Long-term health effects after resolution of acute *Cryptosporidium parvum* infection: a 1-year follow-up of outbreak associated cases

Authors: Rhianwen E Stiff[a,b], Angharad P Davies[a,c], Brendan W Mason[d,a], Hayley A Hutchings[a], Rachel M Chalmers[c,a]

a. Swansea University Medical School, Singleton Park, Swansea, Wales, SA2 8PP, UK
b. Health Protection, Public Health Wales NHS Trust, Temple of Peace, Cathays Park, Cardiff, Wales, CF10 3NW, UK
c. Cryptosporidium Reference Unit, Public Health Wales Microbiology, Singleton Hospital, Swansea, Wales, SA2 8QA, UK
d. Communicable Disease Surveillance Centre, Public Health Wales NHS Trust, Temple of Peace, Cathays Park, Cardiff, Wales, CF10 3NW, UK

Corresponding author:
Prof Rachel Chalmers

Public Health Wales Cryptosporidium Reference Unit/Swansea University Medical School
Swansea, Wales
SA2 8PP
Tel: 01792 285341
Fax: 01792 202320
Email: rachel.chalmers@wales.nhs.uk

Keywords:
Cryptosporidium, cryptosporidiosis, long-term health effects, post-acute health effects, sequelae

Abstract:

We describe a longitudinal study carried out in an adult outbreak-associated cohort to investigate health effects, including post-infectious irritable bowel syndrome, occurring after resolution of acute *Cryptosporidium parvum* infection. New symptoms self-reported up to 12 months included: weight loss (31%), abdominal pain (38%), diarrhoea (33%), eye pain (9%), joint pain (33%), fatigue (22%) and symptoms consistent with irritable bowel syndrome (IBS) (28%). Two people were medically diagnosed with IBS. This study describes for the first time sequelae reported by patients up to twelve months after infection with *C. parvum*, which appear to be similar to those described with *C. hominis.*

Cryptosporidium is a protozoan parasite which causes symptoms of gastroenteritis including diarrhoea, vomiting and abdominal pain. It is the commonest cause of protozoal diarrhoea in the UK with nearly 6000 laboratory notifications in 2012 (Public Health England, 2014), and it particularly affects children aged between 2 and 5 years. Its global significance has become better recognised since publication of the Global Enteric Multi-centre Study (Kotloff et al., 2013), which found it to be the second commonest pathogen causing moderate to severe diarrhoea in children aged under 1 year in low/middle income countries. In immune-competent individuals cryptosporidiosis is often perceived as an unpleasant but relatively mild self-limiting illness. However there is growing evidence to suggest that, like certain bacterial causes of gastroenteritis, it may have longer-term health effects which manifest after resolution of the acute infection. The main two species affecting humans are *C. hominis* (predominantly anthroponotic) and *C. parvum* (both zoonotic and anthroponotic), and as might be expected the epidemiology differs between them. In addition, previous work has suggested that differences may exist in their post-infectious sequelae (Hunter et al., 2004; Bushen et al 2007). However there have been very few studies on this subject.

In the UK, a case-control study of patients who had cryptosporidiosis found that infection with the species *C. hominis* (but not *C. parvum*) was associated with joint pain, eye pains, headaches and fatigue in the two months following infection (Hunter et al., 2004). A seronegative reactive arthritis has been reported in adults (Hay et al., 1987; Ozgul et al., 1999) and children (Shepherd et al., 1989; Cron et al., 1995) including one report of Reiter's syndrome (arthritis, conjunctivitis and urethritis) (Cron et al., 1995). It has also been suggested that *Cryptosporidium* infection may cause Crohn's disease and ulcerative colitis to relapse (Manthey et al., 1997; Colussi et al., 2010; Vadlamudi et al., 2013). A study in Sweden (Rehn et al., 2015) followed up 459 cases who suffered *C. hominis* in two waterborne outbreaks and controls, finding that outbreak cases were more likely to report diarrhoea, abdominal pain and joint pain several months after infection than controls were. In terms of sequelae in developing countries, cryptosporidiosis is now recognized as being associated with

77 stunting of growth, and with persistent diarrhoea. In studies in Brazil (among others), early
78 childhood diarrhoea with *Cryptosporidium* was associated with impaired physical fitness and
79 cognitive function 4-7 years later (Guerrant et al., 1999), and with increased diarrhoea morbidity in
80 subsequent years (Agnew et al., 1998). It is therefore now believed that the effects of
81 cryptosporidiosis go beyond the initial acute diarrhoeal episode, possibly due to an effect on the
82 gastrointestinal epithelium (for example, villous blunting with chronic inflammation, and an
83 association with the poorly understood entity 'environmental enteropathy') (see Bartelt et al.,
84 2013).
85
86 Several agents of infectious gastroenteritis lead to an increased risk of developing irritable bowel
87 syndrome (IBS). IBS is a common condition occurring in about 9-12% of the population in the UK
88 (Jones et al., 2000). A validated diagnostic criteria assessment tool exists for IBS
89 (http://romecriteria.org/). Post-infectious IBS (PI-IBS) is well-documented, occurring in 25-38% of
90 patients with enteritis (Jones et al., 2000), for instance after *Campylobacter*, *Salmonella* or *Shigella*
91 infection. People who have had a laboratory confirmed diagnosis of bacterial gastroenteritis are
92 nearly 12 times more likely to develop new-onset IBS in comparison with people who have not had a
93 laboratory diagnosis of bacterial gastroenteritis (Rodriguez et al., 1999). PI-IBS appears to have a
94 rather better prognosis than non-PI-IBS (Jones et al., 2000). For culture-confirmed bacterial
95 gastroenteritis, a RR of 11.9 of new-onset IBS has been reported (Hanevik et al., 2009). This risk is
96 not confined to bacterial infection but has also been documented with *Giardia lamblia* (Hanevik et
97 al., 2009), another protozoan parasite causing gastroenteritis. Infection of a rat model with *C.*
98 *parvum* triggers long-term jejunal hypersensitivity and mast cell accumulation, pathological changes
99 akin to those found in human patients with IBS (Marion et al., 2006; Khaldi et al., 2009).
100
101 During spring 2012, an outbreak of cryptosporidiosis caused by *C. parvum* occurred in the UK (mainly
102 northern England) associated with consumption of pre-cut bagged salad leaves (McKerr et al., 2012).

Just over 300 cases were identified and due to the nature of the products involved, most cases were adults. This was a rare opportunity to add to Hunter's evidence (Hunter et al., 2004) relating to the sequelae of infection with *C. parvum* in adults. We undertook a longitudinal study among this adult outbreak-associated cohort to investigate health effects, including PI-IBS, occurring after resolution of acute *C. parvum* infection.

Cases with illness onset between 14 May and 3 June 2012, aged ≥16 years, resident in northern England and confirmed as having *C. parvum* infection by the Cryptosporidium Reference Unit were invited to participate in the study. Consenting participants completed a self-administered web-based questionnaire at 6 and 12 months after laboratory diagnosis of cryptosporidiosis (known as 6MQ and 12MQ). We sought information about participants' acute illness, as well as their symptoms and medical diagnoses prior to onset of acute cryptosporidiosis and during the following year. A review of previously reported health effects (Hunter et al., 2004; Hay et al., 1987; Ozgul et al., 1999; Shepherd et al., 1989; Cron et al., 1995; Manthey et al., 1997; Rehn et al., 2015) informed our inclusion of questions on specific symptoms and diagnoses, as well as some with no known association, such as diabetes and chest pain, included for comparison. To investigate reported symptoms consistent with a diagnosis of IBS the Rome III diagnostic criteria assessment tool (http://romecriteria.org/) was used. Participants were asked about the following conditions diagnosed by a medical practitioner: IBS, ulcerative colitis, Crohn's disease, depression, anxiety, arthritis, diabetes or immunosuppression, either preceding their infection with *Cryptosporidium* or developing in the 12 months afterwards. In addition, data were also collected on self-reported symptoms, either consistent with IBS as indicated by Rome III criteria, weight loss, loss of appetite, nausea, recurrent vomiting, abdominal pain, diarrhoea, blood in stool, blurred vision, eye pain, recurrent headache, dizzy spells, fatigue, joint pain, back pain, fever or chest pain. Persons reporting

pre-existing diagnosis or symptoms were excluded when enumerating new diagnosis or symptoms at 6MQ and 12MQ.

The results are shown in Table 1. One hundred and ninety seven potential participants were invited, of whom 54 (27%; 14 males and 40 females) took part. Response rates at 6MQ were significantly better among women than men (32.8 % vs 18.7%; difference 14.1 %, 95% CI 1.3% to 25.5%). There was no statistically significant difference in the mean age of those who did (41.8 years) and did not (41.5 years) participate (p=0.18). The 12MQ was completed by 39 of the original 54 participants (retention rate 72%). Not all participants responded to each question, resulting in reduced denominators for some responses (Table 1). The mean duration of symptoms of acute cryptosporidiosis was 23 days (range 7-84 days). Six of 54 people spent a total of 95 days in hospital (mean hospital stay 15.8 days SD±13.3; range 2-34 days). Self-reported severity was described by 32/53 (60%) as severe, 20/53 (38%) as moderate and 1/53 as mild. Among the cohort's 41 employees, 434 lost working days were reported. Pre-existing medically diagnosed IBS was reported by 11 of 54 (20%) participants, but this did not appear to correlate with the severity of acute cryptosporidiosis. Those with pre-existing IBS reported a mean duration of illness of 24.8 days (SD±21.5; range 14-84 days); 2 of 11 were hospitalized for 2 and 5 days respectively; and 55% self-reported their acute episode as severe. Seven (66%) reported their IBS symptoms were unchanged at 6MQ; 1 reported IBS symptoms had improved; 1 reported IBS symptoms had worsened and 2 reported 'not applicable'. At 12MQ, 4 of 9 (44%) responders with pre-existing IBS reported a worsening in their IBS symptoms. At 6MQ, 4 of 43 (9%) people without pre-existing IBS reported symptoms which fulfilled all Rome III criteria for diagnosing IBS, including one person who received a new IBS diagnosis from a doctor. Combining reports from 6MQ and 12MQ, a total of six (14%) responders had symptoms meeting all Rome III criteria, and a total of two people received a new medical diagnosis of IBS. Additionally, new onset gastrointestinal and non-gastrointestinal symptoms were reported at 6MQ and/or 12MQ by a high proportion of participants including

154 weight loss (31%), abdominal pain (38%), diarrhoea (33%), eye pain (9%), joint pain (33%), fatigue
155 (22%) and dizzy spells (10%) (Table 1).
156
157 This outbreak provided an unusual and fortuitous opportunity to recruit a cohort of adults with *C.*
158 *parvum*, since a large proportion of *Cryptosporidium* cases usually occur in young children.
159 Recruitment delay may have biased towards over-representation of those most adversely affected
160 by, or those who attributed post-acute symptoms to, acute cryptosporidiosis. Hunter et al. (2004)
161 reported new or worsened symptoms following cryptosporidiosis in 40.9% of case patients, including
162 statistically significantly more cases than controls with weight loss (29.5%), appetite loss (23%),
163 abdominal pain (26.2%) and diarrhoea (29.5%) at two months after acute infection. A study of a
164 waterborne cryptosporidiosis outbreak in Milwaukee (Manthey et al., 1997) found that 39% of cases
165 experienced a relapse of diarrhoeal symptoms after initial resolution, but this study did not follow
166 patients up for longer than a maximum of three months, and in most cases for less than that.
167 Evidence pertaining to periods longer than this is very scant. One study of cryptosporidiosis in
168 Sweden (without controls) found persistent gastrointestinal symptoms in up to 8 of 53 (15%)
169 patients 25-36 months post-infection: of interest, in this group, no difference was noted between
170 cases who had been infected with *C. hominis* and those with *C. parvum* (Insulander et al., 2013).
171 Another larger study (Rehn et al., 2015), also in Sweden, followed cases up at various time periods
172 between 2.5 and 11.5 months post-*C. hominis* infection, in two locations, and found that 49% and
173 56% of cases respectively reported symptoms during this period, with fatigue, headache, abdominal
174 pain and diarrhoea being commonest. In our study, following *C. parvum* infection, cases reported
175 new-onset weight loss (30.8%), loss of appetite (25%) abdominal pain (37.8%) and diarrhoea
176 (33.3%). The figures are strikingly similar to Hunter's et al (2004) and furthermore our study suggests
177 persistence of these gastrointestinal symptoms beyond the two months studied by Hunter, and up
178 to a year after acute cryptosporidiosis. Of interest also is that certain non-gastrointestinal sequelae
179 were previously found to be significantly associated only with *C. hominis* (Hunter et al., 2004). For

example, new onset eye pain was reported by 5/54 (9%) of our participants in either 6MQ or 12MQ in comparison with the previously reported nearly 10.9% of *C. hominis* and none of *C. parvum* cases with new or worsening eye pain (Hunter et al., 2004); new or worsening joint pain was found in 22% of cases in Hunter's et al. study and 33% in ours. However, interpretation of our findings requires caution. Case numbers are small; there was no available control population (unlike Hunter's et al. study), with cases simply acting as their own controls before and after infection; symptoms reported are common among the general adult population and recall bias may also have contributed.

Medical diagnoses may underestimate the incidence of post-acute health effects. For example, although 15 of 46 (33%) participants reported new onset of joint pains at 6MQ and/or 12MQ, only three (20%) consulted healthcare services resulting in one new diagnosis of arthritis. Hunter also found that only about a third of those reporting joint pains intended to report it to a medical practitioner, and concluded that therefore the symptoms may have been relatively mild (Hunter et al., 2004). Among our participants 20% disclosed a pre-existing diagnosis of IBS, considerably greater than the reported UK population level of 9-12% (Jones et al., 2000). This could suggest that those with pre-existing IBS may more readily present to health services, or be more likely to have participated in our research study; alternatively, we cannot discount that those with IBS may be pre-disposed to more symptomatic cryptosporidiosis.

Four individuals without pre-existing IBS reported new symptoms that met Rome III criteria at 6MQ and a further 2 at 12MQ (14% in total). This does not necessarily equate to a diagnosis of IBS because other underlying pathological causes of symptoms must be excluded (by history, examination or clinical investigations) before considering IBS, and two people reported alternative plausible causes for their symptoms. The Rome III criteria are validated for the diagnosis of IBS, not specifically for PI-IBS, and require certain symptoms to be present for 6 months or more. It could be argued that duration of symptoms for diagnosing PI-IBS should be amended to 'since resolution of

206 acute infection'. If this were applied in our study, 8/43 (19%) participants without pre-existing IBS
207 reported symptoms that met these modified criteria at 6MQ – there having hardly been a chance for
208 them to have experienced symptoms of sequelae for 6 whole months. At 12MQ, 28% reported
209 symptoms consistent with Rome III criteria for IBS if those with symptom duration shorter than 6
210 months were included - twice as many as the number reporting the same symptoms for 6 months
211 or more. Two patients were diagnosed as having IBS by a medical practitioner during the 12 month
212 period following infection. In summary, we found that within twelve months following acute *C.*
213 *parvum* infection, two adults without pre-existing irritable bowel syndrome received a new medical
214 diagnosis of IBS and up to 28% (12/43) self-reported new onset of IBS-consistent symptoms. PI-IBS
215 appears to have a better prognosis than IBS diagnosed in the absence of recent known
216 gastrointestinal infection (Jones et al., 2000), and usually resolves without medical intervention.
217 Patients may gain reassurance if made aware of this potential longer term health effect and likely
218 clinical course at the time of receiving their cryptosporidiosis diagnosis.

219 The results presented here lend support to the notion of post-infectious sequelae after
220 cryptosporidiosis. In addition this is the first time non-GI symptoms have been reported following *C.*
221 *parvum*, as opposed to *C. hominis* infection. Despite the small number of participants and
222 uncertainties discussed, the outcomes from this follow-up study add to available information on the
223 self-reported and medically diagnosed health effects occurring following resolution of acute
224 cryptosporidiosis. Our ongoing work will compare self-reported symptoms among patients with *C.*
225 *hominis*, *C. parvum* and without *Cryptosporidium* infection. Quantifying post-acute health sequelae
226 assists healthcare and environmental health professionals in providing more complete advice and
227 support to patients with cryptosporidiosis in a timely manner. In turn, this may reduce subsequent
228 use of healthcare services such as repeated GP consultations and laboratory testing. Since, in the EU,
229 there is no licensed treatment for cryptosporidiosis, identifying and increasing awareness of post-
230 acute health effects may assist policy decision-makers to understand the potential longer-term
231 burden of disease and prioritise interventions to prevent *Cryptosporidium* infection.

References

1. Agnew DG, Lima AA, Newman RD, Wuhib T, Moore RD, Guerrant RL, Sears CL. Cryptosporidiosis in northeastern Brazilian children: association with increased diarrhea morbidity. J Infect Dis. 1998;177:754–60.
2. Bartelt LA, Lima AAM, Kosek M, Peñataro Yori P, Lee G, Guerrant RL. ''Barriers'' to Child Development and Human Potential: The Case for Including the ''Neglected Enteric Protozoa'' (NEP) and Other Enteropathy- Associated Pathogens in the NTDs. PLoS Negl Trop Dis. 2013;7:e2125.doi:10.1371/journal.pntd.0002125
3. Bushen OY, Kohli A, Pinkerton RC, Dupnik K, Newman RD, Sears CL, Fayer R, Lima AAM, Guerrant RL. Heavy cryptosporidial infections in children in northeast Brazil: comparison of *Cryptosporidium hominis* and *Cryptosporidium parvum.* Trans R Soc Trop Med Hyg. 2007;101:378-84.
4. Colussi O, Rouen A, Seksik P, Cosnes J, Beaugerie L, Sokol H. Acute cryptosporidiosis as a cause of sudden recurrence of digestive symptoms in patients with_Crohn's disease. J Crohns Colitis. 2010;4:669-70
5. Cron RQ & Sherry DD. Reiter's syndrome associated with cryptosporidial gastroenteritis. J Rheumatol. 1995;22:1962-3.
6. Guerrant DI, Moore SR, Lima AA, Patrick PD, Schorling JB, Guerrant RL. Association of early childhood diarrhea and cryptosporidiosis with impaired physical fitness and cognitive function four-seven years later in a poor urban community in northeast Brazil. Am J Trop Med Hyg. 1999;61:707-13.
7. Hanevik K, Dizdar V, Langeland N, Hausken T. Development of functional gastrointestinal disorders after *Giardia lamblia* infection. BMC Gastroenterol. 2009;9:27doi: 10.1186/1471-230X-9-27.
8. Hay EM, Winfield J, McKendrick MW. Reactive arthritis associated with *Cryptosporidium* enteritis. Br Med J (Clin Res Ed) 1987;295(6592):248.
9. Hunter PR, Hughes S, Woodhouse S, Raj N, Syed Q, Chalmers RM et al. Health sequelae of human cryptosporidiosis in immunocompetent patients. Clin Infect Dis. 2004;39:504-10.
10. Insulander M, Silverlås C, Lebbad M, Karlsson L, Mattsson JG, Svenungsson B. Molecular epidemiology and clinical manifestations of human cryptosporidiosis in Sweden. Epidemiol Infect. 2013;141:1009-20. doi: 10.1017/S0950268812001665.
11. Jones J, Boorman J, Cann P, Forbes A, Gomborone J, Heaton K, et al. British Society of Gastroenterology guidelines for the management of the irritable bowel syndrome. Gut (Suppl II) 2000;47:ii1-ii19.
12. Khaldi S, Gargala G, Le Goff L, Parey S, Francois A, Fioramonti J et al. *Cryptosporidium parvum* isolate-dependent postinfectious jejunal hypersensitivity and mast cell accumulation in an immunocompetent rat model. Infect Immun 2009;77:5163-9.
13. Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet 2013;382: 209–22.
14. MacKenzie WR, Schell WL, Blair KA, Addiss DG, Peterson DE, Hoxie NJ et al. Massive outbreak of waterborne *Cryptosporidium* infection in Milwaukee, Wisconsin: recurrence of illness and risk of secondary transmission. Clin Infect Dis. 1995;21:57-62
15. Manthey MW, Ross AB, Soergel KH. Cryptosporidiosis and inflammatory bowel disease. Experience from the Milwaukee outbreak. Dig Dis Sci. 1997;42:1580-6.

16. Marion R, Baishanbo A, Gargala G, Francois A, Ducrotte P, Duclos C et al. Transient neonatal *Cryptosporidium parvum* infection triggers long-term jejunal hypersensitivity to distension in immunocompetent rats. Infect Immun. 2006;74:4387-9.
17. McKerr C, Adak GK, Nichols G, Gorton R, Chalmers RM, Kafatos G et al. An outbreak of *Cryptosporidium parvum* across England & Scotland associated with consumption of fresh pre-cut salad leaves, May 2012. PLoS One. 2015;10:e0125955
18. Ozgül A, Tanyüksel M, Yazicioglu K, Arpacioglu O. Sacroiliitis associated with *Cryptosporidium parvum* in an HLA-B27–negative patient. Rheumatology 1999;38:288–9.
19. Public Health England. Cryptosporidiosis, guidance, data and analysis. 2014.
20. Rehn M, Wallensten A, Widerstrom M, Lilja M, Grunewald M, Stenmark S et al. Post-infection symptoms following two large waterborne outbreaks of *Cryptosporidium hominis* in Northern Sweden, 2010-2011. BMC Public Health. 2015;15:529. doi: 10.1186/s12889-015-1871-6.
21. Rodriguez LA & Ruigomez A. Increased risk of irritable bowel syndrome after bacterial gastroenteritis. Br Med J. 1999;318:565-6.
22. Shepherd RC, Smail PJ, Sinha GP. Reactive arthritis complicating cryptosporidial infection. Arch Dis Child. 1989;64:743–4.
23. Vadlamudi N, Maclin J, Dimmitt RA, Thame KA. Cryptosporidial infection in children with inflammatory bowel disease. J Crohns Colitis. 2013;7:e337-43