed with enhanced patient data, has demonstrated a predominance of some alleles linked to anthroponotic transmission, and others linked to zoonotic transmission [30].

The temporal distribution, with *C. parvum* predominating in the spring and *C. hominis* in the autumn, which has been reported in some other temperate climates [31] are shown to have changed over time in England and Wales. Although the national reduction in the spring peak in 2001, driven by *C. parvum*, showed strong association with control measures for the foot and mouth disease epidemic that year [32] it clearly continued after the control measures were lifted and data from this archive for the North West of England demonstrated links to improvements in drinking water quality [33]. This demonstrates the value of typing isolates in identifying interventions for disease reduction. The regional differences observed, reflecting population densities, have been further explored in analysis of the socio-economic risk factors [34]. This showed significant association between *C. hominis* and higher social economic status, young children and urban areas, and for *C. parvum* faecal application to land [35].

Although the cases in our dataset were representative of those reported to national surveillance, a higher proportion of our cases reported foreign travel. This is not considered to be submission bias but due to improved reporting since our submission form actively sought this information whereas it is reported passively to national surveillance. Travel data is under-reported in national surveillance and to a lesser extent to CRU, compared with enhanced data collection in a case-control study [29]. Travel-related cryptosporidiosis was mainly caused by *C. hominis* but this is influenced by the most frequently visited areas and differences may reflect variations in the endemic *Cryptosporidium* species of the host countries (about which little is known), or differences in behaviour and exposure during travel to different destinations. It is also possible that outbreaks among holiday makers may occur independently of the indigenous population, particularly if hotel swimming pools are involved [30]. It appears that foreign travel has a role in initiating the autumn peak, although this has not been investigated and should be studied further to investigate community spread and identify risk factors and interventions for disease reduction.

The typing methods used in this study enabled investigation of a vast number of specimens with very little loss in resolution [22]. Potential mis-identifications in the COWP assay that are currently known include the *Cryptosporidium* rabbit genotype confounding for *C. hominis* [6] and the mouse genotype mistaken for *C. parvum* [36]. Enhanced testing of a subset of our isolates indicates that the rabbit genotype is a rare human infection (unpublished data) and there is only one report from elsewhere of human infection with the mouse genotype [5]. *Cryptosporidium* species/genotypes not amplified by the COWP primers were further investigated at the SSU rRNA locus. PCR amplification of isolates not typable in this algorithm may have been inhibited by substances in the faecal samples or represent genotypes not amenable to amplification with the primer sets used in this study.

We identified 40 (0.5%) cases with dual *C. parvum* and *C. hominis* infections. This proportion is comparable with that found previously in England [13]. The disadvantage of any PCR-based system using common primers is the probable under-ascertainment of dual or multiple alleles within a sample. However, a subset of our isolates have been tested using separate species-specific primers and by multi-locus typing and showed little evidence of mixed infection [37]. The likelihood of dual infections is also driven by the endemicity of the parasite and exposure, as higher proportions have been detected in high-prevalence regions of the UK [38]. Unlike studies investigating only immunocompromised patients,

we investigated both immunocompetent and immunocompromised populations and found no difference in the distribution of *C. parvum* and *C. hominis*, and other species/genotypes were not more prevalent in immunocompromised patients (unpublished data).

Conclusion

Cryptosporidium species-specific risk factors have been identified as a result of this work. Although zoonotic risks regarding handling animals have been well described, indirect exposures are less well documented and in January 2004, the focus of national collection was changed to a sentinel laboratory scheme for the study of zoonotic cryptosporidiosis. The work presented in this paper facilitates the development of more rapid methods for *Cryptosporidium* species identification is facilitated by this work, not only providing an archive of material for assay development and evaluation but also by identifying that the key targets in the UK, and probably elsewhere in northern Europe, are *C. parvum* and *C. hominis*. In conclusion, species-level analyses are critical to the investigation and explanation of changes in incidence over time.

Aknowledgements

We thank UK Cryptosporidium Reference Unit staff Stephen Hadfield, Guy Robinson and Cathy Bentley for scientific support, Sian Wood, David Gomez and Rachael Seymour for administrative and technical assistance. Gordon Nichols, Health Protection Agency, for providing national surveillance data, Health Protection Teams and Units in England and Wales for providing outbreak data, Roland Salmon CDSC Wales for helpful comments on the manuscript, and primary diagnostic laboratories in England and Wales for contributing specimens to the National collection.

This study was funded by Department for Environment, Food and Rural Affairs and administered by the Drinking Water Inspectorate.

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This article was published on 15 January 2009.

Citation style for this article: Chalmers RM, Elwin K, Thomas AL, Guy EC, Mason B. Long-term Cryptosporidium typing reveals the aetiology and species-specific epidemiology of human cryptosporidiosis in England and Wales, 2000 to 2003. Euro Surveill. 2009;14(2):pii=19086. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19086