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

2

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16

17 **Abstract**

18

19 **Purpose**

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

22 **Methods**

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

28 **Results**

29 205/505 cases completed questionnaires (40% response rate). At 12 months, over a third of
30 cases reported persistent abdominal pain and diarrhea, 28% reported joint pain and 26%
31 reported fatigue. At both 3 and 12 months, the proportion reporting fatigue and abdominal
32 pain after *C. hominis* infection was statistically significantly greater than after *C. parvum*.
33 Overall, 10% of cases had sufficient symptoms to meet IBS diagnostic criteria. A further 27%
34 met all criteria except 6 months' duration and another 23% had several features of IBS but
35 did not fulfil strict Rome III criteria. There was no significant difference between *C. parvum*
36 and *C. hominis* infection with regard to PI-IBS.

37 **Conclusions**

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

43

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

46

47

48

49

50 Introduction

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

58 Symptomatic cryptosporidiosis in immunocompetent patients is characterized by
59 gastrointestinal symptoms that include sudden-onset, profuse, watery diarrhoea which may
60 be accompanied by abdominal pain or cramps, vomiting and weight loss. Other, more non-
61 specific symptoms include malaise, fatigue, fever, nausea and muscle weakness [3]. The
62 symptomatic period may last for up to 3 weeks, although the mean duration of symptoms
63 has been reported as 12.7 days [1]. In over a third of cryptosporidiosis cases, relapse of
64 diarrheal symptoms can occur within days of the initial symptomatic period resolving [4, 5,
65 6]. In immunocompromised patients *Cryptosporidium* can produce severe symptoms which
66 may persist to cause critical, sometimes life-threatening, illness [1]. *Cryptosporidium*
67 infections in these patients may have an atypical presentation characterized by involvement
68 of the liver, pancreas, gallbladder and, rarely, the respiratory system [7].

69 Clearance of gastrointestinal pathogens, and the subsequent recovery of the
70 gastrointestinal epithelium, usually coincides with the resolution of diarrheal symptoms,
71 however, this is not always the case. Prospective and retrospective studies have shown that
72 4-26% of patients can develop post-infectious irritable bowel syndrome (PI-IBS), following
73 an initial acute gastroenteritis [8]. While the development of PI-IBS usually follows acute
74 bacterial gastrointestinal infections, *C. parvum* in animal models can also induce
75 pathophysiological features consistent with PI-IBS, such as jejunal hypersensitivity to
76 distension and the accumulation of active mast cells, that is present 50 days after infection
77 [9]. More recently, a study which followed up *C. parvum* outbreak cases found that 28% of
78 cases still reported 'IBS-like' symptoms up to 12 months after the initial infection [10].

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

80 However, there is growing evidence to suggest that, rather like some bacterial causes of
81 gastroenteritis, *Cryptosporidium* infection may have longer-term consequences [4, 10, 11,
82 12, 13].

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

86

87 **Methods**

88 *Data Collection:*

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

104

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

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

112

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

120

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

127

128 *Laboratory diagnosis*

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

132

133 *Genotyping*

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

138

139 *Data analysis*

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

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

143 **Results**

144 *Study Population*

145 From July 2013 to July 2015, 586 cases of *Cryptosporidium* were notified in Wales, of which
146 515 were confirmed at the Reference Unit, genotyped and reported to our study team. 52%
147 were < 18 years old. The predominant infecting species was *C. parvum* (n=300), followed by
148 *C. hominis* (n=200), *C. cuniculus* (n=9), *C. felis* (n=3), both *C. hominis* and *C. parvum* (n=2)
149 and *C. ubiquitum* (n=1). Infecting species by age group is shown in Table 1. 505 of these
150 cases agreed to be contacted about the study and were sent questionnaires.

151
152 205 case patients completed study questionnaires, a 40% response rate. Complete data sets
153 for analysing sequelae (baseline, 3 months and 12 months questionnaires) were obtained
154 from 89 participants, while partial data sets were obtained from a further 43 participants
155 (Table 2). A further 73 participants were ineligible for inclusion in the analysis of sequelae as
156 no follow-up questionnaires were completed after baseline. However, they were included in
157 the analysis of the presenting symptoms of acute cryptosporidiosis.

158
159 Overall, the proportion of female to male participants was higher throughout the duration
160 of this study: 60.6% female at baseline, 58.2% female at 3 months and 66.3% female at 12
161 months. A higher proportion of females was represented amongst the participants than
162 among the 515 eligible case patients who were initially contacted, of whom 274 (53%) were
163 female. In terms of age, there were 42 respondents from 113 cases age 6 months-4 years
164 (response rate 37%), 63 respondents from 156 cases age 5-17 years (response rate 40%) and
165 100 respondents from 246 cases aged over 18 (response rate 41%). Therefore there was
166 little difference in response rates between the different age groups. At all time-points, the
167 18+ years age group accounted for approximately half of all the responses received (50.8%
168 at baseline, 46.4% at 3 months, 50.6% at 12 months), with 5-17 years being the next most
169 represented age group and 6 months - 4 years being the least represented. The proportion
170 of under-18s participating was similar to the proportion of eligible cases contacted (52%).

171 Female participants were significantly older than male participants, chi squared for trend
172 $p < 0.001$ (Figure 1).

173
174 *Acute Symptoms*

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

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

187 *3 month and 12 month sequelae*

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

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

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

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

204 *IBS*

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

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

213 **Discussion**

214 A small number of previous studies have investigated post-acute symptoms after
215 cryptosporidiosis [4, 10, 11, 12, 13]; however, most did not include both *C. hominis* and *C.*
216 *parvum* infections, and some did not have a follow-up period of sufficient duration to be
217 able to identify PI-IBS, a diagnosis of which requires symptoms to have been present for at
218 least 6 months [14]. Anecdotally, *Cryptosporidium* infection has also been associated with
219 the development of reactive arthritis [18, 19, 20], Reiter's syndrome [21], acute pancreatitis
220 [22, 23] and haemolytic uraemic syndrome [24].

221

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

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

242

243 A formal diagnosis of IBS requires that symptoms have been present over a period of time
244 greater than 6 months. Cases reporting all features required for an IBS diagnosis were
245 categorised as IBS RIII. At the time of the 3 month questionnaire, it was not possible for
246 participants to report new symptoms diagnostic of IBS that had been present for more than
247 6 months. Therefore cases who at 3 month follow-up reported all features of IBS *except*
248 duration >6 months were categorised as 'IBS RIII <6m'. In some cases enough information
249 was given to identify a functional change in bowel habit consistent with IBS, but the
250 information given was not specific enough to assign them with certainty to the IBS group.
251 The problematic criteria in these instances were: how often they had pain – the Rome III
252 criteria specify 3 times per month minimum, but some replies, whilst specifying pain at least
253 monthly or more often, did not specify whether the pain reached the threshold of three
254 times per month; the duration of the IBS-like symptoms – some replies did not specify
255 whether the pain had been experienced for at least 6 months, only that it was for several
256 months. Cases who could not definitely be shown to meet the formal definition of Rome III
257 for one of these reasons were categorised as 'IBS-like'.

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

262

263 Previous work has suggested that children rarely develop sequelae, in contrast to adults
264 [25]. The findings of this study do not support this. The manifestations of IBS are different in
265 paediatric practice, and this is reflected in differences in the Rome III criteria for different
266 age groups. This study analysed the Rome III diagnostic criteria for each age group in detail
267 and this is likely to account for differences compared to previous work.

268

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

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

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

287

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290 data, and the Communicable Disease Surveillance Centre for identifying and recruiting
291 cases.

292

293 **Compliance with Ethical Standards**

294
295 Funding: This study received no external funding
296
297 Conflict of Interest: The authors have no conflicts of interest to declare
298
299 Ethical approval: Ethical approval was in place for this study. UK REC reference
300 12/LO/1659; IRAS project ID 94686
301 Informed consent: All participants gave their signed informed consent to be included in
302 this study.
303

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