Health Sequelae of Human Cryptosporidiosis – a 12 month prospective follow-up study

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Abstract

Purpose

To investigate long-term health sequelae of cryptosporidiosis, with especial reference to post-infectious irritable bowel syndrome (PI-IBS).

Methods

A prospective cohort study was carried out. All patients with laboratory-confirmed, genotyped cryptosporidiosis in Wales, UK, aged between 6 months and 45 years of age, over a two year period were contacted. 505 patients agreed to participate and were asked to complete questionnaires (paper or online) at baseline, 3 and 12 months after diagnosis. Presence/absence of IBS was established using the Rome III criteria for different age groups.

Results

205/505 cases completed questionnaires (40% response rate). At 12 months, over a third of cases reported persistent abdominal pain and diarrhea, 28% reported joint pain and 26% reported fatigue. At both 3 and 12 months, the proportion reporting fatigue and abdominal pain after *C. hominis* infection was statistically significantly greater than after *C. parvum.* Overall, 10% of cases had sufficient symptoms to meet IBS diagnostic criteria. A further 27% met all criteria except 6 months' duration and another 23% had several features of IBS but did not fulfil strict Rome III criteria. There was no significant difference between *C. parvum* and *C. hominis* infection with regard to PI-IBS.

Conclusions

Post-infectious gastrointestinal dysfunction and fatigue were commonly reported after cryptosporidiosis. Fatigue and abdominal pain were significantly more common after *C. hominis* compared to *C. parvum* infection. Around 10% of people had symptoms meriting a formal diagnosis of IBS following cryptosporidiosis. Using age-specific Rome III criteria, children as well as adults were shown to be affected.

Keywords: cryptosporidiosis, sequelae, *Cryptosporidium hominis, Cryptosporidium parvum*, irritable bowel syndrome

Introduction

Cryptosporidium is the commonest protozoal cause of acute gastroenteritis in the UK [1], with between 3,500 and 5,500 laboratory-confirmed cases reported annually in England and Wales from 2013 to 2015 [2]. The actual incidence of *Cryptosporidium* infection is almost certainly underestimated as asymptomatic carriage is possible [1] and diagnosis often requires a request for specific laboratory stool sample analysis. More than 90% of human cryptosporidiosis cases can be attributed to two species; *Cryptosporidium parvum*, a zoonotic species, and *Cryptosporidium hominis*, mainly adapted to humans [3].

Symptomatic cryptosporidiosis in immunocompetent patients is characterized by gastrointestinal symptoms that include sudden-onset, profuse, watery diarrhoea which may be accompanied by abdominal pain or cramps, vomiting and weight loss. Other, more non-specific symptoms include malaise, fatigue, fever, nausea and muscle weakness [3]. The symptomatic period may last for up to 3 weeks, although the mean duration of symptoms has been reported as 12.7 days [1]. In over a third of cryptosporidiosis cases, relapse of diarrheal symptoms can occur within days of the initial symptomatic period resolving [4, 5, 6]. In immunocompromised patients *Cryptosporidium* can produce severe symptoms which may persist to cause critical, sometimes life-threatening, illness [1]. *Cryptosporidium* infections in these patients may have an atypical presentation characterized by involvement of the liver, pancreas, gallbladder and, rarely, the respiratory system [7].

Clearance of gastrointestinal pathogens, and the subsequent recovery of the gastrointestinal epithelium, usually coincides with the resolution of diarrheal symptoms, however, this is not always the case. Prospective and retrospective studies have shown that 4-26% of patients can develop post-infectious irritable bowel syndrome (PI-IBS), following an initial acute gastroenteritis [8]. While the development of PI-IBS usually follows acute bacterial gastrointestinal infections, *C. parvum* in animal models can also induce pathophysiological features consistent with PI-IBS, such as jejunal hypersensitivity to distension and the accumulation of active mast cells, that is present 50 days after infection [9]. More recently, a study which followed up *C. parvum* outbreak cases found that 28% of cases still reported ‘IBS-like’ symptoms up to 12 months after the initial infection [10].

Relatively little is known about the longer-term health effects of *Cryptosporidium* infection.

However, there is growing evidence to suggest that, rather like some bacterial causes of gastroenteritis, *Cryptosporidium* infection may have longer-term consequences [4, 10, 11, 12, 13].

This is a prospective cohort study of laboratory-confirmed cryptosporidiosis cases in Wales sought to investigate the development of potential post-infection sequelae of both *C. parvum* and *C. hominis* over a 12-month time period, with particular attention to PI-IBS.

Methods

Data Collection:

Cryptosporidium is a notifiable causative agent and all persons diagnosed with cryptosporidiosis in Wales are routinely contacted by an Environmental Health Officer (EHO). From July 2013 to July 2015, the EHO informed case patients, or their parents/guardians, that a study was in progress, and that our study team would contact them via post to seek to recruit them into the study. To be considered for recruitment into our study, participants had to be more than 6 months old, under 45 years old, and resident in Wales, with *Cryptosporidium* infection having been confirmed and genotyped from a faecal specimen by the national Cryptosporidium Reference Unit (CRU), Swansea within the two year study period. The submission of *Cryptosporidium*-positive stools by primary diagnostic laboratories to the CRU for genotyping is part of the routine diagnostic pathway. Any cases (or parents/guardians of a child case) who informed the EHO that they did not wish to take part in the research study were not considered as a potential study participant and were not contacted. The 45 year upper limit for age was used since patients over the age of 45 cannot be given a diagnosis of IBS without investigations to exclude other pathologies, according to the Rome III criteria.

Those who consented to be contacted were sent an age-appropriate letter, information sheet and baseline questionnaire in either paper or internet-based format, depending on participant preference. The study questionnaire included questions aimed at establishing the presence or absence of symptoms of irritable bowel syndrome (IBS), as defined by the Rome III criteria for diagnosis of IBS [14]. The study questionnaires also enquired about

other symptoms related to potential post-cryptosporidiosis health sequelae previously reported in the literature.

Questionnaires were specifically designed for three age groups; 6 months – 4 years, 5 – 17 years and 18+ years. Different Rome III criteria for diagnosis of IBS apply to each of these age-groups. Study questionnaires were administered to each consenting participant/guardian on three occasions: baseline (as near to laboratory diagnosis of cryptosporidiosis as feasible); 3 months after diagnosis; and 12 months after diagnosis. In the baseline questionnaire, cases were asked about symptoms over the 6 months pre-dating their episode of cryptosporidiosis as well as their symptoms during their acute illness.

Reminder letters were sent if there was no response within 2 weeks of a questionnaire being sent. If no response was received after 2 weeks, an additional questionnaire was sent. After this time, if there was no response, it was assumed that the person did not want to participate in the study, and no further contact was made by the study team. All returned paper questionnaires were quality checked and transferred into a central, secure electronic database, along with the online questionnaire data.

Laboratory diagnosis

Cryptosporidium was diagnosed in primary diagnostic laboratories using commercially-available enzyme linked immunosorbent assays (ELISA) or auramine phenol or modified Ziehl-Neelsen stained microscopy.

Genotyping

Cryptosporidium-positive stools were genotyped at the CRU by real-time PCR incorporating *C. parvum*- and *C. hominis*-specific primers and probes based on the LIB13 and A135 genes respectively (15, 16). Other *Cryptosporidium* species were determined by sequencing part of the ssu rRNA gene (17).

Data analysis

Confidence intervals for proportions, risk ratios, 95% confidence intervals for risk ratios, chi

squared, and chi squared for trend were calculated used Stata 13 (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP).

Results

Study Population

From July 2013 to July 2015, 586 cases of *Cryptosporidium* were notified in Wales, of which 515 were confirmed at the Reference Unit, genotyped and reported to our study team. 52% were < 18 years old. The predominant infecting species was *C. parvum* (n=300), followed by *C. hominis* (n=200), *C. cuniculus* (n=9), *C. felis* (n=3), both *C. hominis* and *C. parvum* (n=2) and *C. ubiquitum* (n=1). Infecting species by age group is shown in Table 1. 505 of these cases agreed to be contacted about the study and were sent questionnaires.

205 case patients completed study questionnaires, a 40% response rate. Complete data sets for analysing sequelae (baseline, 3 months and 12 months questionnaires) were obtained from 89 participants, while partial data sets were obtained from a further 43 participants (Table 2). A further 73 participants were ineligible for inclusion in the analysis of sequelae as no follow-up questionnaires were completed after baseline. However, they were included in the analysis of the presenting symptoms of acute cryptosporidiosis.

Overall, the proportion of female to male participants was higher throughout the duration of this study: 60.6% female at baseline, 58.2% female at 3 months and 66.3% female at 12 months. A higher proportion of females was represented amongst the participants than among the 515 eligible case patients who were initially contacted, of whom 274 (53%) were female. In terms of age, there were 42 respondents from 113 cases age 6 months-4 years (response rate 37%), 63 respondents from 156 cases age 5-17 years (response rate 40%) and 100 respondents from 246 cases aged over 18 (response rate 41%). Therefore there was little difference in response rates between the different age groups. At all time-points, the 18+ years age group accounted for approximately half of all the responses received (50.8% at baseline, 46.4% at 3 months, 50.6% at 12 months), with 5-17 years being the next most represented age group and 6 months - 4 years being the least represented. The proportion of under-18s participating was similar to the proportion of eligible cases contacted (52%).

Female participants were significantly older than male participants, chi squared for trend p<0.001 (Figure 1).

Acute Symptoms

All cases reported diarrhea. The proportions of cases reporting other symptoms are shown in Table 3. Abdominal pain, anorexia, nausea, fatigue, weight loss and fever were each seen in over half of all cases. Joint pain was reported in over a quarter. Table 3 also shows the acute symptoms analysed by those reported with *C. hominis* and with *C. parvum*. The only symptom for which a statistically significant difference in incidence was found between the species was eye pain, which was commoner with *C. hominis* (p=0.03).

Table 4 shows the acute symptoms broken down by age-group. Vomiting was reported by over half of under-18s but only by just over a third of adults, and occurred more frequently in children (p=0.01), an observation which is consistent with our anecdotal clinical experience. Fatigue was more commonly reported in adults, over three-quarters vs just over half (p=0.01). Joint pains, headache, dizzy spells, eye pain and blurred vision may be difficult to identify in young children which may explain why they were infrequently reported in the under-5s in this study.

3 month and 12 month sequelae

Symptoms reported at 3 months and/or 12 months were only recorded if they were reported as having been absent prior to the acute illness. Symptoms reported at 3 months and 12 months which were not reported as present prior to the acute illness are shown in Table 5. At 3 months, 43% of cases reported abdominal pain and 40% reported fatigue. Diarrhoea was still reported by 36% and 32% reported loss of appetite. At 12 months, abdominal pain and diarrhea were still being reported in over a third of cases overall. The other most commonly reported symptoms at 12 months which were not present prior to infection were joint pain (28%) and fatigue (26%).

When comparing cases who had *C. hominis* with those who had *C. parvum*, at 3 months (Table 6), symptoms tended to be more frequent with *C. hominis.* The numbers reporting fatigue (p=0.003), vomiting (p=0.04) and abdominal pain (p=0.045) after *C. hominis* infection were all statistically significantly greater.

At 12 months (Table 7), a comparison of symptoms reported after *C. hominis* with *C. parvum* again found a statistically significant higher reported incidence of fatigue (p=0.002) and abdominal pain

(p=0.04) associated with *C. hominis.* The difference in reported incidence of vomiting was no longer marked at 12 months (p=0.76).

IBS

Prior to this part of the analysis, nine participants were excluded: seven of these already had a doctor's diagnosis of IBS prior to the acute cryptosporidiosis, the other two met the Rome III criteria at baseline. All excluded participants belonged to the 18+ age group. None of the participants who already had a diagnosis or evidence of IBS at baseline reported that their pre-existing IBS worsened in the 12 months following their *Cryptosporidium* infection.

For identifying IBS the Rome III diagnostic criteria were used. Overall, 10% of cases had features diagnostic for IBS. No significant difference was seen between *C. parvum* and *C. hominis* with regard to IBS (Table 8). The distribution by age group is shown in Figure 2.

Discussion

A small number of previous studies have investigated post-acute symptoms after cryptosporidiosis [4, 10, 11, 12, 13]; however, most did not include both *C. hominis* and *C. parvum* infections, and some did not have a follow-up period of sufficient duration to be able to identify PI-IBS, a diagnosis of which requires symptoms to have been present for at least 6 months [14]. Anecdotally, *Cryptosporidium* infection has also been associated with the development of reactive arthritis [18, 19, 20], Reiter's syndrome [21], acute pancreatitis [22, 23] and haemolytic uraemic syndrome [24].

Hunter *et al.* [4], found that loss of appetite, vomiting, abdominal pain and diarrhoea were more commonly reported in both *C. hominis* and *C. parvum* cases than in non-cases after a 2 month follow-up period, while joint pain, fatigue, dizzy spells, recurrent headache and eye pain were more commonly reported by those with *C. hominis* infections. Among presenting complaints during acute illness with *C. parvum* and *C. hominis,* our study also found an increased proportion of cases reporting eye pain during acute *C. hominis* infection compared with *C. parvum* (p=0.03), and identified that vomiting is more common in children than in adults in acute cryptosporidiosis. Similarly, another study of *C. hominis* and *C. parvum* cases found that dizziness, fatigue, weight loss, diarrhea and abdominal pain were commonly reported up to 4 months post-infection, but did not identify any difference in

sequelae between the two species [13]. In contrast, here, fatigue and abdominal pain was reported at both 3 months and 12 months significantly more often after C*. hominis* than *C. parvum.* Vomiting was also seen more commonly at 3 months after *C. hominis* infection but this difference was no longer apparent at 12 months. Intermittent diarrhoea, persistent abdominal pain, myalgia/arthralgia and fatigue, have been reported up to 3 years post-*Cryptosporidium* infection [12]. A study of *C. hominis* outbreak cases found a significant incidence of fatigue, nausea and joint pain in cases, when compared to non-cases, up to 11 months post-infection [11]; and even persisting up to 28 months later [25]. A 2017 study of a *C. parvum* outbreak [10] found that abdominal pain, diarrhoea, joint pain, weight loss, fatigue and eye pain were still being reported up to 12 months post-infection.

A formal diagnosis of IBS requires that symptoms have been present over a period of time greater than 6 months. Cases reporting all features required for an IBS diagnosis were categorised as IBS RIII. At the time of the 3 month questionnaire, it was not possible for participants to report new symptoms diagnostic of IBS that had been present for more than 6 months. Therefore cases who at 3 month follow-up reported all features of IBS *except* duration >6 months were categorised as 'IBS RIII <6m'. In some cases enough information was given to identify a functional change in bowel habit consistent with IBS, but the information given was not specific enough to assign them with certainty to the IBS group. The problematic criteria in these instances were: how often they had pain – the Rome III criteria specify 3 times per month minimum, but some replies, whilst specifying pain at least monthly or more often, did not specify whether the pain reached the threshold of three times per month; the duration of the IBS-like symptoms – some replies did not specify whether the pain had been experienced for at least 6 months, only that it was for several months. Cases who could not definitely be shown to meet the formal definition of Rome III for one of these reasons were categorised as 'IBS-like'.

A further 27% displayed all the Rome III criteria except that at 3 months post-infection they could not report >6months duration of symptoms. Thus in total 37% of cases fell into the categories IBSRIII *or* IBSRIII<6m. Another 23% had several features of IBS but lacked one symptom according to Rome III criteria.

Previous work has suggested that children rarely develop sequelae, in contrast to adults [25]. The findings of this study do not support this. The manifestations of IBS are different in paediatric practice, and this is reflected in differences in the Rome III criteria for different age groups. This study analysed the Rome III diagnostic criteria for each age group in detail and this is likely to account for differences compared to previous work.

Overall, persistent gastrointestinal symptoms were noted in 59% of the cases during the study period and 10% of cases had features diagnostic for IBS at 12 months. No significant difference was seen between *C. parvum* and *C. hominis* with regard to IBS. In a previous study of *C. parvum* outbreak cases [10], 28% had symptoms consistent with IBS over the course of 1 year follow-up and two of 54 patients received a medical diagnosis of IBS. Similarly, among patients who have suffered bacterial gastroenteritis, persistent bowel dysfunction has been recorded in around 25% [26]. Around 7% of patients were found to have developed IBS following bacterial gastroenteritis [27], a figure not dissimilar to that found in our study.

A limitation of this study was that the number of respondents dropped off somewhat at each timepoint, as might be expected. Of the 205 cases who completed the baseline survery, ninety-six completed the 12 month survey.

This study adds weight to the recent body of evidence that post-infectious gastrointestinal dysfunction after cryptosporidiosis is common, and patients should be given realistic expectations regarding recovery. Fatigue and abdominal pain were frequently reported up to 12 months after acute illness and was significantly more common after *C. hominis* infection compared to *C. parvum* infection. Around 10% of people had symptoms which merited a formal diagnosis of IBS following cryptosporidiosis.

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Compliance with Ethical Standards

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Conflict of Interest: The authors have no conflicts of interest to declare

Ethical approval: Ethical approval was in place for this study. UK REC reference 12/LO/1659; IRAS project ID 94686

Informed consent: All participants gave their signed informed consent to be included in this study.

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