The epidemiology of sporadic human infections with unusual cryptosporidia detected during routine typing in England and Wales, 2000–2008

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SUMMARY

Routine typing of 14 469 isolates from human cryptosporidiosis cases between 2000 and 2008 revealed that 7439 (51·4%) were *Cryptosporidium* (*C.*) *hominis*, 6372 (44·0%) *C. parvum*, 51 (0·4%) both *C. hominis* and *C. parvum*, 443 (3·1%) were not typable and 164 (1·1%) were other *Cryptosporidium* species or genotypes. Of the latter, 109 were *C. meleagridis*, 38 *C. felis*, 11 *C. ubiquitum*, one *C. canis*, two horse, two novel and one skunk genotype. *C. hominis* monkey genotype and *C. cuniculus* were identified in a separate study. Patients with unusual infections were older than those with *C. hominis* ($P < 0.01$) or *C. parvum* ($P < 0.01$) and were more likely to be immunocompromised (Fisher's exact $P < 0.01$). Forty-one percent of unusual cases had travelled abroad, mainly to the Indian subcontinent. Significant risk factors in those with unusual species were travel abroad (*C. meleagridis*, $P < 0.01$), being immunocompromised (*C. felis*, Fisher's exact $P = 0.02$), and contact with cats (*C. felis*, Fisher's exact $P = 0.02$).

Key words: *Cryptosporidium*, emerging infections, epidemiology.

INTRODUCTION

The epidemiology of human cryptosporidiosis caused by *Cryptosporidium* (*C.*) *hominis* and *C. parvum*, and parasite species-specific risk factors have been well described [1–4]. Two UK studies covering a 7-year period between them showed that nearly all typed cases were caused by *C. hominis* (54%) or *C. parvum* (45%). Other species and genotypes were found more rarely, being identified in the remaining 1% [3, 4]. These species and genotypes which are unusual in the UK included *C. meleagridis*, *C. ubiquitum*, *C. felis*, *C. canis*, horse genotype, skunk genotype and novel genotypes. *C. hominis* and *C. parvum* were especially prevalent in children, and there was no difference

in the distribution of these two species in immunocompetent and immunocompromised patients [3, 4]. Significant risk factors, identified in a case-control study of sporadic cases, for *C. hominis* infection included area of UK residence, travel outside the UK, and changing nappies [1]. For *C. parvum*, significant risk factors were contact with farm animals [1], and in a study where 84% of cases were *C. parvum*, increasing volume of tap water drunk and short visits to farms [5]. Little is known about the epidemiology of infections with other *Cryptosporidium* spp. in the UK, although individual case reports and descriptions of affected patients have been published [6–8]. In contrast, epidemiological studies of cryptosporidiosis in developing countries have involved more cases with other species and genotypes. In a study of children in a shanty town in Lima, Peru no difference in associations between drinking-water supply, sanitation, presence of animals or socioeconomic status

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and unusual cryptosporidiosis compared to *C. hominis* were found [9]. In the same setting, Cama and colleagues [10, 11] described *Cryptosporidium* infections in children and HIV-positive adults and found a high frequency of zoonotic species (*C. meleagridis, C. felis, C. canis*) in an urban area leading to their suggestion that there may be anthroponotic transmission of species with seemingly animal origins. It is clear from the available literature that, like *C. hominis* and *C. parvum, C. meleagridis, C. canis* and *C. felis* are found worldwide, but appear to be more common in developing countries [9–12]. In contrast, with the exception of three children in Nigeria [13], infections with *C. ubiquitum* (syn. cervine genotype [14]) have only so far been reported in developed nations [15–17]. It is therefore important for us to understand the occurrence and epidemiology of these species and genotypes in the UK as they have a higher prevalence elsewhere and are deemed to be pathogenic to humans. This information will help interpret risk presented by, for example, their finding during water monitoring, and in understanding potential reservoirs of these infections.

To investigate the epidemiology of sporadic, unusual cases in England and Wales we compared their demographics and exposure data with those of *C. hominis* and *C. parvum* cases from the national dataset, from the same 9-year period, followed by comparison of data between the unusual species cases.

METHODS

Species and genotype characterization

Unusual *Cryptosporidium* species and genotypes were identified routinely by the UK Cryptosporidium Reference Unit (CRU) under the national typing scheme between January 2000 and December 2008 [3, 4] as isolates having PCR–RFLP profiles of the *Cryptosporidium* oocyst wall protein (COWP) gene using *Rsa*I [18], other than those matching *C. parvum* or *C. hominis*. Additionally, isolates from cases confirmed by microscopy but not amplifiable using the COWP locus were investigated further. First, DNA was re-amplified and analysed using a small sub-unit (SSU) rDNA PCR–RFLP using *Ssp*I and *Vsp*I [19, 20] and then identity was confirmed by bi-directional PCR sequence analysis (Geneservice, UK: ABI 3730xl; in-house: CEQ8000, Beckman Coulter, UK). The exception to this routine test algorithm was that the identities of *C. canis* and a novel genotype were

established using an HSP70 PCR [21] as amplicons suitable for further analysis could not be generated from the first choice loci. Representative sequences were deposited into GenBank using the online submission protocol.

Enhanced surveillance of unusual cases

An unusual case was defined as a person who had submitted a stool sample for diagnosis of gastrointestinal symptoms and had *Cryptosporidium* oocysts identified routinely as a species other than *C. parvum* or *C. hominis*. To obtain descriptive epidemiological data about each case, a questionnaire was sent to the lead consultant of the local Health Protection Team. Data from initial trawling questionnaires, based on routine investigation of sporadic cases [22] containing personal details and basic exposure data on known *Cryptosporidium* risk factors, were transferred to an enhanced questionnaire. This was augmented with clinical data from the patient on immune status, diagnosis of other gastrointestinal pathogens, and further animal exposures. Case occupation, derived from an open question, was also recorded and grouped according to risks of transmission, i.e. those that worked on a farm, with food, with children (e.g. carers at home, nursery staff, school staff), or had close contact with other people (e.g. care workers, hospital) were categorized as 'high risk'. Other occupations were categorized as 'low risk'.

Data analysis

Exposure data were entered and stored in a Microsoft Excel spreadsheet with the *Cryptosporidium* typing results and transferred to Epi Info (version 6, Centers for Disease Control and Prevention, USA) for analysis. Demographic and epidemiological data for all unusual cases were compared to those for *C. parvum* and *C. hominis* cases and to each other using χ^2 test to compare patient's sex, seasonal quarter of specimen receipt, foreign travel and immune status (the latter for cases occurring during 2000–2003 only). Mann–Whitney *U* test was used to compare case age. To investigate foreign travel case ages were arranged into groups stratified by 10-year bands. Basic statistical data, describing significance of differences between groups for each variable are given in Tables 1 and 2 and more detailed analyses are described in the text. Analysis of data within the unusual case group was only carried out with *C. meleagridis, C. felis* and

Table 1. *Comparison of unusual cases with* Cryptosporidium hominis *and* C. parvum *cases*

Variable	Unusual Cryptosporidium spp. ($n=164$)	C. hominis ($n=7439$)	C. parvum ($n=6372$)	Unusual Cryptosporidium spp. compared to C. hominis	Unusual Cryptosporidium spp. compared to C. parvum
10-year age group					
0–9 years	41 (25·0%)	3572 (48·0%)	3279 (51·5%)	**Kruskal–Wallis**	**Kruskal–Wallis**
10–19 years	20 (12·2%)	982 (13·2%)	1074 (16·9%)	**$H=34·28$, $P<0·01$**	**$H=53·72$, $P<0·01$**
20–29 years	46 (28·0%)	831 (11·2%)	679 (10·7%)		
30–39 years	24 (14·6%)	1134 (15·2%)	644 (10·1%)		
40–49 years	19 (11·6%)	410 (5·5%)	319 (5·0%)		
50–59 years	8 (4·9%)	207 (2·9%)	184 (2·9%)		
⩾60 years	6 (3·7%)	213 (2·9%)	136 (2·1%)		
Not known	0	90	57		
Sex					
Female	78 (47·6%)	3808 (51·2%)	3244 (50·9%)	$\chi^2=2·42$, $P=0·298$	$\chi^2=1·54$, $P=0·462$
Male	86 (52·4%)	3570 (48·0%)	3100 (48·7%)		
Not known	0	61	28		
Immunodeficiency reported*					
Yes	4 (5·8%)	29 (0·8%)	21 (0·5%)	**Fisher's exact**	**Fisher's exact**
No	65 (94·2%)	3789 (99·2)	3545 (99·5%)	**$P=0·0024$**	**$P=0·0011$**
Specimen quarter					
January–March	32 (19·5%)	664 (8·9%)	833 (13·1%)	**$\chi^2=72·37$, $P<0·01$**	**$\chi^2=19·17$, $P<0·01$**
April–June	32 (19·5%)	498 (6·7%)	2241 (35·2%)		
July–September	61 (37·2%)	3051 (41·0%)	1931 (30·3%)		
October–December	39 (23·8%)	3226 (43·4%)	1367 (21·5%)		
Government Office Region (% regional total)					
East	27 (1·6%)	1002 (58·8%)	674 (39·6%)	$\chi^2=17·44$, $P=0·07$	**$\chi^2=107·83$, $P<0·01$**
East Midlands	20 (1·3%)	949 (62·2%)	557 (36·5%)		
London	11 (4·7%)	191 (78·3%)	42 (17·2%)		
North East	3 (1·2%)	134 (54·5%)	109 (44·3%)		
North West	30 (1·1%)	1261 (47·0%)	1391 (51·9%)		
South East	16 (1·1%)	937 (68·9%)	408 (30·0%)		
South West	11 (0·6%)	734 (41·0%)	1044 (58·4%)		
Wales	21 (0·9%)	1087 (47·5%)	1180 (51·6%)		
West Midlands	7 (0·6%)	501 (45·1%)	604 (54·3%)		
Yorkshire and Humber	18 (1·8%)	643 (62·8%)	363 (34·5%)		
Foreign travel reported					
Yes	67 (41·0%)	1169 (15·7%)	479 (7·5%)	**$\chi^2=74·48$, $P<0·01$**	**$\chi^2=232·05$, $P<0·01$**
No	97 (59·0%)	6270 (84·3%)	5893 (92·5%)		
Foreign travel destination†					
Europe	13 (7·9%)	559 (7·5%)	264 (4·1%)	**$\chi^2=127·46$, $P<0·01$**	**$\chi^2=113·18$, $P<0·01$**
Indian Subcontinent	32 (19·5%)	217 (2·9%)	44 (0·7%)		
South East Asia and the Far East	8 (4·9%)	6 (0·08%)	7 (0·1%)		
Sub-Saharan and Southern Africa	6 (3·7%)	68 (0·9%)	26 (0·4%)		
North America, Australia and New Zealand	2 (1·2%)	16 (0·2%)	11 (0·2%)		
South America	2 (1·2%)	9 (0·1%)	8 (0·1%)		
Other or destination not stated	4 (2·4%)	294 (4·0%)	119 (1·9%)		
Outbreak cases					
Yes	0	710 (9·5%)	345 (5·4%)	**$\chi^2=17·26$, $P<0·01$**	**$\chi^2=9·37$, $P<0·01$**
No	164	6729 (90·5%)	6027 (94·6%)		

* Data from 2000 to 2003 only.
† Yellow book list of countries by continental group (National Travel Health Network and Centre, NaTHNaC, HPA UK).
Significant statistics shown in bold.

Table 2. *Description of all unusual cases and comparison of variables between* Cryptosporidium meleagridis, C. felis *and* C. ubiquitum

Variable	C. meleagridis (n = 109)	C. felis (n = 38)	C. ubiquitum (n = 11)	Comparison between C. meleagridis, C. felis and C. ubiquitum	C. canis (n = 1)	Horse genotype (n = 2)	Skunk genotype (n = 1)	Novel genotype (n = 1) (W13314)	Novel genotype (n = 1) (W14532)
10-year age group									
0–9 years	20 (18·3%)	13 (34·2%)	7 (63·6%)	$\chi^2 = 26.15$, **P = 0·01**	0	0	0	0	0
10–19 years	14 (12·8%)	6 (15·8%)	0		0	0	0	0	1
20–29 years	38 (34·9%)	6 (15·8%)	0		0	0	1	0	0
30–39 years	16 (14·7%)	6 (15·8%)	0		1	1	0	1	0
40–49 years	14 (12·8%)	3 (7·9%)	1 (9·1%)		0	1	0	0	0
50–59 years	4 (3·7%)	2 (5·3%)	2 (18·2%)		0	0	0	0	0
≥60 years	3 (1·8%)	2 (5·3%)	1 (9·1%)		0	0	0	0	0
Sex									
Female	55 (50·5%)	16 (42·1%)	2 (18·2%)	$\chi^2 = 4.53$, P = 0·10	0	2	1	1	1
Male	54 (49·5%)	22 (57·9%)	9 (81·8%)		1	0	0	0	0
Occupation risk									
Farm	1 (0·9%)	0	0	$\chi^2 = 6.39$, P = 0·27	0	0	0	0	0
Food	4 (3·7%)	0	0		0	1	0	0	0
Child	1 (0·9%)	1 (2·6%)	0		0	0	0	0	0
Other person	7 (6·4%)	4 (10·5%)	0		0	0	1	0	0
Low	30 (27·5%)	5 (13·2%)	0		0	0	0	1	0
Not stated or child case	66 (60·6%)	28 (73·7%)	11		1	1	0	0	1
Immunodeficiency stated									
Yes	4 (3·7%)	6 (15·8%)	1 (9·1%)	$\chi^2 = 6.47$, **P = 0·04**	0	0	0	0	0
No	105 (96·3%)	32 (84·2%)	10 (90·9%)		1	2	1	1	1
Household contact									
Yes	5 (4·6%)	1 (2·6%)	2 (18·2%)	$\chi^2 = 4.46$, P = 0·11	0	0	0	0	0
No	104 (95·4%)	37 (97·4%)	9 (81·8%)		1	2	1	1	1
Specimen quarter									
January–March	22 (20·2%)	7 (18·4%)	2 (18·2%)	$\chi^2 = 6.13$, P = 0·41	0	0	0	0	1
April–June	22 (20·2%)	7 (18·4%)	1 (9·1%)		1	1	0	0	0
July–September	40 (36·7%)	11 (28·9%)	7 (63·6%)		0	1	1	1	0
October–December	25 (22·9%)	13 (34·2%)	1 (9·1%)		0	0	0	0	0
Government Office Region									
East	20 (18·3%)	4 (10·5%)	4 (36·4%)	$\chi^2 = 23.25$, P = 0·18	0	1	0	0	0
East Midlands	14 (12·8%)	6 (15·8%)	0		0	0	0	0	0
London	9 (8·3%)	1 (2·6%)	1 (9·1%)		0	0	0	0	0
North East	3 (2·8%)	0	0		0	0	0	0	0
North West	20 (18·3%)	8 (21·1%)	1 (9·1%)		0	0	0	0	1

Table 2 (*cont.*)

Variable	C. meleagridis (n=109)	C. felis (n=38)	C. ubiquitum (n=11)	Comparison between C. meleagridis, C. felis and C. ubiquitum	C. canis (n=1)	Horse genotype (n=2)	Skunk genotype (n=1)	Novel genotype (n=1) (W13314)	Novel genotype (n=1) (W14532)
South East	8 (7·3%)	6 (15·8%)	0		0	0	0	0	0
South West	7 (6·4%)	0	2 (18·2%)		0	1	1	0	0
Wales	12 (11%)	7 (18·4%)	2 (18·2%)		0	0	0	0	0
West Midlands	6 (5·5%)	0	1 (9·1%)		0	0	0	0	0
Yorkshire and Humber	10 (9·2%)	6 (15·8%)	0		1	0	0	1	0
Other pathogens detected									
Yes	16 (14·7%)	2 (5·3%)	0	$\chi^2=3.99$, $P=0.14$	0	0	0	1	1
No	93 (85·3%)	36 (94·7%)	11		1	2	1	0	0
Foreign travel reported									
Yes	55 (50·5%)	7 (18·4%)	0	$\chi^2=\mathbf{19.77}$, $\mathbf{P<<0.01}$	1	2	0	1	1
No	54 (49·5%)	31 (81·6%)	11		0	0	1	0	0
Foreign travel destination*									
Europe	10 (9·2%)	3 (7·9%)	0	$\chi^2=7.34$, $P=0.39$	0	0	0	0	0
Indian Subcontinent	28 (25·7%)	2 (5·3%)	0		1	0	0	0	1
South East Asia and the Far East	8 (7·3%)	0	0		0	0	0	0	0
Sub-Saharan and Southern Africa	4 (3·7%)	1 (2·6%)	0		0	0	0	1	0
North America, Australia and New Zealand	1 (0·9%)	1 (2·6%)	0		0	0	0	0	0
South America	2 (1·8%)	0	0		0	0	0	0	0
Other or destination not stated	2 (1·8%)	0	0		0	1	0	0	0
Animal contact									
Yes	26 (23·9%)	8 (21·1%)	2 (18·2%)	$\chi^2=1.56$, $P=0.46$	0	0	0	1	0
No	83 (76·1%)	30 (78·9%)	9 (81·8%)		1	2	1	0	1
Cat contact									
Yes	4 (3·7%)	6 (15·8%)	1 (9·1%)	$\chi^2=\mathbf{6.47}$, $\mathbf{P=0.03}$	0	0	0	0	0
No	105 (96·3%)	32 (84·2%)	10 (90·9%)		1	1	1	1	1
Dog contact									
Yes	2 (1·8%)	3 (7·9%)	1 (9·1%)	$\chi^2=0.18$, $P=0.92$	0	0	0	0	0
No	107 (98·2%)	35 (92·1%)	10 (90·9%)		1	1	1	1	1
Ill pet contact									
Yes	1 (0·9%)	3 (7·9%)	0	$\chi^2=5.87$, $P=0.05$	0	0	0	0	0
No	108 (99·1%)	35 (92·1%)	11		1	1	1	1	1
Bird contact									
Yes	11 (11%)	3 (7·9%)	3 (27·3%)	$\chi^2=3.50$, $P=0.17$	0	0	0	1	0
No	98 (89%)	35 (92·1%)	8 (72·7%)		1	1	1	0	1

Table 2 (cont.)

Variable	C. meleagridis (n=109)	C. felis (n=38)	C. ubiquitum (n=11)	Comparison between C. meleagridis, C. felis and C. ubiquitum	C. canis (n=1)	Horse genotype (n=2)	Skunk genotype (n=1)	Novel genotype (n=1) (W13314)	Novel genotype (n=1) (W14532)
Swimming pool contact									
Yes	21 (19·3%)	9 (23·7%)	2 (18·2%)	$\chi^2=0·37$, $P=0·83$	0	2	1	1	0
No	88 (80·7%)	29 (76·3%)	9 (81·8%)		1	0	0	0	1
Water sports									
Yes	13 (11·9%)	5 (13·2%)	0	$\chi^2=1·56$, $P=0·46$	0	1	0	1	0
No	96 (88·1%)	33 (86·8%)	11		1	1	1	0	1
Mains water									
Yes	105 (96·3%)	38	10 (90·9%)	$\chi^2=2·59$, $P=0·27$	1	2	1	1	0
No	4 (3·7%)	0	1 (9·1%)		0	0	0	0	0

* Yellow book list of countries by continental group (National Travel Health Network and Centre, NaTHNaC, HPA UK).
Significant statistics shown in bold.

C. ubiquitum as separate groups due to numbers of other species/genotypes being very small. Demographic and clinical data on the very small numbers of other species or genotypes are provided in Table 2.

RESULTS

Species and genotype identification

Of 14 469 confirmed *Cryptosporidium* isolates from individual patients (one per patient) submitted to CRU during the 9-year period, 164 (1·1%) were unusual *Cryptosporidium* species or genotypes, 7439 (51·4%) were *C. hominis*, 6372 (44·0%) *C. parvum*, 51 (0·4%) both *C. hominis* and *C. parvum*, and 443 (3·1%) were not typable. Of the unusual cases, 109 were *C. meleagridis*, 38 *C. felis*, 11 *C. ubiquitum*, two horse genotype, two novel genotypes and one each of *C. canis* and skunk genotype. One *C. hominis* monkey genotype case and 37 *C. cuniculus* cases were identified by a look-back exercise during 2007 and 2008 using a different test algorithm, targeting species/genotypes closely related to *C. hominis* and the data presented elsewhere [23]; these cases have been removed from this dataset as they were not identified routinely.

Representative sequences have been deposited into GenBank under accession numbers EU437414-EU437418, HM191258-HM191264 and HM485431-HM485435.

Enhanced surveillance of unusual cases of cryptosporidiosis and comparison with C. hominis and C. parvum

Completed questionnaires were returned from 103/164 (63%) unusual cases, although a basic dataset of demographic data was available for all cases from the specimen request forms (Tables 1 and 2). There were no differences in age group or sex distribution between cases who did and did not return a questionnaire ($\chi^2=3·28$, $P=0·77$; $\chi^2=0·42$, $P=0·52$, respectively). Regional variation approached significance ($\chi^2=16·73$, $P=0·053$) which may be due to local differences in case follow-up procedures and therefore independent of the case. Specimen date was incomplete for 12/164 (7·3%) unusual cases and for 882/13 811 (6·4%) *C. parvum* and *C. hominis* cases.

Almost half the unusual cases were female (47·6%) which is comparable with cases of *C. hominis* and *C. parvum*. Unusual case age ranged from 0 to 82 years

(median 24 years) and was significantly older than cases with *C. hominis* (median 10 years, $\chi^2 = 34\cdot28$, $P < 0\cdot01$) or *C. parvum* (median 9 years, $\chi^2 = 53\cdot72$, $P < 0\cdot01$). Only 17% of cases with unusual species were aged $\leqslant 5$ years compared to 35% of *C. hominis* cases and 37% of *C. parvum* cases.

Data about patient immune status were collected for *C. parvum* and *C. hominis* up to the end of 2003: being immunocompromised was significantly more frequent in unusual cases (4/69, 5·8%) compared to C. hominis cases (29/3818, 0·8%) (Fisher's exact $P = 0\cdot002$) and *C. parvum* cases (21/3566, 0·6%) (Fisher's exact $P = 0\cdot001$) during this period.

Unusual species were recorded throughout the year, but the highest proportion of specimens was received between July and September (61/164, 37·2%). This is significantly different from *C. hominis* samples, most of which were received between October and December (3226/7439, 43·4%; $\chi^2 = 72\cdot37$, $P < 0\cdot01$) and *C. parvum* samples, most of which were received between April and June (2241/6372, 35·2%; $\chi^2 = 19\cdot17$, $P < 0\cdot01$).

Samples were submitted from cases living in all ten Government Office Regions of England and Wales. There were regional differences in the distribution of unusual cases compared to *C. hominis* and *C. parvum*. The highest proportion of unusual cases was in Yorkshire and the Humber (18/1024, 1·8%); the highest proportion of *C. hominis* was in the South East (937/1361, 68·8%) and *C. parvum* in the South West (1044/1789, 58·4%). Data from the London region were omitted from regional analysis as few samples were sent for typing.

Foreign travel was reported by 67/164 (41·0%) of the unusual cases which is significantly higher than reported for *C. hominis* (1169/7439, 15·7%; $\chi^2 = 74\cdot48$, $P < 0\cdot01$) and *C. parvum* (479/6372, 7·5%; $\chi^2 = 232\cdot05$, $P < 0\cdot01$) infections. Of the 67 unusual cases that indicated travel, 65 (97·0%) stated the destination. These were predominantly the Indian subcontinent (India, $n = 24$; Pakistan, $n = 3$; Nepal, $n = 5$) (total 32/65, 49·2%). Destination was stated by 1082/1169 (92·6%) *C. hominis* cases and by 428/479 (89·4%) *C. parvum* cases. Unusual cases were more likely to have travelled to the Indian subcontinent than returning travellers with *C. hominis* (223/1082, 20·6%; $\chi^2 = 29\cdot08$, $P < 0\cdot01$) or *C. parvum* (51/428, 11·9%; $\chi^2 = 56\cdot00$, $P < 0\cdot01$). Returning travellers with *C. hominis* or *C. parvum* were more likely to have come from Europe than unusual cases (823/1510, 54·5%; $\chi^2 = 29\cdot77$, $P < 0\cdot01$) (Table 1). Of the unusual cases, those who had travelled abroad were more likely to have used a swimming pool (22/67, 32·8%), than those who had not travelled abroad (14/97, 14·4%; $\chi^2 = 7\cdot79$, $P < 0\cdot01$).

None of the unusual cases which we identified through routine testing were part of a recognized outbreak of cryptosporidiosis compared to 710/7439 (9·5%) *C. hominis* cases ($\chi^2 = 17\cdot26$, $P < 0\cdot01$) or 345/6372 (5·4%) *C. parvum* cases ($\chi^2 = 9\cdot37$, $P < 0\cdot01$).

Comparisons between *C. meleagridis*, *C. felis* and *C. ubiquitum*

When case data for individual unusual species were compared (Table 2), no difference in the sex distribution was observed ($\chi^2 = 4\cdot53$, $P = 0\cdot1$) but there was a notable excess of males in *C. ubiquitum* cases (9/11, 81·8%) compared to all other unusual cases ($\chi^2 = 3\cdot71$, $P = 0\cdot05$). Case ages varied according to species ($\chi^2 = 26\cdot15$, $P = 0\cdot01$). *C. ubiquitum* cases peaked in young children (mode 1 year), *C. meleagridis* and *C. felis* cases were spread across all ages but *C. meleagridis* peaked in young adults (mode 24 years), with significantly more aged 20–29 years than any other unusual species ($\chi^2 = 8\cdot55$, $P = 0\cdot003$). *C. felis* peaked in children with 34·2% in those aged < 9 years (mode 1 year).

In adult cases, occupation group was not significantly associated with any unusual species ($\chi^2 = 6\cdot39$, $P = 0\cdot27$); most occupations were categorized as low risk. Where the case was a child, parental occupation group was not linked to a specific species or genotype ($\chi^2 = 11\cdot77$, $P = 0\cdot30$).

Being immunocompromised was more common in cases with *C. felis* (6/38, 15·8%, Fisher's exact $P = 0\cdot02$) than other unusual cases. Where stated, HIV infection was the most common cause of immunodeficiency in the unusual cases (6/9 cases, 66·7%).

The presence of diarrhoeal illness in household contacts was not associated with any particular unusual *Cryptosporidium* spp. ($\chi^2 = 1\cdot56$, $P = 0\cdot46$).

No significant difference in seasonal quarter of specimen receipt between unusual species was evident ($\chi^2 = 6\cdot13$, $P = 0\cdot41$).

There were no significant differences in the regional distribution of unusual species ($\chi^2 = 23\cdot25$, $P = 0\cdot18$), although the East of England had more *C. ubiquitum* cases (4/11, 36·4%) than any other region and the North West had more *C. felis* cases (8/38, 21·1%).

The presence of other gastrointestinal pathogens was not associated with any single unusual species of

Cryptosporidium ($\chi^2 = 3 \cdot 99$, $P = 0 \cdot 14$). Co-infections were reported with *Campylobacter* spp. ($n = 14$), *Escherichia coli* O157 ($n = 1$), *Salmonella* sp. ($n = 1$) and one case with multiple co-infections (*Campylobacter* spp., *Entamoeba coli*, *Giardia* spp.). Of the 15 unusual cases with *Campylobacter* spp., 13 were *C. meleagridis* cases (86·7%).

Travel abroad varied in the unusual cases ($\chi^2 = 19 \cdot 77$, $P < 0 \cdot 01$) and in particular was undertaken more frequently by cases infected with *C. meleagridis* (55/109, 50·5%) than with other unusual *Cryptosporidium* spp. infections (7/49, 14·3%) ($\chi^2 = 18 \cdot 43$, $P < 0 \cdot 01$). None of the 11 cases with *C. ubiquitum* had travelled. Where destination was stated more cases with *C. meleagridis* had travelled to the Indian subcontinent (28/54, 51·9%) than had *C. felis* cases (2/7, 28·6%) but this was not a significant difference (Fisher's exact $P = 0 \cdot 23$). Foreign travel was reported by all age groups ($\chi^2 = 12 \cdot 51$, $P = 0 \cdot 05$) and peaked in the 20–29 years group. The choice of destination (where stated) was significantly linked to case age group ($\chi^2 = 47 \cdot 59$, $P < 0 \cdot 01$); 15/30 (50%) travellers to the Indian subcontinent were aged 20–29 years ($\chi^2 = 5 \cdot 26$, $P = 0 \cdot 02$).

Contact with any animals was not associated with infection with an unusual *Cryptosporidium* sp. ($\chi^2 = 0 \cdot 27$, $P = 0 \cdot 87$). However, contact with cats was reported by significantly more *C. felis* cases (6/38, 15·8%) than cases with other unusual species (5/120, 4·2%, Fisher's exact $P = 0 \cdot 02$). Contact with dogs was not associated with an unusual species ($\chi^2 = 0 \cdot 18$, $P = 0 \cdot 92$). Contact with an unwell pet was associated with an unusual species ($\chi^2 = 5 \cdot 87$, $P = 0 \cdot 05$), with three of the four unusual cases who reported contact with an unwell pet identified as having *C. felis*, all of whom specifically reported kittens or cats that were unwell (Fisher's exact $P = 0 \cdot 04$). The fourth unusual case had *C. meleagridis* and reported contact with an unwell dog, a host from which *C. meleagridis* has been previously reported [24]. Contact with birds was not associated with infection with an unusual species ($\chi^2 = 3 \cdot 50$, $P = 0 \cdot 17$).

The use of swimming pools ($\chi^2 = 0 \cdot 37$, $P = 0 \cdot 83$) or participation in water sports ($\chi^2 = 1 \cdot 56$, $P = 0 \cdot 46$) were not associated with infection with an unusual species. Twenty percent of unusual cases reported contact with swimming pools which is less than that reported by 790 sporadic *C. hominis* and *C. parvum* cases (44·8%) during a 3-year study in the UK [4].

Drinking-water source (whether or not mains water was consumed) was not associated with any particular unusual species ($\chi^2 = 2 \cdot 59$, $P = 0 \cdot 27$); however, 5/158 (3·2%) cases consumed water from a private supply.

DISCUSSION

Enhanced surveillance of unusual cases of cryptosporidiosis has provided insight into the epidemiological and behavioural factors associated with risk of infection with unusual *Cryptosporidium* species or genotypes in the population of England and Wales. The median age of unusual cases was 24·5 years, higher than that of *C. hominis* and *C. parvum* cases, and the age distribution showed a much lower proportion of cases aged <5 years than *C. hominis* and *C. parvum* cases. It is probable that behavioural factors are important here; for example foreign travel, especially to developing countries in the Indian subcontinent, is associated with unusual infections, particularly *C. meleagridis*, and such travel is more often undertaken by those aged 20–29 years than any other age group [25]. Although it could be argued that foreign travel may be used as a selection criterion for primary testing for *Cryptosporidium* spp., thus increasing detection rates in this age group, the unusual case-case comparison showed that the age distribution was unique to *C. meleagridis* in this dataset, and is unlikely to be a surveillance artefact. Unusual infections have been reported to occur in residents of developing countries such as India and elsewhere in Asia, where they may be endemic [12, 26]. It is not known whether prior exposure to *C. parvum* or *C. hominis* is cross-protective against further illness caused by a heterologous isolate [27], so the risk of infection for travellers to these destinations may not be mitigated by previous domestically acquired infection.

Being immunocompromised was associated with infection with an unusual species or genotype, but it is possible that our data are biased by better information on immune status actively sought for the unusual cases than that received passively for *C. hominis* and *C. parvum* cases on the routine genotyping request form.

When date of receipt of unusual case samples was compared with *C. hominis* and *C. parvum* differences were observed. These contrast to the well documented albeit recently reduced 'spring peak' [28] of *C. parvum* cases and the post-summer holiday period peak in the last quarter of the year of *C. hominis* cases [3]. Unusual cases peaked in the July–September period which may reflect slightly earlier travel dates, or

seasonal exposure to other unknown risks. Specimen date was incomplete for a substantial number of cases; therefore seasonality was measured using date of receipt in our laboratory. Our previous experience using both specimen and receipt dates has shown that the latter differs from the former by an average of 5 days [3] which would not significantly affect seasonality reported here.

When the demographics and risk factors for specific unusual species or genotypes were compared an excess of young male cases of *C. ubiquitum* was seen which cannot be explained by data collected and may warrant further investigation. Most cases of *C. meleagridis* occurred in young adults, although in this scenario, age is probably acting as a confounder for foreign travel as discussed above.

In contrast none of the 11 cases with *C. ubiquitum* had travelled abroad and this suggests that *C. ubiquitum* in humans may be indigenous to the UK, which is supported by frequent identification in sheep and during environmental investigations here [29–31]. Elsewhere *C. ubiquitum* has been found in a wide variety of hosts [14], but in the UK sheep are a likely source of environmental contamination due to their high density [32]. However, the number of human cases with *C. ubiquitum* is small and its pathogenicity is not known.

The association between cats and infection with *C. felis* is perhaps unsurprising as this is the predominant species in cats [33]. Although contact with companion animals has not been associated with cryptosporidiosis in epidemiological or microbiological studies [1, 34], individual infection risks may be present and hygiene advice should be followed. Since *Cryptosporidium* infection is by the faecal–oral route, either directly or indirectly, the most appropriate intervention is thorough hand-washing with soap and warm running water before preparing and eating food, after handling raw food, after going to the toilet or changing a baby's nappy, after working, feeding, grooming or playing with pets and other animals [34].

Within unusual cases, concomitant infection with *Campylobacter* was commonly reported. This concurs with previous findings in developed and developing nations [35, 36] (CRU, unpublished observations). *Campylobacter* is the most commonly detected bacterial gastrointestinal pathogen in the UK [37] and is routinely sought in addition to *Salmonella* spp., *Escherichia coli* O157 and *Shigella* spp. in the diagnosis of gastrointestinal infections in the UK [38].

Conversely, the diagnosis and reporting of parasites such as *Giardia* spp. and *Entamoeba* spp. are often limited by selective testing of patients who have travelled abroad [38]. Nevertheless, the potential association between *C. meleagridis* and *Campylobacter* spp. is intriguing as both species have either recently or in the past been associated with poultry [39, 40]. However, no significant association between bird contact and infection with *C. meleagridis* was established from the data presented here.

No unusual species or genotypes were the cause of a recognized outbreak of cryptosporidiosis through the routine test algorithm described here; however, it should be noted that *C. cuniculus* (known at the time as the rabbit genotype) was identified by CRU as the causative agent of a waterborne outbreak in 2008 through a separate test algorithm using SSU rDNA PCR–RFLP as the first-line test [41].

This study describes for the first time the epidemiology and risk factors for infection with unusual *Cryptosporidium* spp. in a developed country. Some of the unusual species are probably indigenous to the UK while others more likely to be acquired abroad. To properly understand the infectivity and pathogenicity of different *Cryptosporidium* spp. and genotypes more information is needed about the spectrum of illness of all causes of cryptosporidiosis and should therefore be the focus for future work of the kind presented here.

DECLARATION OF INTEREST

None.

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