

Exploring transmission pathways for cryptosporidiosis in England and Wales

Thesis submitted in accordance with the requirements of the University of Liverpool
for the degree of Doctor in Philosophy by Caoimhe McKerr (June 2020)

This thesis is based on research carried out in the Department of Epidemiology and
Population Health, Institute of Infection and Global Health, University of Liverpool.

Except for where indicated, this thesis is my own work.

Caoimhe McKerr

June 2020

Dedication

For Daire.

“It’s a long way to the top, if you wanna rock and roll”

Young, Young, & Scott
1975

Acknowledgements and thanks

As we all know, no research is undertaken in isolation, and the work presented here represents many hours of teamwork, support, and dedication from others. In fact, too many to mention. I have added specific project thanks at the end of my chapters, but I would like to shine a particular light on those without whom so much of this work could not have been started, pursued, and certainly not delivered.

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Glossary of main terms and abbreviations

Asymptomatic	Not displaying any (comparable) symptoms
Carrier	A person or animal that harbours a specific infectious agent in the absence of discernible clinical disease, and serves as a potential source of infection.
Causality	The relating of causes to the effects they produce.
Determinant	Any factor, whether event, characteristic, or other definable entity, that brings about change in a health condition or other defined characteristic.
Endemic	The constant presence of a disease or infectious agent within a given geographic area or population group.
Exposure	Contact with a source of a disease agent in such a manner that effective transmission of the agent or harmful effects of the agent may occur.
Household	One or more persons who occupy a dwelling, i.e. a place that provides shelter, cooking, washing, and sleeping facilities. This may or may not be a family. The term is also used to describe the dwelling unit in which the persons live.
Incubation Period	The time interval between invasion by an infectious agent and appearance of the first sign or symptom of the disease in question.
Infectiousness	A characteristic of a disease that concerns the relative ease with which it transmits to other hosts.
Infectivity Period	The time during which an infectious agent may be transferred directly or indirectly from an infected person to another person, from an infected animal to humans, or from an infected person to an animal.
Isolate	A population of microorganisms that has been isolated from a (in this case) host.
Mode of infection	How transmission of infection happens - by direct contact, droplet spread, or contaminated fomites.
Pathogenicity	The property of an organism that determines the extent to which overt disease is produced in an infected population, or the power of an organism to produce disease.
Pilot	A small-scale test of the methods and procedures to be used on a larger scale if the pilot study demonstrates that these methods and procedures can work.
Rate	A measure of the frequency of occurrence of a phenomenon. In epidemiology, demography, and vital statistics, a rate is an expression of the frequency with which an event occurs in a defined population in a specified period of time.
Reservoir	Places where the pathogen lives (this includes people, animals and insects, medical equipment, and environments such as soil and water).
Risk Factor	An attribute or exposure that is associated with an increased probability of a specified outcome, such as the occurrence of a disease. Not necessarily a causal factor. May be modifiable (intervention which decreases risk of disease).
Sample	A specimen taken for analysis or testing.

Secondary spread	The spread of disease to others from cases exposed through the original source of infection.
Specimen	A sample of tissue, blood, urine, or in this case, faeces, used for analysis and diagnosis.
Surveillance	The systematic collection of data for information and public health action.
Transmission of infection	Spread of an infectious agent from a source or reservoir to another person.
Transmission pathway	The underlying personal behaviours or characteristics which help the organism enter the body by supporting the mode of transmission. This can be person-to-person, foodborne, vector-borne, waterborne.
Zoonosis	A disease which can be transmitted to humans from animals.

Abbreviations	
%	Percent
AIDS	Acquired Immunodeficiency Syndrome
BSS	Bristol Stool Scale
CDC	Centres for Disease Control (US)
CI	Confidence interval
CRU	<i>Cryptosporidium</i> Reference Unit
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
GP	General practitioner
GRADE	Grading of Recommendations, Assessments, Development and Evaluation
HIV	Human Immunodeficiency Virus
HPU	Health Protection Unit
IBD	Inflammatory bowel disease
IBS	Irritable bowel syndrome
IMD	Index of multiple deprivation
LA	Local authority
NIHR	National Institute for Health Research
NOS	Newcastle-Ottawa Scale
OECD	Organisation for Economic Co-operation and Development
OR	Odds ratio
PCR	Polymerase chain reaction
PHE	Public Health England
PHW	Public Health Wales
PRISMA-P	Preferred Reporting Items for Systematic Reviews and Meta-Analyses for Protocols
RFLP	Restriction fragment length polymorphism
ROBINS-I	Risk Of Bias In Non-randomized Studies of Interventions
RR	Relative risk/Risk ratio
SD	Standard deviation
TX	Transmission
UK	United Kingdom
WHO	World Health Organization

Abstract

Caoimhe McKerr

Exploring transmission pathways for cryptosporidiosis in England and Wales

Globally, *Cryptosporidium* is thought to exact a considerable burden of disease with the highest prevalence observed among children under five years old, in particular the under twos. Annually, over 10,000 cases are detected and reported in Europe each year and the UK cases contribute around 40% of this.

The risk factors and exposures for *Cryptosporidium* infection are often identified from outbreak investigations. Several main transmission pathways are repeatedly examined, including water, animal contact, food contamination, and contact with other cases. Despite studies that have investigated differences in risk for sporadic and outbreak disease, exposures and transmission pathways for sporadic disease are still unclear. Thus, eliciting the pathways to infection is important to assess the burden of *Cryptosporidium*, and in considering strategies to reduce risk and mitigate spread in certain groups or settings.

In the work contributing to this thesis, I have taken three steps to further examining *Cryptosporidium* in England and Wales:

1. Using the latest data to describe the distribution of this infection in the UK, as well as undertaking an audit describing laboratory detection methods and approaches to testing that underpin this.
2. Conducting a literature review to describe the exposures examined and associated with sporadic disease.
3. Designing and implementing an analytical observational study that examines transmission of *Cryptosporidium* in the home environment.

I conclude that we are likely under ascertaining cases of sporadic illness, with local laboratories still testing short of the recommended amount, and stool consistency is a common selection criterion. The systematic review revealed that we might be under examining person-to-person spread as a pathway responsible for sporadic infection. I go on to suppose that secondary spread of infection from an initial case might a) represent a burden of sporadic disease and, b) have underlying associated exposures not yet well examined. In addition, these may differ by species of infection.

The analytical study demonstrates that additional cases of *Cryptosporidium* occur in over a quarter of homes with a case. This is likely to affect up to a third of the home and cause considerable burden of illness. This is especially common where the index is a young child, with mums and other siblings most at risk of secondary infection, and where homes have cases of *C. hominis*.

Feasible recommendations from these investigations include:

- To support *Cryptosporidium* testing in all stools submitted for the investigation of gastrointestinal pathogens regardless of consistency
- Systematic changes to surveillance to support the inclusion of speciation data for *Cryptosporidium* samples in England and Wales
- The consideration of specific clinical advice on prevention for high-risk homes; those with cases of *C. hominis* and/or children under five years old

The work supports recognition of this pathogen as burdensome and of considerable significance in immunocompetent individuals, and further work proposed includes designing methods that can identify true secondary transmission with increased accuracy.

Chapter 1

General introduction and thesis outline

1 **Infectious intestinal disease and an introduction to**

2 ***Cryptosporidium***

3

4 Infectious intestinal disease (IID) is an umbrella term often use to categorise
5 symptomatic disease, usually with symptoms of diarrhoea and/or vomiting, caused by
6 infection with microorganisms (World Health Organization, 2019). Agents of IID can
7 include viruses, bacteria, and protozoa. Globally it represents a common cause of
8 morbidity, and the impact on patients and services can be considerable, sometimes
9 with significant economic burden in the less industrialised countries (Gauci *et al.*,
10 2007). The distribution of IID varies worldwide, and it is likely that there are wider
11 determinants at play influencing this: variations in exposure might be due to sanitation,
12 availability of clean drinking water, infrastructure, housing, and health factors such as
13 acquired immunity and nutrition (Putignani and Menichella, 2010; Fletcher, McLaws
14 and Ellis, 2013).

15 Human cryptosporidiosis usually presents as gastrointestinal disease, transmitted via
16 the faecal-oral route, although there is some evidence for respiratory cryptosporidiosis
17 in specific populations (Sponseller, Griffiths and Tzipori, 2014; Morris *et al.*, 2019).
18 This thesis examines research likely only to relate to the faecal-oral transmission
19 pathway and gastrointestinal disease.

20 Globally, *Cryptosporidium* is thought to exact a considerable burden of disease
21 (Putignani and Menichella, 2010; Kotloff *et al.*, 2013): infection is reported in 1-3
22 percent of immunocompetent patients with diarrhoea in industrialised countries and
23 7-20 percent in developing countries (Leder and Weller, no date; Casemore, 1990;
24 Current and Garcia, 1991; Jelinek *et al.*, 1997; Mulyil *et al.*, 2010). The highest
25 prevalence is observed among children under five years old, in particular the under
26 twos (Ajampur *et al.*, 2010; Kotloff *et al.*, 2013). Like general IID, the distribution of
27 *Cryptosporidium* varies geographically and it is a parasite of some socio-economic
28 importance (Putignani and Menichella, 2010), joining the World Health Organization's
29 list of neglected tropical diseases in 2004 (Savioli, Smith and Thompson, 2006). A
30 plethora of literature exists describing acute disease and longer-term sequelae
31 including growth faltering and cognitive defects in developing countries. In
32 industrialised countries significant impact has also been described including
33 persistent gastrointestinal upset, irritable bowel syndrome (IBS), and a possible
34 association between infection and colon cancer in cases as well as highlighting the
35 considerable economic impact of outbreaks (Innes *et al.*, 2020). Most of the

36 knowledge round transmission and exposures for *Cryptosporidium* is generated from
37 outbreak investigations, and this is particularly true of the more industrialised
38 countries, and in the UK. Therefore, the background work presented in this thesis
39 considers infection mostly from industrialised countries, in order to better support the
40 investigations and results described from an England and Wales-based study and to
41 propose public health recommendations from a UK perspective.

42 Annually, over 10,000 cases are detected and reported in Europe each year; UK
43 cases contribute around 40% of this (n=~4,000), which is partly due to ascertainment
44 and reporting differences (European Centre for Disease Control, 2017). Testing
45 practices are changing and offering increased resolution, but these can differ locally
46 and country-wide (Cacciò and Chalmers, 2016). In order to understand laboratory
47 approaches, selection criteria, and capture of cases by our surveillance systems, I
48 have undertaken an audit of a subset of local laboratories, which outlines testing
49 practices in England and Wales.

50 The risk factors and associated exposures for *Cryptosporidium* are often identified
51 from outbreak investigations but we cannot be certain that transmission routes for
52 sporadic disease are the same as those which drive outbreaks (Bouزيد *et al.*, 2013).
53 Several main transmission pathways are repeatedly examined and reported including
54 water exposures, animal contact, food contamination, and contact with other cases.
55 Despite case control studies which have investigated differences in risk for endemic
56 and outbreak disease (Hunter and Thompson, 2005; Yoder, Harral and Beach, 2010),
57 exposures and transmission pathways for sporadic disease are still unclear. Thus,
58 eliciting the pathways to infection is important to assess the burden of
59 *Cryptosporidium* and in considering strategies to reduce risk and mitigate spread in
60 certain groups or settings. I have undertaken a systematic review in order to examine
61 relevant literature on sporadic disease and understand which exposures are
62 examined and contribute to disease burden.

63 The highest burden of cryptosporidiosis is observed in our most vulnerable
64 populations, but sporadic disease in immunocompetent people is probably more
65 common than we recognise (Food Standards Agency, 2000a; Adak, Long and
66 O'Brien, 2002; Briggs *et al.*, 2014; R. M. Chalmers *et al.*, 2016). Symptoms can range
67 from severe to negligible, and asymptomatic infections have been reported (Davies
68 *et al.*, 2009; Johansen *et al.*, 2014). Outbreaks are commonplace with this type of
69 illness, probably given the range of transmission pathways and the infectivity potential
70 of the responsible pathogens. However, it is well recognised that despite sensitive

71 case definitions to increase capture, official reports of IID fall way below the true
72 incidence (Doorduyn, Van Pelt and Havelaar, 2012; Tam *et al.*, 2012). Many
73 outbreaks, particularly those within families/homes go unrecognised (Bloomfield,
74 2001; Day, 2001), and sporadic cases may remain uncaptured (Tam *et al.*, 2012). I
75 have undertaken an analytical study to explore transmission in the home environment.
76 This also seeks to describe burden and longevity of additional illness in the home.

77 **Next steps and objectives of the work**

78 In the work contributing to this thesis, I have taken three steps to further examining
79 *Cryptosporidium* in England and Wales:

80 Objective 1

81 To describe the distribution and burden of this infection in the UK, as well as
82 considering detection and approaches to testing that underpin the surveillance
83 data. I will approach this by presenting the most recent surveillance data and
84 undertaking a laboratory practices audit.

85 Objective 2

86 To examine and present exposures most associated with sporadic disease,
87 and calculate how much these contribute to infection. I have conducted a
88 literature review to describe the reported exposures examined and associated
89 with sporadic disease in industrialised countries, and highlight any pathways
90 to infection that should be further considered.

91 Objective 3

92 To explore transmission in the home environment, and calculate the burden
93 this might have on people in the home, considering longevity and severity of
94 illness. I carried out an analytical study in homes where there were known
95 cases of *Cryptosporidium*. I calculated the burden of disease and examined
96 those most at risk and the factors that might contribute to spread. I also
97 attempted to describe longevity and severity of illness, and detect any
98 asymptomatic infections.

99 **A note on terminology**

100 On reviewing papers in the course of this work, it became clear that terminology
101 relating to means of transmission were not always standard, or indeed even intuitive.
102 As I began to embark on the systematic review element of the thesis, it became more
103 important to use clear, and standard, definitions, regardless of the terms used by

104 authors. For this reason, I use set definitions throughout the thesis when talking about
105 exposures and the pathways under which infection occurs.

106 **Table 1** below shows how I use these terms in the thesis and these are designed to
107 be specifically relevant to *Cryptosporidium* infection. Other relevant terms are defined
108 in the Glossary.

109 **Table 1: Definitions used in the thesis to describe elements of exposure and transmission**

Transmission route	Faecal-oral			
Mode of entry	Ingestion			
Transmission mode	Direct		Indirect	
Transmission pathway	Person-person contact	Animal contact	Water contamination	Food contamination
Some examples of exposures	Nappy changing <i>(Changing nappies, helping with potty training)</i>	Pet contact <i>(Petting, feeding, cleaning domestic pets)</i>	Recreational water <i>(Consuming or swallowing water during activities, taking part in water sports, both outdoor (e.g. lakes) and indoor (e.g. treated swimming pools))</i>	Fresh produce <i>(Salad and fruit contaminated at picking or processing, from contaminated water or from handlers, or fruit juices made with contaminated water)</i>
	Childcare activities <i>(Domestic care for a child, working in a school or</i>	Attending petting farms <i>(Feeding animals e.g. bottle-feeding lambs, eating on farm site, poor hand</i>	Drinking water <i>(Consuming drinking water, either treated (i.e. from a municipal tap) or untreated</i>	Meat <i>(Meat from infected animals, contaminated by</i>

	<i>nursery, feeding, assisting with toileting, bathing)</i>	<i>hygiene, handling animals directly)</i>	<i>(e.g. private water supply, drinking from a stream)</i>	<i>staff or handlers, or from water)</i>
	<i>Case contact (Lack of personal hygiene following contact with a case, caring for a case, general social contact, sexual contact)</i>	<i>Wild animal contact (Petting, feeding, touching animals in the wild)</i>	<i>Droplets and spray (Inhalation from contamination or event, e.g. cleaning up an animal faeces spillage)</i>	<i>Poor kitchen hygiene (Food that gets contaminated, especially in restaurant, linked to surface contamination from produce or staff because of poor hygiene)</i>
	<i>Sexual contact (Sexual contact with another person, or a case)</i>	<i>Living on a farm (Direct contact with the animals – petting, feeding, caring, or indirect, such as walking around the farm, eating on the farm)</i>	<i>Water used in food production and processing (Contaminated water runoff from vegetation where land is manured)</i>	<i>Milk and dairy (Contaminated produce from infected cattle, or contaminated at processing)</i>

111 *Thesis approach and outline*

112 **In Chapter 2**, I outline general information about *Cryptosporidium* including the
113 clinical picture, treatment, management, and detection.

114 I then go on in **Chapter 3** to describe the epidemiology and burden of disease, using
115 the most recently available England and Wales *Cryptosporidium* surveillance data. I
116 outline the main transmission pathways hypothesised from the existing evidence. I
117 use this chapter to set the scene for the work.

118 **In Chapter 4**, I describe the surveillance capture of *Cryptosporidium* in the UK, and I
119 incorporate into this chapter results from a laboratory audit, which explores testing
120 practices used in England and Wales.

121 **In Chapter 5**, I outline the methods and results of a systematic literature review
122 describing exposures for sporadic *Cryptosporidium* reported in the literature. I
123 combine results to report on the main transmission routes for infection, various
124 genotypes reported, and any differences between them. I conclude by considering
125 person-to-person spread as a pathway responsible for sporadic infection, and
126 suppose that secondary spread of infection from an initial case might a) represent a
127 burden of sporadic disease and, b) have underlying associated risks and exposures
128 not yet well examined. I use this chapter to inform the design and approach of the
129 subsequent analytical study.

130 **In Chapter 6**, I present the methods and results of the epiCrypt Study - an
131 investigation of transmission of *Cryptosporidium* in the home environment. This work
132 tries to estimate the amount of spread of infection that happens in the home
133 environment, describe any factors associated with spread in the homes in which this
134 happens, and detect any asymptomatic infections. I present species-specific data
135 from participants recruited into the study and examine the molecular characterisation
136 of the cases. I make recommendations for further work and practice, which include
137 considering specific clinical advice on prevention of spread in homes identified as
138 high-risk and propose further work to identify true secondary transmission more
139 accurately.

140 **In Chapter 7**, I conclude the work with a short summary of discussions from each
141 chapter, considering my thesis overall. I reiterate my recommendations for further
142 work and include my own reflections on the overall programme of research.

Chapter 2

Background to *Cryptosporidium*, infection, and human disease

144 **Chapter 2: *Cryptosporidium* background**

145 **History**

146 *Cryptosporidium* has a somewhat short history compared to many other pathogens.
147 The first real recorded observation of infection, and its association with disease, was
148 in 1907, by Edward Tyzzer, who characterised the life-cycle of the parasite in mice
149 (Tyzzer, 1907; Tzipori and Widmer, 2008). There were a handful of publications after
150 that, but it wasn't until the 1950s and later that the parasite began to be described
151 more widely, albeit associated with infection in animals (Current and Garcia, 1991;
152 Chalmers, 2013) such as turkeys (Slavin, 1955) and cattle (Panciera, Thomassen and
153 Garner, 1971; Tzipori, 1983). In 1976 the first human cases were documented, both
154 describing patients with watery diarrhoea and severe disease. One of these reports
155 described infection and severe disease in a three year old child (Nime *et al.*, 1976)
156 and the other in an immunosuppressed adult male (Meisel *et al.*, 1976): the sources
157 of infection were never determined but both had contact with farm animals. A decade
158 later, still fewer than 60 human cases had been published, reported in people with
159 immune deficiencies, although these reports did at least demonstrate that this was a
160 pathogen capable of causing enteric disease in humans. By 1980, it was still widely
161 held that "cryptosporidiosis does not appear to cause a problem and tentatively it can
162 be regarded, therefore, as an opportunistic pathogenic parasite" (Bird and Smith,
163 1980).

164 In the early 1980s the advent of a growing AIDS epidemic highlighted *Cryptosporidium*
165 as a very serious pathogen of immunocompromised patients (Tzipori and Widmer,
166 2008) and it was quickly labelled as an AIDS defining diagnosis (directly associated
167 with advanced HIV infection (Centers for Disease Control, 2008)) (Hunter and
168 Nichols, 2002). Survival in this patient group was significantly impacted by infection
169 with *Cryptosporidium*, with a large longitudinal study in San Francisco reporting a
170 relative hazard of death two times that of other AIDS-defining diagnoses (Colford *et*
171 *al.*, 1996). This association with AIDS was a significant milestone in our understanding
172 of this infection. Disease began to be better described, and for the first time it became
173 more widely recognised as a pathogen of clinical importance: not only chronic and
174 often life-threatening in vulnerable patients, but also more generally wide-spread than
175 first thought (Navin and Juranek, 1984).

176 Additionally at this time, studies began to emerge that suggested that
177 *Cryptosporidium* was not as host-specific as once thought, and that indeed infection
178 could cross species, which had important implications for our understanding of the

179 pathways to human infection (Tzipori *et al.*, 1980). An investigation in 1981 implicated
180 cryptosporidiosis in 4%–7% of sporadic cases of acute gastroenteritis in humans
181 (Tzipori *et al.*, 1983). This was one of the first studies to recognise that there might
182 also be a burden of infection in immunocompetent populations, and to demonstrate
183 that children were at greater risk. Additionally, this work began to describe the
184 symptom profile in immunocompetent humans and was one of the first papers to
185 suggest that that person-to-person transmission might occur. Supplementary in-depth
186 descriptive epidemiology published in 1985 synthesised literature that supported
187 these findings, adding that continued infectivity of cases made contact hazardous to
188 other people with particular risk among immune-compromised populations
189 (Casemore, Sands and Curry, 1985). Moreover, diagnosis in humans thus far had
190 depended upon histological examination, but the advent of this increasing interest in
191 human disease brought with it collaboration between veterinary and human health
192 professionals. This initiated the application of veterinary techniques to determine and
193 identify infection in humans. Staining techniques such as modified Ziehl-Neelsen were
194 fast becoming the method of choice at this time, allowing for quicker and better
195 identification and capture of cases (Henriksen and Pohlenz, 1981; Garcia *et al.*, 1983).

196 By the 1990s it was established that whilst still a clinical and devastating illness among
197 the HIV population, this infection carried a considerable burden of illness in the
198 general population. It was in the late 80s and early 90s that we began to see large
199 outbreaks documented in the industrialised countries, such as Australia, USA, Europe
200 and the UK, with those from drinking water sources receiving the most attention (Mac
201 Kenzie *et al.*, 1995; Cicirello *et al.*, 1997; Percival, Walker and Hunter, 2000). These
202 events led to massive public health responses (I. R. Lake *et al.*, 2007) for
203 *Cryptosporidium* generally, including changes in water regulations, and increased
204 efforts in surveillance and detection (Nichols *et al.*, 2006). Additionally during this time,
205 several seminal papers were published which emphasised the diversity of
206 transmission pathways for this infection, including changes to taxonomy of
207 *Cryptosporidium* species and knowledge of host specificity (McLauchlin *et al.*, 1999;
208 Goh *et al.*, 2004, 2005; Hunter, Hughes, Woodhouse, Syed, *et al.*, 2004; Chalmers *et al.*,
209 *et al.*, 2009). Most notable among these works was the recognition of the *C. parvum*
210 genotypes 1 and 2 as *C. hominis* and *C. parvum*, respectively, with different
211 epidemiology, and *C. hominis* largely restricted to human hosts (Chalmers, 2013).

212 Recognising the changes in burden and epidemiology of this pathogen, from 2000
213 onwards many European countries introduced mandatory reporting and some
214 surveillance for *Cryptosporidium* (European Centre for Disease Control, 2017). In the

215 UK *Cryptosporidium* was made notifiable in 2010 (Public Health England, 2019). This
216 decade brought with it developments in our knowledge of species-specific pathways
217 to infection and recognition of the separate epidemiology of *Cryptosporidium* species
218 and genotypes, very notably in the UK (McLauchlin *et al.*, 2000; Nichols and
219 McLauchlin, 2003; Hunter, Hughes, Woodhouse, Syed, *et al.*, 2004; Insulander *et al.*,
220 2013). Advances in laboratory techniques for oocyst detection began to enhance
221 ascertainment and detection of this pathogen. Furthermore, moves to molecular
222 methods for pathogen characterisation has allowed greater resolution of isolates (R
223 M Chalmers, Elwin, *et al.*, 2002).

224 **Biology and life cycle**

225 *Cryptosporidium* is a protozoal parasite: a single-celled microscopic organism that
226 can live and multiply within animal cells, including humans. *Cryptosporidium* can infect
227 the intestinal tract of all mammals, along with several species of birds, fish, reptiles,
228 and amphibians, displaying some host-species specificity (Yaeger, 1996; Bouzid *et al.*,
229 *et al.*, 2013; Thompson, Koh and Clode, 2016).

230 Protozoa that are infectious to humans are classified into four groups, one of which is
231 'Sporozoa' – groups of protozoa, including *Cryptosporidium*, in which the life cycle
232 includes a spore-forming stage (CDC, 2014). This oocyst-forming quality renders
233 cryptosporidia particularly hardy, facilitating persistence in both hosts and the
234 environment which has implications for transmission (Thompson, Koh and Clode,
235 2016). The oocysts become infective during a process known as 'sporulation' and,
236 quite uniquely, *Cryptosporidium* oocysts are sporulated *in vivo*, and thus are infective,
237 by the time they are shed in faeces (Ryan and Caccio, 2010). These features have
238 implications for understanding the endurance, transmissibility, and management of
239 these organisms.

240 *The cycle of infection*

241 The cycle of infection begins with the ingestion (usually) of oocysts. These will already
242 be infective and the transmission route is most likely to be faecal-oral (Cacciò *et al.*,
243 2005). Figure 1

244 Following ingestion, inside the gastrointestinal tract of the host, the parasite emerges
245 from the oocyst (Box 1) and undergoes a complex life cycle, distinctively characterised
246 by both sexual and asexual stages. Because of these reproductive stages, oocysts
247 develop and subsequently sporulate inside the infected host. Two different types of
248 oocysts are produced: thick-walled oocysts - excreted in the faeces of the infected

249 host, infective and ready to be transmitted to another susceptible host, and thin-walled
250 oocysts, which remain inside the host, prompting another cycle of infection (known as
251 autoinfection).



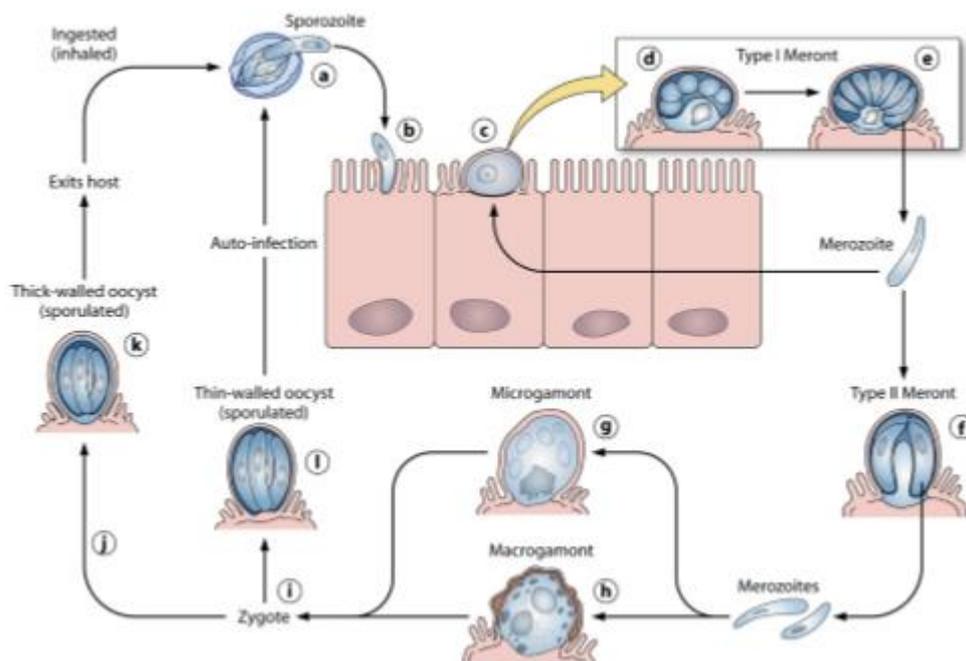
252 Box 1: *Cryptosporidium* parasites emerging from an infective oocyst

253 Image courtesy of Public Health Image Library, www.cdc.gov

254 The process from ingestion of oocysts to symptomatic disease can take anywhere
255 from 2 to 26 days (Jokipii and Jokipii, 1986; DuPont *et al.*, 1995; Hawker *et al.*, 2020)
256 and our understanding of the pathogenesis of this organism is still lacking. The
257 presentation of disease following *Cryptosporidium* infection can vary from
258 asymptomatic (but with shedding of oocysts) (DuPont *et al.*, 1995; Chappell *et al.*,
259 2016) to very severe illness, and sometimes even be life-threatening (Bouzid *et al.*,
260 2013). An infected human might shed as many as 10^9 oocysts in one bowel movement
261 (DuPont *et al.*, 1995) and some animals even more than this (Nydam *et al.*, 2001).
262 Only a small number of these (10-30 oocysts) would be needed to cause infection in
263 another person (Okhuysen and White Jr, 1999).

264 Once oocysts are released into the environment via the host faeces, they are able to
265 be immediately transmitted to another host and cause infection via a faecal-oral route.
266 This might occur in a direct mode such as close contact with an infected person or
267 animal. Alternatively, oocysts may contaminate the immediate environment, be
268 transferred, and ultimately reach another host via an indirect mode such as food or
269 water contamination. Mechanical transmission may also occur (e.g. on flies) although
270 evidence is still varied, but this is not addressed in this thesis (Graczyk *et al.*, 2000;
271 Nichols, 2005).

272 Figure 1 shows the main elements of the life cycle of *Cryptosporidium*, followed by a
 273 brief narrative accompaniment.



274 Figure 1: An overview of the main points along the life cycle of *Cryptosporidium*

275 Amended from <https://www.cdc.gov/parasites/crypto/pathogen.html> (Copyright: Centers for Disease
 276 Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID),
 277 Division of Foodborne, Waterborne, and Environmental Diseases (DFWED)) and (Bouzid *et al.*, 2013)

278 **Brief description to accompany figure** (Nichols, 1992; Nydam *et al.*, 2001; P.
 279 Hunter and Thompson, 2005; Chalmers and Davies, 2010; Bouzid *et al.*, 2013; CDC,
 280 2014; Checkley *et al.*, 2015; Thomson *et al.*, 2017)

281 Once a host has ingested oocysts, these oocysts reside in the lumen of the intestine.
 282 A process of excystation occurs, triggered by low pH and body temperature in the
 283 host, and sporozoites are released (a). These sporozoites are the infective agents.

284 The sporozoites attach to and penetrate the epithelial cells (b) of the gastrointestinal
 285 tract and develop into trophozoites (c). Trophozoites absorb nutrients from the host's
 286 body.

287 In the epithelial cells, the parasites undergo asexual multiplication (d, e, f), forming
 288 merozoites.

289 These merozoites differentiate and initiate a phase of sexual multiplication producing
290 the male microgamonts (g) and the female macrogamonts (female).

291 (i) Macrogamonts are fertilised by the microgametes. This creates zygotes.

292 (j) This zygote can develop into an oocyst. Two different types of oocysts are
293 produced (k & l).

294 Thin-walled oocysts (k), primarily involved in autoinfection, develop and sporulate in
295 the infected host, taking the cycle back to the beginning (a).

296 Thick-walled oocysts (l) develop and are excreted from the host in (usually) faeces.
297 Oocysts are sporulated (infective) upon excretion.

298 **A note on genetic recombination**

299 It is increasingly recognised that our epidemiological understanding of this parasite
300 could be clouded by genetic recombination that happens within hosts (Morris *et al.*,
301 2019). Recent work has demonstrated host infections with a genetically mixed
302 population of oocysts, both intra- and inter-species (Grinberg and Widmer, 2016).
303 Potentially, the sexual stage of the life cycle could result in an increased genetic
304 variation in offspring: different fertilization scenarios could occur, where either the
305 same genotypes are replicated, resulting in clonal, identical cells, or a random
306 panmixia occurs, resulting in a range of genetic heterogeneity (Alizon, de Roode and
307 Michalakis, 2013). It is still unclear what impact these mixed populations may have
308 on transmission, or how this might colour our understanding of the pathways to
309 infection, given that dominant populations are those most likely detected. This thesis
310 is not focused on detailed molecular characterisation, but nonetheless it is important
311 to note this phenomenon when making conclusions about transmission routes by
312 species, or at any greater resolution.

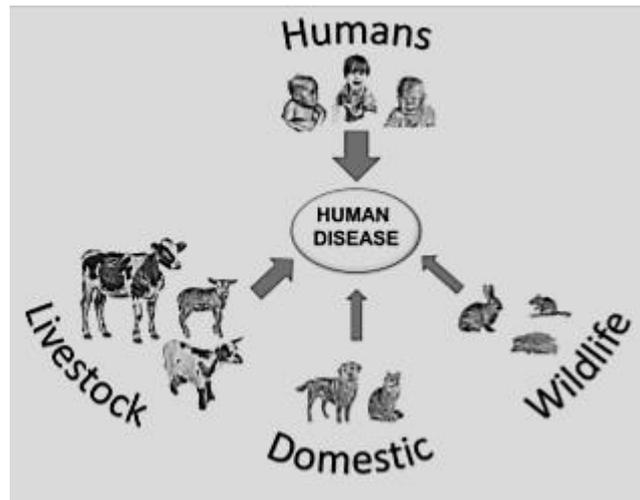
313 *Conclusions on the life cycle*

314 When we consider the complex life cycle of this parasite, and the numbers of oocysts
315 generated, it is easy to see how oocysts become so ubiquitous, and how they
316 effectively make their way into almost any environment. The hardy oocysts can persist
317 in even harsh environments, including a wide range of temperatures, flooding, and
318 disinfection, their thick wall protecting them (Robertson, Campbell and Smith, 1992;
319 Leitch and He, 2011).

320 The main sources of human infection are wild and farm animals, possibly domestic
321 pets, although to a lesser extent, and other humans (Figure 2). These are different for
322 the two main species: sources for *C. parvum* include animals and humans, and human
323 hosts for *C. hominis*. Infection from oocysts derived from these sources may happen
324 on any of the transmission pathways already mentioned: person-to-person contact,
325 animal contact, water contamination, food contamination. Outbreaks via person-to-
326 person (*C. hominis* and *C. parvum*) or animal-to-person (*C. parvum*) contact are
327 common, as well as more indirect transmission routes through ingestion of water and
328 food or contact with objects contaminated with infectious oocysts (Cacciò *et al.*, 2005).
329 Pathways and risks for infection are explored in more detail later ([Transmission](#)
330 [pathways and the underlying exposures for infection](#)).

331 In conclusion, several characteristics of *Cryptosporidium* render it effective, and
332 particularly suited to a faecal-oral transmission route:

- 333 • *Cryptosporidia* can infect the intestinal tract of a wide range of animals, making
334 for a large reservoir, and exposure can come from many sources
- 335 • The production of oocysts makes it a hardy pathogen, facilitating persistence
336 in both hosts and the environment, challenging disinfection processes, and
337 increasing disease and exposure
- 338 • Excreted oocysts are immediately infective, as soon as they are shed, making
339 direct contact with faeces of infected animals or humans very risky for infection
- 340 • The organism has both sexual and asexual stages and can re-infect their host,
341 leading to persistent infection and prolonged shedding, increasing the length
342 of time a host can transmit the infection
- 343 • A long incubation period between infection and disease might inadvertently
344 support ongoing transmission
- 345 • Oocysts are shed in large numbers but only a few are needed to cause
346 infection in a new susceptible host



347 Figure 2: Main reservoirs of infection that can affect humans, for *C. parvum* and *C.*
 348 *hominis*.

Adapted from (Morris *et al.*, 2019) with kind permission: Guy Robinson, *Cryptosporidium* Reference Unit, Swansea

349 The thickness of the arrow represents the likely contribution of that exposure to
 350 human disease.

351 ***Cryptosporidium* infection and human disease**

352 *Species that infect humans*

353 At the time of writing this, 39 species of *Cryptosporidium* are recognised, with 24 of
 354 these acknowledged as infecting humans, although with varying frequency (Morris *et*
 355 *al.*, 2019).

356 Table 2 documents the species, demonstrating the major hosts and their detection in
357 humans.

358 Although *C. parvum* and *C. hominis* are the most prevalent species causing disease
359 in humans (McLauchlin *et al.*, 2000; Xiao, 2010; Morris *et al.*, 2019), infections by *C.*
360 *felis* (Caccio *et al.*, 2002; Beser *et al.*, 2015), *C. meleagridis* (Gatei *et al.*, 2002), *C.*
361 *canis* (Fayer *et al.*, 2001; Xiao *et al.*, 2007), and *C. muris* (Palmer *et al.*, 2003) have
362 also been reported. There is evidence that species infection patterns vary globally,
363 which could be linked to differences in exposures. North and South America,
364 Australia, and Africa tend to record more *C. hominis*, and *C. parvum* is more often
365 reported in European countries, particularly the UK (Cacciò *et al.*, 2005; Putignani and
366 Menichella, 2010). An analysis of UK cases elicited differences between infecting
367 species and the distribution of disease (Chalmers *et al.*, 2009). *C. parvum* is
368 commoner among younger cases, and *C. hominis* is observed in infants, and females
369 between 15-44 years old.

370 Differences in transmission pathways between species

371 Increasingly, research has demonstrated heterogeneity in the transmission pathways
372 between species of *Cryptosporidium* (Hunter, Hughes, Woodhouse, Syed, *et al.*,
373 2004; Nic Lochlainn *et al.*, 2019). *C. hominis* is most often associated with human
374 transmission pathways while *C. parvum* mostly zoonotic (Tzipori and Widmer, 2008).
375 Despite this continual and increasing evidence for difference in species' epidemiology
376 (Xiao, 2010; Ryan, Fayer and Xiao, 2014) major shortcomings still exist in the
377 reporting of this in the published literature (Introduction). Most basic laboratory
378 methods for the detection of *Cryptosporidium* in stool samples will only identify to the
379 genus level, and where it is employed as a specialist service, this is mainly in
380 industrialised countries, with varied frameworks. In the UK, positive isolates from local
381 laboratories are voluntarily submitted to the national *Cryptosporidium* Reference Unit
382 (CRU) for speciation (Chalmers *et al.*, 2019).

383 Species sub-types

384 Further sub-typing of isolates for greater discrimination is usually only undertaken in
385 specific research, and practices are variable across Europe. The 60-kDa glycoprotein
386 gene (gp60) is the most commonly used marker for subtyping of *Cryptosporidium*,
387 mainly *C. parvum* and *C. hominis*, and is a useful tool to explore sources of infection
388 and host diversity (Rojas-Lopez *et al.*, 2020). In the UK, gp60 sub-typing has
389 sometimes been undertaken to supplement epidemiological and outbreak data,
390 allowing a better understanding of exposures with greater resolution in case definition

391 and identifying changes in the circulation of predominant subtypes (McKerr *et al.*,
392 2015; Chalmers and Caccio, 2016).

393 Throughout Europe the prevalent *C. hominis* alleles are IbA10G2 and IbA12G3
394 (Cacciò, 2005; Fournet *et al.*, 2013). This is reflected in the UK using outbreak data
395 (Chapter 3) and these types have most often been associated with water transmission
396 pathways (Chalmers *et al.*, 2019). *C. parvum* demonstrates greater diversity (Cacciò
397 and Chalmers, 2016) and the predominant circulating types are IIaA15G2R1 and
398 IIaA17G1R1, also most prevalent in England and Wales (outbreak data) and mostly
399 associated with animal contact (Chalmers *et al.*, 2019; Morris *et al.*, 2019). Within
400 country variation is observed and it is thought that, rather than being species-specific,
401 variability is related to local exposures and host factors (Chalmers and Caccio, 2016).

402 Further discrimination can be had using multi-locus genotyping (MLG) techniques
403 which have typically only been employed in special studies so far (Morris *et al.*, 2019).
404 Using several loci can discriminate specific sub-populations within a gp60 sub-type
405 and has previously been used to demonstrate concurrent outbreaks in Sweden
406 (Mattsson *et al.*, 2008) and in England (Hunter *et al.*, 2008). As of yet no standard
407 exists, making comparisons difficult, and the need to account for genetic
408 recombination enhances the requirement for a standard sub-typing and multi-locus
409 protocol (Feng *et al.*, 2014).

410 Table 2: *Cryptosporidium* species, their major hosts, and reported human infections

411 (adapted with kind permission from: Morris, Robinson, Swain and Chalmers (Morris *et al.*, 2019)

412 Copyright © 2019)

Cryptosporidium species	Major host(s)	Infections reported in humans
<i>C. alticolis</i>	Voles	No
<i>C. apodemi</i>	Mice	No
<i>C. andersoni</i>	Cattle	Yes (rarely)
<i>C. avium</i>	Birds	No
<i>C. baileyi</i>	Birds	No
<i>C. bovis</i>	Cattle	Yes (rarely)
<i>C. canis</i>	Canids	Yes (occasionally)
<i>C. cuniculus</i>	Lagomorphs, Humans	Yes (occasionally)
<i>C. ditrichi</i>	Mice	Yes (rarely)
<i>C. ducismarci</i>	Tortoises	No
<i>C. erinacei</i>	Hedgehogs	Yes (rarely)
<i>C. fayeri</i>	Marsupials	Yes (rarely)
<i>C. felis</i>	Felids	Yes (occasionally)
<i>C. fragile</i>	Toads	No
<i>C. galli</i>	Birds	No
<i>C. homai</i>	Guinea Pigs	No
<i>C. hominis</i>	Humans	Yes (commonly)
<i>C. huwi</i>	Fish	No
<i>C. macropodum</i>	Marsupials	No
<i>C. meleagridis</i>	Birds, Mammals	Yes (occasionally)
<i>C. microti</i>	Voles	No
<i>C. molnari</i>	Fish	No
<i>C. muris</i>	Rodents	Yes (rarely)
<i>C. occultus</i>	Rodents	Yes (rarely)
<i>C. parvum</i>	Mammals	Yes (commonly)
<i>C. proliferans</i>	Rodents, maybe Equids	No
<i>C. proventriculi</i>	Birds	No
<i>C. rubeyi</i>	Squirrels	No
<i>C. ryanae</i>	Cattle	No
<i>C. scrofarum</i>	Pigs	Yes (rarely)
<i>C. serpentis</i>	Reptiles	No
<i>C. suis</i>	Pigs	Yes (rarely)
<i>C. testudinis</i>	Tortoises	No
<i>C. tyzzeri</i>	Rodents	Yes (rarely)
<i>C. ubiquitum</i>	Mammals	Yes (occasionally)
<i>C. varanii</i>	Reptiles	No
<i>C. viatorum</i>	Humans, Rodents	Yes (occasionally)
<i>C. wrairi</i>	Guinea Pigs	No
<i>C. xiaoi</i>	Sheep, Goats	No

413 **Clinical manifestation**

414 The subsequent disease following infection with *Cryptosporidium* is cryptosporidiosis.
415 Symptoms of cryptosporidiosis are fairly general to IID, and include non-bloody and
416 watery diarrhoea, abdominal cramps, vomiting and/or nausea, low grade fever,
417 lethargy and general malaise (Adler *et al.*, 2017). Diarrhoea can persist longer than
418 that seen in most other IID (Robertson *et al.*, 2002b; Shirley, Moonah and Kotloff,
419 2012). This seemingly generic initial presentation can make identification of
420 cryptosporidiosis difficult, and a confirmed laboratory test is required for diagnosis (R.
421 Chalmers and Giles, 2010; Chalmers and Katzer, 2013). There is some evidence that
422 prior infection with *Cryptosporidium* confers some protective immunity to subsequent
423 infection or disease, particularly in reducing symptoms (Quilliam *et al.*, 2013).

424 Several studies have suggested differences not just in the presentation of
425 cryptosporidiosis versus other gastrointestinal illness, but also between symptoms
426 following infection with different *Cryptosporidium* species or genotypes. The clinical
427 presentation of *C. hominis* cases specifically may include more non-gastrointestinal
428 symptoms such as joint pain, lethargy, and headaches and eye pain (Kortbeek, 2009;
429 Carter *et al.*, 2019), and overall may be associated with more severe disease (Bushen
430 *et al.*, 2007). A large study of sporadic disease in the UK reported a mean duration
431 of symptoms of 13.5 days for patients with *C. hominis* and 11.3 days for *C. parvum*.
432 Additionally, both *C. hominis* and *C. parvum* infection can cause some significant and
433 often long-term health effects including joint pain, weight loss, and symptoms
434 consistent with irritable bowel syndrome (IBS) (Hunter, Hughes, Woodhouse, Raj, *et*
435 *al.*, 2004; Stiff *et al.*, 2017). In immunocompetent patients, persistent diarrhoea has
436 been reported up to three months post-infection, and a follow-up study of
437 *Cryptosporidium* cases after an outbreak reported this up to three years after original
438 disease (Insulander *et al.*, 2013).

439 *C. hominis* cases in particular report post-infection joint pain, headache and fatigue
440 (Hunter, Hughes, Woodhouse, Raj, *et al.*, 2004; Carter *et al.*, 2019) compared to *C.*
441 *parvum* cases. Similar work in the Netherlands reported no differences in long-term
442 symptoms by species, but did uncover symptoms lasting four months after initial
443 diagnosis highlighting again the significant long lasting burden of this infection (Iglói
444 *et al.*, 2018).

445 Immunocompromised patients commonly experience chronic and persistent disease
446 and are an at-risk group for these relentless, burdensome infections. Those
447 particularly vulnerable are cases with T-cell immune deficiency, including those with
448 haematological malignancies (particularly children), and patients with HIV infection

449 and CD4 counts lower than 200 (Chalmers and Davies, 2010; Cacciò and Chalmers,
450 2016). These sentinel patient groups report similar presentations to those described
451 above, with more severe and protracted disease reported with *C. hominis* infections
452 in particular and the more anthroponotic *C. parvum* subtypes (Cama *et al.*, 2008; Xiao,
453 2010; Adamu *et al.*, 2014).

454 *Recrudescence*

455 Enteric symptoms can last up to three weeks, often with a recrudescence of illness
456 after a period of recovery (Hunter and Nichols, 2002; Hunter, Hughes, Woodhouse,
457 Raj, *et al.*, 2004; Chalmers and Davies, 2010; R. Chalmers *et al.*, 2016). A case-
458 control study of over 200 cases of cryptosporidiosis in England and Wales noted
459 almost 41% of cases reported recurrence of at least one of their enteric symptoms
460 after recovery from the initial infection (Hunter, Hughes, Woodhouse, Raj, *et al.*,
461 2004).

462 This recurrence of symptoms can exact a heavy burden on the individual, but crucially
463 can also amplify the chances of onward transmission, by increasing the length of time
464 the oocysts are shed. It is well recognised that oocyst shedding does occur after
465 cessation of symptoms, and this might go on for one or two weeks, sometimes longer
466 (Jokipii and Jokipii, 1986; Chappell *et al.*, 1996; Chalmers; *et al.*, 2016). Oocyst
467 shedding can, although this is less often documented, also occur in people without
468 any symptoms of enteric disease. Add to this the complexity of recrudescence of
469 illness, and it is clear that an infected person could be shedding infective oocysts
470 intermittently for some time.

471 *Asymptomatic infection*

472 Asymptomatic infection is probably likely (Cicirello *et al.*, 1997; Collinet-Adler and
473 Ward, 2010), but only occasionally documented, and general carriage rates in the
474 general population in industrialised countries seem to be low at between 0.1-1.3%
475 (Tompkins *et al.*, 1999; Davies *et al.*, 2009). Identification of carriage is difficult, as we
476 tend to capture diarrhoeal cases, so this requires special studies. Several manuscripts
477 have documented the heavy toll of onward spread following outbreaks which included
478 asymptomatic infections (Heijbel *et al.*, 1987; Mac Kenzie *et al.*, 1995; Johansen *et al.*,
479 2014). In Brazil a prospective cohort study examined the transmission of
480 *Cryptosporidium* infection in households where there was an identified case (Newman
481 *et al.*, 1994). Secondary cases of infection occurred in 58% of households, and only
482 a quarter of the identified secondary cases had diarrhoea, indicating the presence of
483 asymptomatic infection in almost three-quarters of the participants. Similar results

484 were reported from a longitudinal study in Bangladesh, where asymptomatic infection
485 was more prevalent than diarrhoeal disease (Korpe *et al.*, 2016). Potentially, this
486 finding is due to consistent sample collection and use of PCR detection with strict
487 case definitions, and has been demonstrated before using these methods (Sarkar *et*
488 *al.*, 2014).

489 Spread of this parasite on some of the transmission pathways may be driven in some
490 part by the presence of asymptomatic infection. We know that there can be a period
491 of recovery, followed by a recurrence of symptoms, and that shedding may occur
492 anywhere along this phase (Jokipii and Jokipii, 1986; Chappell *et al.*, 1996; Chalmers;
493 *et al.*, 2016).

494 *Incubation period*

495 Usually, acute diarrhoea follows an incubation period of between two and ten days,
496 averaging about seven (DuPont *et al.*, 1995; Hunter, Hughes, Woodhouse, Syed, *et*
497 *al.*, 2004; Adler *et al.*, 2017). Incubations of over a month have been reported, albeit
498 mostly in the developing countries, where results might be influenced by host features,
499 and possibly by the infecting species (Bouzid *et al.*, 2013; Chalmers and Caccio,
500 2016). This comparatively long period of incubation might allow the parasite to spread
501 more effectively, if oocysts are shed before symptoms arise, although this is not well
502 understood, and evidence varies.

503 *Conclusions on the clinical presentation*

504 These clinical characteristics of *Cryptosporidium*; long incubation, asymptomatic
505 shedding, and recrudescence of illness, are particularly suitable to a persistent and
506 ubiquitous nature. Despite most cases in the UK occurring in immunocompetent
507 populations, with a self-limiting recovering illness, this is not an inconsequential
508 infection. Disease can be long, nasty, and protracted, with long-term health effects
509 and considerable morbidity. Ongoing shedding of infective oocysts with or without
510 symptoms means onward spread is possible.

511 **Treatment and management**

512 Currently, there is no specific licensed treatment for cryptosporidiosis in the UK and
513 advice is that antibiotics should not routinely be administered for any gastrointestinal
514 infection (NICE, 2009; R. Chalmers *et al.*, 2016). In general, because most illness is
515 self-limiting in immunocompetent persons, treatment focuses on supportive therapies
516 to help restore and replace fluids and electrolytes (Sparks *et al.*, 2015).

517 In the USA, nitazoxanide is the only drug approved by the Food and Drug
518 Administration (FDA) for the treatment of cryptosporidiosis, and only in
519 immunocompetent cases older than one year (Checkley *et al.*, 2015). Nitazoxanide
520 has been shown to decrease the duration and severity of symptoms in
521 immunocompetent patients (Abubakar *et al.*, 2007; Shirley, Moonah and Kotloff,
522 2012) but is not indicated in the immunocompromised population (Amadi *et al.*, 2009).

523 The lack of available treatment means that strategic and population-level approaches
524 to management are critical. The geographic distribution and the setting of cases
525 determine the management of general outbreaks of cryptosporidiosis. Such events
526 are usually managed by the appropriate local health protection team, according to
527 locally existing procedures, normally involving local authorities, e.g. environmental
528 health. In the UK, general gastrointestinal infection protocol is followed: these will
529 include risk assessment, provision of hygiene advice, exclusion of symptomatic cases
530 from school or work as necessary and the introduction of control measures where
531 indicated (Public Health Wales, 2012; Public Health England, 2014). Sporadic cases
532 in the UK are asked to detail possible exposures and clinical signs in a general
533 gastrointestinal questionnaire which is then referred to the relevant health protection
534 team for surveillance, but practice has historically been variable (R M Chalmers,
535 Hughes, *et al.*, 2002).

536 **Summary and next steps**

537 In this chapter, I presented background information on *Cryptosporidium* infection and
538 disease, relevant to this thesis. This disease presents in all groups of people, although
539 the greatest burden occurs in the most vulnerable: the very young and old, and the
540 immunocompromised. I have discussed how some parasite characteristics might
541 influence the ways in which people get infected.

542 We know that the burden of this infection in England and Wales is significant: there
543 are over 4,000 diagnosed cases each year across these two countries (Public Health
544 England, 2017a). We are also confident that there are likely to be significantly more
545 than this (Food Standards Agency, 2000a; Adak, Long and O'Brien, 2002).
546 Surveillance practice, including patient presentation, mechanisms of the systems, and
547 laboratory detection, contributes to this disparity in ascertainment. In the next two
548 chapters, I go on to first present the surveillance data for England and Wales, with
549 some analyses to describe the burden of this infection. Later I examine and describe
550 surveillance and detection. I consider the impact this has, including presenting a
551 laboratory audit of a small selection of local laboratories in England and Wales.

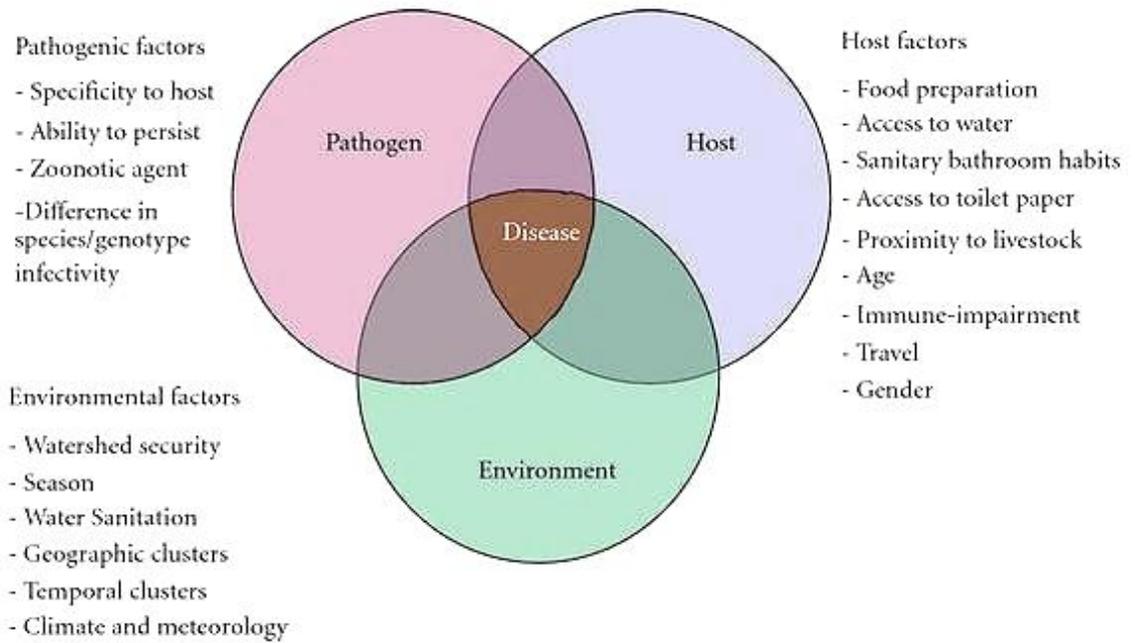
Chapter 3

Epidemiology of *Cryptosporidium* in humans in the UK

552 **Introduction**

553 This section details the most recent epidemiological data for cases in the UK, and
554 highlights some of the main transmission pathways likely to drive infection. Data are
555 derived from Public Health England and Public Health Wales surveillance systems,
556 with additional context added using pre-prepared work from colleagues at PHE's
557 National Infection Service, Colindale, London, United Kingdom, and Wales'
558 *Cryptosporidium* Reference Unit. These are the best available data at the time of
559 writing, at the best resolution that I could access. (PHE, 2017; Public Health Wales
560 NHS, 2018; Douglas *et al.*, 2019)

561 Figure 3 shows some of the global contributory factors which can lead to human
562 infection with this parasite. Common factors associated with infection in the
563 industrialised countries are age of case, season, and intrinsic pathogen factors, and
564 less often, socioeconomic indices (Cacciò and Putignani, 2014). As commented on in
565 Chapter 2, *Cryptosporidium* infection presents in all groups of people. The greatest
566 burden occurs in the most vulnerable: the very young and old, and the
567 immunocompromised. This is true of most of the industrialised countries, where
568 epidemiological patterns are comparable to that in the UK. I touched on some of the
569 factors that are likely contributing to variations in disease epidemiology including
570 differences in ascertainment, prevalence of infecting species and variations in
571 detection, and the severity of illness of cases. In addition to these, specific exposure
572 risks and contributory factors also play a part.



573 Figure 3: Venn diagram of factors leading to *Cryptosporidium* infection. Parasite,
 574 host, and environmental indexes

575 Reproduced from Putignani and Menichella, 2010 (Putignani and Menichella, 2010)

576 **Incidence and distribution in England and Wales**

577 *Included data are from submitted faecal specimens. Data are courtesy of Richard*
 578 *Elson and Amy Douglas, National Infection Service, PHE.*

579 Figure 4 shows laboratory reports of *Cryptosporidium* in England and Wales from
 580 2008 to 2017.

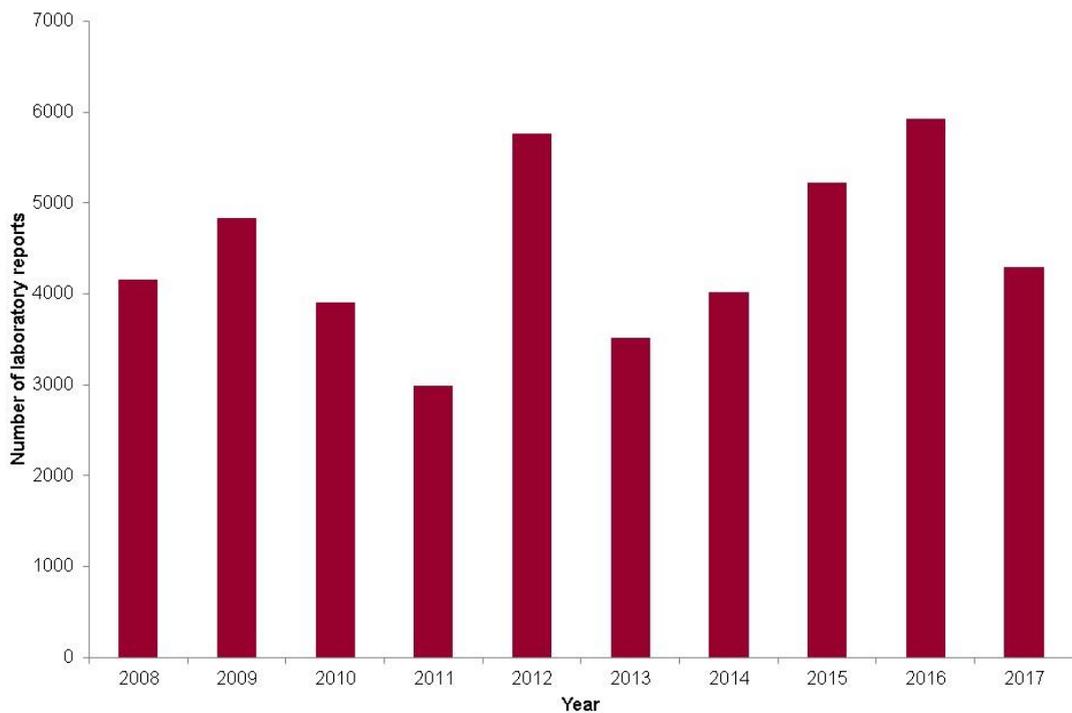
581 **Overall incidence**

582 The number of annually reported cases ranged from 2,990 in 2011 (5.3/100,000) to
 583 5,925 in 2016 (10.1/100,000)¹. The peak years were 2012, 2015 and 2016, all of which
 584 were had large identified outbreaks. the 2012 increase may have been part of a UK-
 585 wide outbreak associated with ready-to-eat salad leaves (McKerr *et al.*, 2015); in
 586 2015, a drinking water contamination event occurred (Drinking Water Inspectorate,

¹ SGSS was introduced in England on 1st Dec 2014. Prior data were generated by the previous system (LabBase2).

587 2017) and 2016 saw large outbreaks associated with recreational water in England
588 (Horne *et al.*, 2017) and a petting farm in Wales (Public Health Wales, 2016). On
589 average, thirteen outbreaks a year are reported (range 3-23).

590 The chart indicates a general increase from 2013 onwards, although this could be due
591 to better detection given the increasing use of more sensitive and specific detection
592 methods (Chalmers *et al.*, 2015).



593 Figure 4: Annual laboratory reports of *Cryptosporidium* in England and Wales (2008
594 to 2017)

595 Reporting region

596 Table 3 shows the number of reports, and rates, of *Cryptosporidium* in England and
597 Wales in 2017. Across England, the contribution of each region to the overall numbers
598 of cases varies considerably. The highest number of reports (n=590), and the highest
599 rate at 10.6/100,000, was from the South West. The next greatest contributor was the
600 South East (not including London) (n=582). The second highest rate (10.4/100,000)
601 was reported from the North East, despite smaller absolute numbers. London reports
602 the fewest numbers of cases. In 2017, the North West rate was 7.6/100,000.
603 Historically, this area has reported sizable numbers of cases.

604 In Wales², rates fluctuated from 8/100,000 to their highest in 2012 at 16/100,000. The
 605 mean number of cases reported annually is 300. This represents about 10% of all
 606 England and Wales cases, although due to lower populations, rates in Wales are
 607 higher.

608 Table 3: Regional distribution of laboratory reports of *Cryptosporidium* in England
 609 and Wales: 2017

Country	Region	Number of laboratory reports	Rate per 100,000 population ³
England	London	250	2.8
England	North East	275	10.4
England	East Midlands	378	7.9
England	West Midlands	414	7.1
England	Yorkshire and the Humber	450	8.3
England	East of England	539	8.7
England	North West	554	7.6
England	South East	582	6.4
England	South West	590	10.6
Wales	Wales	260	8.3

610 Multiple factors might contribute to geographical differences in disease and these data
 611 are not standardised for population structure or reporting practices. The geographic
 612 variation is probably linked to the risk of exposures on specific transmission pathways.
 613 For example, rural areas may be disproportionately affected because of increased
 614 livestock presence (Goh *et al.*, 2004; Lake *et al.*, 2009) or indeed, the susceptibility of
 615 the local population might be influenced by consistent, long-term exposures
 616 (Casemore, 1990).

617 Differences in spatial distribution are sometimes confounded by socioeconomic
 618 factors. In low- and middle-income countries, *Cryptosporidium* infection is often
 619 associated with poverty and aspects of social inequality (Bouزيد, Kintz and Hunter,
 620 2018) but in the higher income countries (as considered in this thesis), sporadic illness
 621 is more often observed among the less deprived areas and communities (Reeve, no
 622 date; Snel, Baker and Venugopal, 2009). Whilst relative poverty alone is unlikely to
 623 directly cause disease it is certain to expose cases to particular pathways or

² Wales' most recent data are for 2007-2016

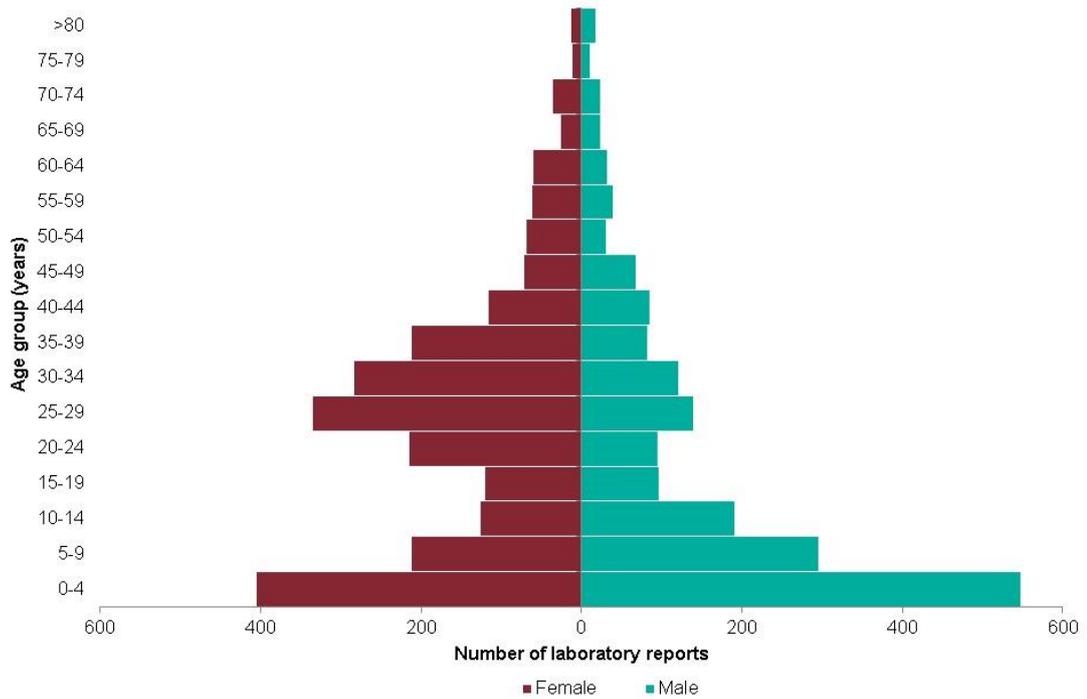
³ Incidence rates were calculated using the relevant Office for National Statistics (ONS) mid-year population estimates for each year

624 transmission risks, or indeed reduce the availability of resources required to avoid
625 exposure, either by personal behaviour/engagement, general health status or access
626 to infrastructure (Snel, Baker and Venugopal, 2009; Ellis *et al.*, 2017). A large USA-
627 based serological surveillance study reported correlates to seropositivity that included
628 poverty and inequality measures. Prior work further strengthens this extract, by
629 reporting that Hispanics, African Americans, and women, all had greater odds of
630 reporting *Cryptosporidium* seropositivity (Frost *et al.*, 2004). An understanding of the
631 physical or social environment can lend context to individual-level relationships,
632 behaviour, or risks, but this is difficult to elicit, and not really a focus of this thesis.

633 **Age and sex**

634 *Cryptosporidium* follows a clear bimodal age-related pattern in most industrialised
635 countries; a peak in young children, and a peak in adulthood, different by gender with
636 females in the 30-44 age range most affected and males in the infant age groups
637 (Dietz and Roberts, 2000a; Nichols *et al.*, 2006; Yoder and Beach, 2007). A Canadian
638 2009 surveillance study confirmed a younger profile of *Cryptosporidium* cases versus
639 other infectious intestinal diseases (Pintar *et al.*, 2009), and the most reported age
640 group in UK are 0-4 year olds, representing 30% of all reported cases in just a small
641 age group (Public Health England, 2017a).

642 Figure 5 is a population pyramid representing the age and sex distribution of
643 laboratory reports of *Cryptosporidium* reported in England and Wales in 2017. The
644 under tens, and in particular the under-fives, represent the highest burden of disease,
645 with a preponderance of males. An additional analysis of these data confirmed
646 diversity in the rates of cryptosporidiosis per 100,000 population between adults and
647 children: higher rates were observed in male, compared to female, children (<1-14
648 years) and the opposite relationship in teenagers (15-19 years) and adults (20 years
649 and older). Overall, the of greatest burden of *Cryptosporidium* infection in children is
650 in the 1-4 year-old age group, specifically males, and among the adult population is
651 centred in the female population aged 20-34 years (Douglas *et al.*, 2019).



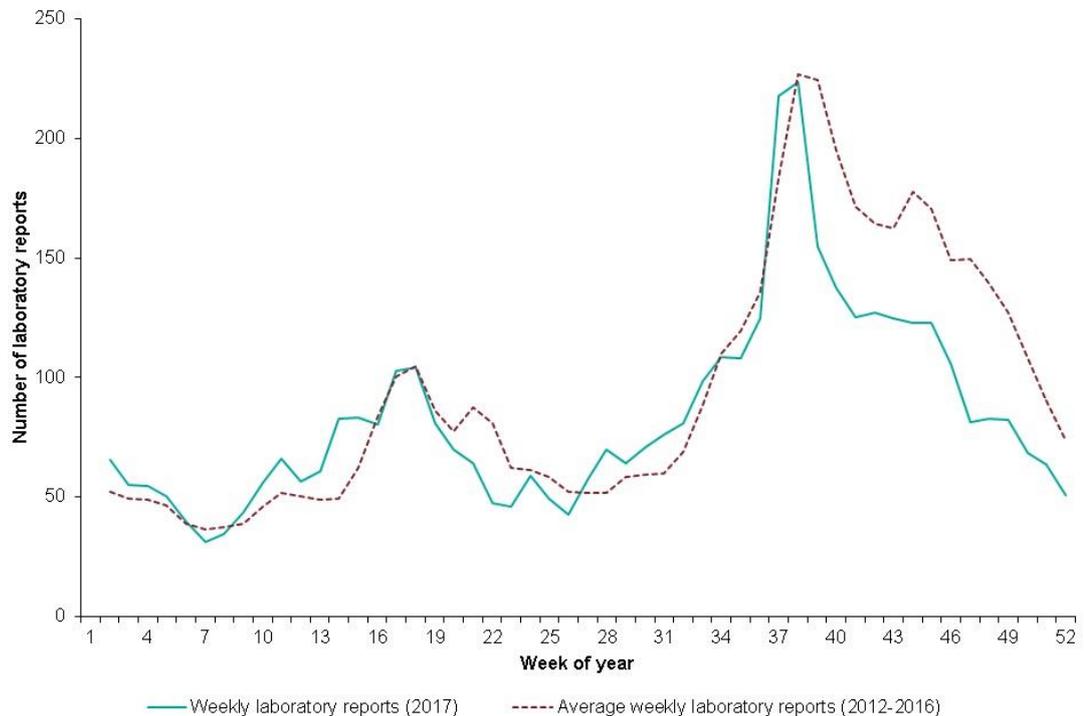
652 Figure 5: Age and sex distribution of laboratory reports of *Cryptosporidium* reported
 653 in England and Wales, 2017

654 Age-related risks are difficult to disentangle and may be due to greater risk of
 655 exposure or susceptibility to infection, both of which are poorly understood and hard
 656 to collect information on. Case reports may also represent an ascertainment bias due
 657 to differences in severity or health-seeking behaviour among parents, or they might
 658 be over-represented due to criteria used for laboratory testing practices (Chapter 4).
 659 Certainly, the consistent profile of a preponderance in cases in both young children
 660 and women in the thirty-plus bracket does suggest a behavioural aspect. This
 661 distribution could be linked to person-to-person transmission, possibly associated with
 662 nappy use and caring responsibilities, which has previously been demonstrated
 663 (Hunter, Hughes, Woodhouse, Syed, *et al.*, 2004), and the gender disparity among
 664 adult cases may reflect gender roles in the home (World Health Organization, 2007).
 665 The contribution of this pathway to this particular profile is still not entirely understood
 666 and there are only a few well-executed studies that have investigated this.

667 **Seasonality**

668 Figure 6 compares 2017 numbers to the previous five years, and illustrates the
 669 tendency for a small peak in spring, around April and May, followed by a much larger

670 increase beginning in August and stretching through September/October (Douglas *et*
671 *al.*, 2019).



672 Figure 6: Number of laboratory reports of *Cryptosporidium* in England and Wales by
673 week in 2017 and average number of reports by week in 2012 to 2016

674 *Cryptosporidium* exhibits a seasonal pattern that is likely to be related to the most
675 appropriate transmission pathway for the infecting species: *C. parvum* occurs more
676 often in the spring and *C. hominis* more often in the autumn (Chalmers *et al.*, 2009).
677 In the UK, this was fairly equal until recently, and following the introduction of strict
678 water control regulations, more *C. hominis* is now seen overall. Although it is accepted
679 that the species exhibit different epidemiological profiles, seasonality may be linked
680 to the preponderance of different sources and environmental factors at different times.
681 Exposure to sources may be made more likely by behavioural factors which vary by
682 season, e.g. taking part in recreational activities (Dale *et al.*, 2009; Lake *et al.*, 2009;
683 Dufour *et al.*, 2017), and the transmission pathways driving this seasonal distribution
684 are still unclear.

685 **Travel among cases**

686 In the available data, 78% of records were not populated with case travel information.
687 Of those that were populated, 6% reported recent travel abroad. With such a high

688 level of missing data, it is difficult to proceed with any meaningful analyses, and any
689 differences in numbers are likely to be due to biases in data collection. Other sources
690 report that foreign travel is captured by routine surveillance in about 10% of cases,
691 often less, but additional work suggests that the true proportion of cases that have
692 recently travelled might be as high as a quarter. Nonetheless, travel has consistently
693 been associated with *Cryptosporidium* infection in the UK, often linked to outbreaks
694 abroad. *Cryptosporidium* is often observed as a non-viral cause of gastrointestinal
695 illness in returning travelers (Okhuysen, 2001) and travel might subject people to
696 exposures for infection. As such it is not an exposure for disease intrinsically, and
697 susceptibility may be linked to immunity, or lack thereof, of travelers on short term
698 holidays in endemic areas (Shlim *et al.*, 1999) as well as increased exposures in areas
699 with lower hygiene or more frequent use of swimming pool in holiday resorts
700 (Introduction). Given the relatively long incubation period of *Cryptosporidium* it is
701 feasible that the autumn peak is somewhat driven by foreign-acquired cases returning
702 to the UK after the summer break (McLauchlin *et al.*, 2000; Hunter, Hughes,
703 Woodhouse, Syed, *et al.*, 2004).

704 **Transmission pathways and the underlying exposures for** 705 **infection**

706 In an earlier part of the thesis (The cycle of infection) I described how the parasite is
707 transmitted via the faecal-oral route, because of ingesting *Cryptosporidium* oocysts.
708 The main transmission pathways are generally shared by *C. hominis* and *C. parvum*
709 although some aspects are different. For *C. hominis*, humans are the major host,
710 whereas for *C. parvum*, both humans and animals can act as reservoirs (Species that
711 infect humans). Outbreaks via person-to-person transmission (*C. hominis* and *C.*
712 *parvum*) or animal-to-person transmission (*C. parvum*) contact have been reported in
713 various settings, as well as more indirect pathways such as ingestion of contaminated
714 water and food, or contact with objects contaminated with infectious oocysts (Cacciò
715 *et al.*, 2005; Chalmers *et al.*, 2019).

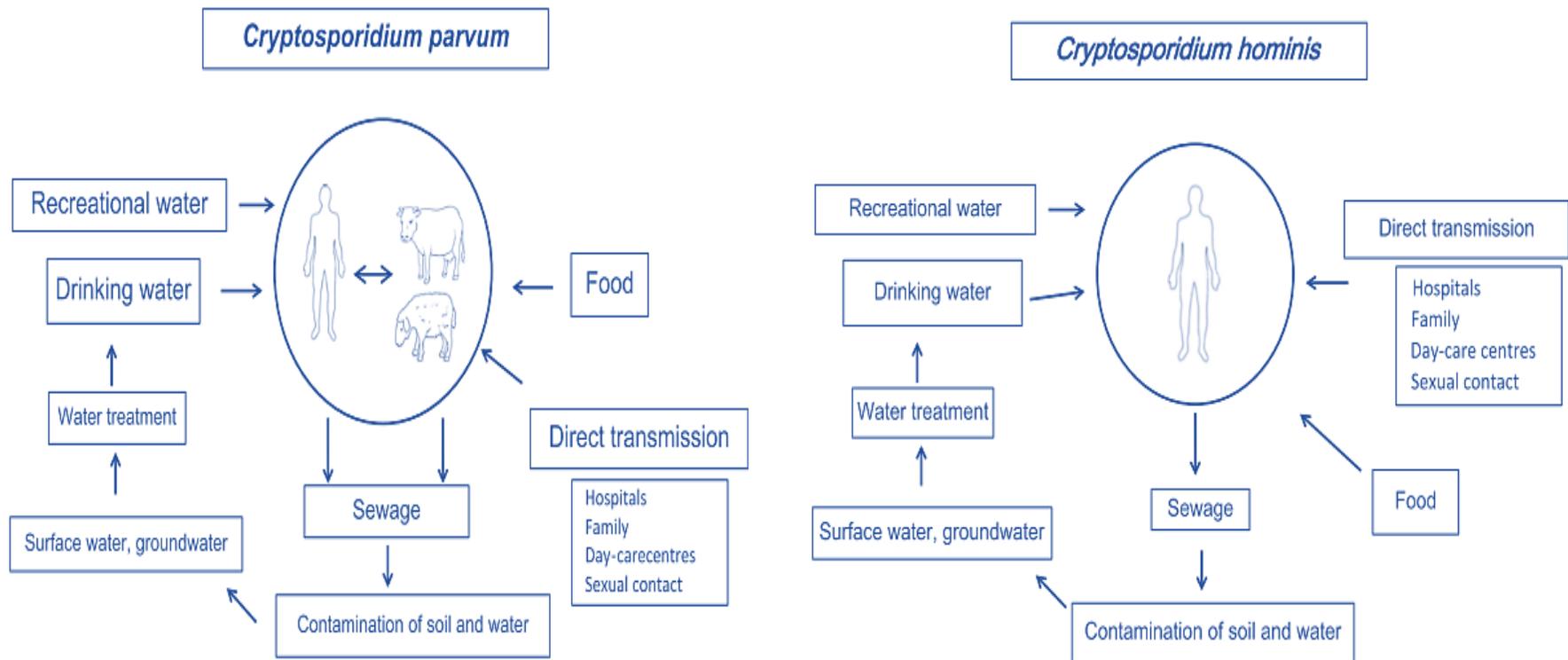


Figure 7: Main host and transmission pathways for *C. parvum* and *C. hominis*

Amended and reproduced from Caccio and Putignani (Cacciò and Putignani, 2014)

716 Reported exposures are often estimated from outbreak investigations and the most
717 well known pathways for *C. parvum* and *C. hominis* include:

718 **Water**

719 Water exposures for both *C. hominis* and *C. parvum* can include consumption of
720 contaminated drinking water (Goh *et al.*, 2004, 2005; Hunter, Hughes, Woodhouse,
721 Syed, *et al.*, 2004; Pollock *et al.*, 2008, 2014) and exposure to recreational waters
722 (Stafford *et al.*, 2000; Louie *et al.*, 2004; McCann *et al.*, 2014). *C. parvum* is found in
723 sources of water abstracted for producing drinking water often contaminated from
724 nearby animals. Agricultural run-off from grazing land, slurry and discharge of
725 effluents from the treatment of wastewaters can lead to outbreaks following heavy
726 rainfall (Lake *et al.*, 2005). Outbreaks linked to mains drinking water can cause
727 considerable illness: a 1993 drinking water outbreak in Milwaukee affected more than
728 400,000 residents. Low quality standards most likely led to *Cryptosporidium* oocysts
729 passing through the filtration system of the city's water-treatment plant (MacKenzie *et*
730 *al.*, 1995). In the UK drinking water outbreaks are now less common, following
731 additional regulations for public water supplies that were introduced in 1999 (Sopwith
732 *et al.*, 2005; Nichols *et al.*, 2006). Outbreaks caused by drinking water consumption
733 only now account for around 1% of outbreak disease (Chalmers *et al.*, 2019).

734 A few studies have examined drinking water in connection with sporadic disease, but
735 results are varied; well executed studies are few and exposures are often difficult to
736 disentangle (Robertson *et al.*, 2002b; Khalakdina *et al.*, 2003; Abubakar *et al.*, 2004;
737 Goh *et al.*, 2004; Hunter, Hughes, Woodhouse, Syed, *et al.*, 2004). Studies in
738 Scotland (Pollock *et al.*, 2014) and England (Goh *et al.*, 2005) have determined
739 decreases in cases following enhanced filtration of water.

740 In addition to drinking water, recreational water use, often in connection with foreign
741 travel, is a common source of outbreaks of illness, especially in summer and autumn
742 (Stafford *et al.*, 2000; Louie *et al.*, 2004; McCann *et al.*, 2014; de Gooyer *et al.*, 2017).
743 Common risk factors linked to this are swallowing untreated outdoor waters
744 (Valderrama *et al.*, 2009), swimming in a treated pool, (Causer *et al.*, 2006;
745 Vythelingum, Cheesbrough and Woywodt, 2012; McCann *et al.*, 2014), and time
746 spent in pools (with children at highest risk) (Suppes *et al.*, 2016). Faecal
747 contamination, inadequate filtration, or breaks in equipment or suitable management
748 are the likely events that lead to infection (Public Health Laboratory Service, 2000).

749 **Animal contact**

750 *C. parvum* is most frequently associated with exposure to farm animals as they carry
751 this zoonotic species (Hoek *et al.*, 2008; Utsi *et al.*, 2016). Farm animals, especially
752 the new-borns of goats, sheep, and cows, are recognised sources (Ng *et al.*, 2012).
753 Both outbreak and sporadic cases have been associated with petting farms where
754 visitors may touch the animals, and occur more often in the springtime when young
755 animals are born (P. Hunter and Thompson, 2005; McGuigan, Steven and Pollock,
756 2010). Outbreaks related to this exposure are detected and reported more commonly
757 than sporadic cases. However, in a recent study in the UK, the proportion of *C.*
758 *parvum* cases acquired from direct contact with farm animals was estimated to be
759 25% but explained only 10% of all the reported *Cryptosporidium* cases (R M
760 Chalmers, R Smith, *et al.*, 2011).

761 Contributory factors include direct contact with lambs, calves, kids, or animal faeces
762 (R. M. Chalmers and Giles, 2010). Additionally, indirect contact with faecal material
763 might occur via the environment; inadequate hygiene practice or hand-washing
764 facilities on a farm might increase the risk of infection after exposure (Gormley *et al.*,
765 2011), and an almost double risk of infection was identified previously during an
766 outbreak in farm goers who habitually bit their nails or sucked their thumbs (Evans
767 and Gardner, 1996). Contamination of food from animals is documented (Bud-
768 Amoako *et al.*, 2011), and even mechanical transmission on items such as farm
769 vehicles, pram wheels, shoes etc. is possible. There has been some suggestion that
770 the shift of large populations of young, susceptible children into often rural areas
771 during the spring season might contribute to cases (Stefanogiannis, McLean and Van
772 Mil, 2001; LeJeune and Davis, 2004).

773 It is not thought that contact with domestic pets are a significant risk for
774 *Cryptosporidium* infection at a population level (Chalmers and Giles, 2010) and in the
775 UK studies confirm this (Hunter, Hughes, Woodhouse, Syed, *et al.*, 2004; Smith *et*
776 *al.*, 2009). Individuals may however be at risk. A handful of studies have reported
777 infections with cat (Caccio *et al.*, 2002; Beser *et al.*, 2015) and dog (Fayer *et al.*,
778 2001; Xiao *et al.*, 2007) associated species, but most identified cases have been
779 reported in immunocompromised patients (Glaser *et al.*, 1998) or in developing
780 countries where the epidemiology and exposure risks differ (Cama *et al.*, 2008).

781 Having said that, many of the decent case-control studies were undertaken before
782 laboratory methods improved in sensitivity and specificity. The lack of species
783 differentiation might lead to invalid assumptions about the risk posed by domestic

784 animals. Further to this, recent work to characterise the gp60 sequence for *C. felis*
785 isolates confirmed two accounts of zoonotic transmission, one of which revealed a
786 variability in the sequence from the host (cat) to the human case (Rojas-Lopez *et al.*,
787 2020). A better understanding of infecting species, and their subtypes would help
788 support ongoing evaluation of the risks of companion animal transmission (Irwin,
789 2002).

790 **Food items**

791 Food-related outbreaks have been reported for both *C. parvum* and *C. hominis*
792 species (Ethelberg *et al.*, 2009; Robertson and Chalmers, 2013; Åberg *et al.*, 2015;
793 McKerr *et al.*, 2015) and are likely contaminated via water or by food handlers. These
794 are not yet particularly common, but the potential for foodborne transmission exists,
795 and reports are increasing in frequency (Putignani and Menichella, 2010; Robertson
796 and Chalmers, 2013). Globally, these are more likely to present in the industrialised
797 countries: USA, Canada, Netherlands, Scandinavia, and the UK most frequently
798 report risks associated with fresh produce and drinks.

799 A recent global analysis reported that a fifth of outbreaks could be considered food
800 related, and represented the more recent years' reports (Putignani and Menichella,
801 2010). Conversely, some studies of sporadic illness have identified decreased risks
802 of illness associated with certain raw food/salad items, such as tomatoes (Goh *et al.*,
803 2004; Hunter, Hughes, Woodhouse, Syed, *et al.*, 2004; Nic Lochlainn *et al.*, 2019)
804 raw carrots (Robertson *et al.*, 2002b), and lettuce and mixed salad (Goh *et al.*, 2004).
805 A high prevalence of *Cryptosporidium* parasites in packaged salads and leafy greens
806 has been detected and so the potential for infection exists (Dixon *et al.*, 2013), as
807 proven by outbreaks. Better detection, reporting and molecular resolution (Dixon *et*
808 *al.*, 2013; Robertson and Chalmers, 2013; McKerr *et al.*, 2015) may increase our
809 capture of these exposures in the future.

810 **Contact with other cases**

811 Contact with other cases has previously been highlighted as a possible factor in the
812 transmission of *Cryptosporidium*, both in outbreak and sporadic cases, and often for
813 *C. hominis*, the more anthroponotic of the human species (Hannah and Riordan,
814 1988; Newman *et al.*, 1994; Nichols *et al.*, 2006; Johansen *et al.*, 2014). An analysis
815 of outbreak reports taken from surveillance data in Ireland reported that ingestion of
816 water and person-to-person spread both represented the most important
817 mechanisms of transmission in outbreaks (Garvey and McKeown, 2009). In the

818 United States, a case-control study evaluating sporadic cryptosporidiosis among
819 immunocompetent persons, found one of the main risk factors associated with
820 increased odds of illness was contact with a child with diarrhoea (Abubakar *et al.*,
821 2004). This result was supported in the UK, in a case-control study conducted in the
822 North West of England examining species-specific risk factors for sporadic
823 cryptosporidiosis (Hunter, Hughes, Woodhouse, Syed, *et al.*, 2004). Crucially, this
824 work found that changing children's nappies was a specific risk factor for infection
825 with *C. hominis*, whether the child was symptomatic or not. This suggested some
826 contribution of asymptomatic infection to spread. A later case-control study in the
827 Netherlands (Nic Lochlainn *et al.*, 2019) found similar results, specifying that for *C.*
828 *hominis*, cases were more likely than controls to have been exposed to a case in the
829 home. Additionally, the authors reported other indicators for both species, including
830 living in smaller homes, and living with children. These findings are corroborated in
831 the literature and even anecdotally, and could be not only because young children
832 are at higher risk of *Cryptosporidium* (i.e. higher incidence), but also that caring
833 responsibilities in the home put adults, more likely females (World Health
834 Organization, 2007), at greater risk of exposure and thus infection.

835 Contact with a case is likely to represent a person-to-person transmission pathway
836 (Nic Lochlainn *et al.*, 2019), and can happen in various settings including the home,
837 nurseries, and healthcare institutions (J Hannah and Riordan, 1988; Gardner, 1994).

838 **Summary**

839 The data indicate that the age and sex profile of cases is comparable here to the main
840 industrialised countries, and that seasonal differences and travel do contribute to
841 disease patterns in the UK. When we consider these patterns, we can conclude that
842 the variables are not always considered intrinsic risks for infection and instead are
843 related to the behaviours of people that likely put them at risk of specific exposures.
844 Examples of these have already been demonstrated, such as a female parent
845 changing an infant's nappy.

846 Following on from this, I ended this chapter with a brief overview of the main
847 transmission pathways, considering some of the underlying exposures and how they
848 contribute to infection. The main pathways reported are water, with both drinking and
849 recreational exposures, animal contact, food, and person-to-person, in various
850 settings. Outbreaks related to all of these have been reported, and the frequency of

851 some are changing. However, we are not entirely sure if the contribution of these
852 appears the same for sporadic disease.

853 As part of my effort to describe and understand *Cryptosporidium* infection in the UK,
854 I follow this chapter with a presentation of a laboratory audit, which helps us to
855 understand how these data are captured and what we might do to better detect
856 sporadic cases. This helps to meet my first objective, to understand the distribution
857 and burden of this infection in the UK, as well as considering detection and
858 approaches to testing that underpin the surveillance data.

859 Later in Chapter 5, I follow up on these suppositions with a systematic literature
860 review to describe the main exposures reported for sporadic cases. This approach
861 allows us to understand the detail underneath some of these pathways and explore
862 differences between species.

Chapter 4

Surveillance, detection, and diagnosis of *Cryptosporidium* infection in England and Wales

863 **Surveillance**

864 Much like testing practices, surveillance approaches differ geographically. In Europe,
865 notification of cryptosporidiosis is mandatory in 20 EU Member States, Iceland, and
866 Norway. No surveillance system exists in Austria, Denmark, France, Greece or Italy
867 (European Centre for Disease Control, 2017). In the USA and Australia, systems
868 similar to the UK models exist: in the states a National Notifiable Diseases
869 Surveillance System (NNDSS) managed by the CDC and in Australia, a National
870 Notifiable Diseases *Surveillance* System, overseen by the *Australian* Government
871 Department of Health (Lal *et al.*, 2015; Painter *et al.*, 2015).

872 In the UK, *Cryptosporidium* has been classed as a 'statutory reportable causative
873 agent' since 2010 (Public Health England, 2019) meaning that should a laboratory
874 identify it in a human sample, they have a duty to notify the relevant public health
875 authority, usually within seven days (Public Health (Control of Disease) Act 1984,
876 1984; The Health Protection (Notification) Regulations 2010, 2010). Systematic
877 national surveillance of laboratory confirmed *Cryptosporidium* in England and Wales
878 has been established for many years (Wall *et al.*, 1996). Both Public Health England
879 and Public Health Wales collect these notifications and publish analyses of local and
880 national trends. Positive samples identified in the diagnostic laboratories are routinely
881 forwarded to the national *Cryptosporidium* Reference Unit (CRU) that provides expert
882 management, prevention and control advice as well as *Cryptosporidium* typing and
883 confirmation services for speciation and surveillance (Public Health England, no
884 date).

885 *Surveillance system descriptions*

886 I have included a brief overview of the systems in both England and Wales to provide
887 some insight into how laboratory case data are reported, and subsequently counted.

888 **England**

889 Laboratory confirmations of *Cryptosporidium* sp. are reported to the surveillance
890 system, Second Generation Surveillance System (SGSS), an application that stores
891 and manages data on laboratory isolates and notifications. Laboratories use their own
892 information management systems (LIMS), which may vary locally, to directly feed to
893 SGSS, which is then updated in real-time for PHE end-users (Public Health England,
894 2016). The data are stored in a central database within PHE and made available to a
895 wide range of users within and out with the organisation, subject to robust access
896 control.

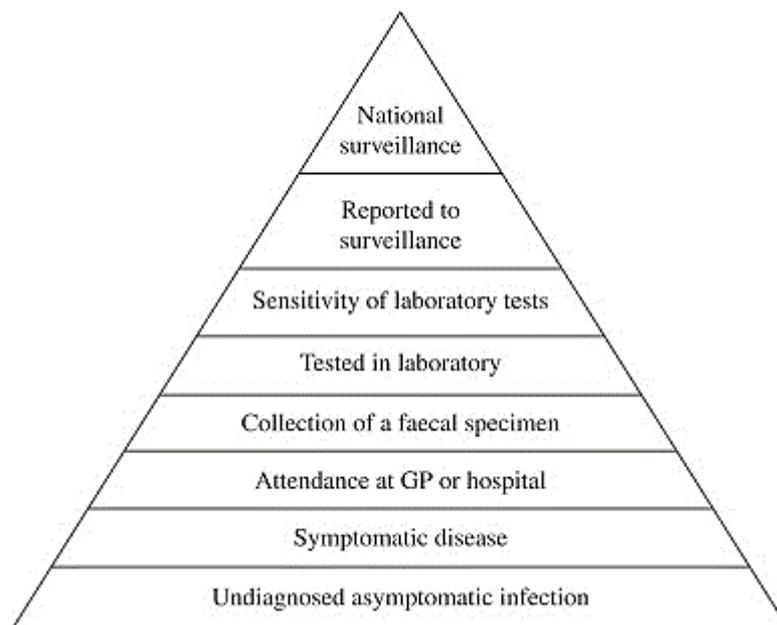
897 Wales

898 Laboratory confirmations of *Cryptosporidium* sp. detected in samples are reported to
899 the Welsh surveillance system, Tarian. Tarian also provides a case and incident
900 management system, which links to relevant information in the Wales LIMS. The
901 pathology systems are all-Wales and compatible with LIMS. (The Welsh Government,
902 2017).

903 *Speciation data*

904 Species identification reports are returned to the relevant submitting laboratories by
905 paper and are captured by Tarian. In Wales, this means the local health protection
906 teams can access the updated results. (Tarian also feeds into SGSS so informs PHE
907 at national surveillance level, although this process that currently still being quality
908 checked.)

909 *The burden of illness pyramid*



910 Figure 8: The burden of illness pyramid

911 Adapted from (FoodNet Surveillance | FoodNet | CDC, no date)

912 The burden of illness pyramid (Figure 8) is a model for understanding disease
913 reporting, and the steps in the process that lead from true infection to ascertainment
914 of cases.

915 At the bottom of the pyramid, we imagine all the true infection that exists in the
916 population or community of interest, and at the peak are the cases that reach the

917 national surveillance dataset and are reported. At each stage in this process, true
918 cases drop out of the system, usually due to myriad reasons.

919 Data from a study of infectious intestinal diseases (IID) estimated that, in the UK,
920 there are likely to be up to around 15 true cases in the community for every case that
921 makes it to a lab diagnosis (Adak, Long and O'Brien, 2002). This could potentially
922 mean that there are about 64,000 *Cryptosporidium* cases per year (range 45,000-
923 88,000) at the bottom of the pyramid, compared to the four thousand or so cases
924 actually captured by surveillance at the top (O'Brien *et al.*, 2010).

925 We know that outbreaks are not always the biggest contributor to disease, despite
926 being well detected, and so the true burden in the UK is not well-understood (Tam *et*
927 *al.*, 2012). The cases of *Cryptosporidium* that are reported only represent diagnosed
928 and usually symptomatic illness (O'Brien *et al.*, 2010). Importantly, *Cryptosporidium*
929 infected diarrhoea patients often exhibit a recrudescence of symptoms, and the
930 spectrum of severity of symptoms may vary (Hunter, Hughes, Woodhouse, Raj, *et al.*,
931 2004; Hunter, Hughes, Woodhouse, Syed, *et al.*, 2004). Thus, cases presenting to
932 healthcare may have had symptoms for some time, or be in an interim asymptomatic
933 period, and testing only diarrhoeic stools would certainly underestimate incidence.
934 Additionally, variations in whether samples are tested and in primary diagnostic test
935 used might contribute to differences in detection in stools after the acute clinical
936 episode has passed (Chalmers *et al.*, 2016).

937 **Detection and diagnosis in humans**

938 *Cryptosporidium* oocysts are small, about 5 µm in diameter (Leitch and He, 2011) and
939 primary diagnosis of infection is usually by determining the presence of these in stool
940 using microscopy. Sometimes small bowel aspirates, biopsies, or tissue samples may
941 be required. More rarely, and usually as part of studies, exposure to *Cryptosporidium*
942 might be examined by serological tests, although this does not detect the presence
943 of current infection or indeed disease.

944 In the UK, Standards for Microbiological Investigations (SMIs) recommend that
945 routine laboratory diagnosis of *Cryptosporidium* in stools should be undertaken by
946 either stained microscopy, using auramine phenol (AP) or modified Ziehl–Neelsen
947 (mZN), or antigen detection by enzyme immunoassay (EIA) or DNA by PCR (UK
948 Standards for Microbiology Investigations, 2017). Following EIA positive reaction, the
949 diagnosis should be confirmed with a method of choice, and AP microscopy
950 confirmed with mZN. From 2008, use of EIA has been reported increasingly (RM.,

951 2008; Chalmers *et al.*, 2015; Alexander *et al.*, 2017). EIA has been shown to have
952 better diagnostic sensitivity for *Cryptosporidium* than mZN microscopy, while PCR is
953 most sensitive (R. M. Chalmers *et al.*, 2011). Because none of these diagnostic tests
954 identify *Cryptosporidium* species, laboratories are requested to send all
955 *Cryptosporidium*-positive stools to the national *Cryptosporidium* Reference Unit
956 (CRU) in Swansea for species identification, and subtyping.

957 Despite SMI recommendations that ‘all faecal samples from symptomatic individuals
958 should be tested for *Cryptosporidium* oocysts’, previous audits in the UK have
959 reported differences in completeness of testing that varied geographically (RM., 2008;
960 Chalmers *et al.*, 2015; Alexander *et al.*, 2017). Selection criteria for testing were
961 reported to differ, were inconsistent, and laboratories frequently used submitted stool
962 consistency as a criterion, even though this is an unreliable predictor of
963 *Cryptosporidium* positivity (D P Casemore, Armstrong and Sands, 1985).

964 As part of the overall PhD work, I undertook a contemporary audit of some
965 laboratories in England and Wales to describe the methods used and selection criteria
966 applied. Continued understanding of laboratory practice is fundamental to
967 understanding and interpreting surveillance data, estimating true disease burden, and
968 implementing control measures for patient management and at the population level.
969 The aim of this audit was to assess approaches and monitor changes to
970 *Cryptosporidium* testing, reporting and referral, including methods used and selection
971 criteria applied, among clinical microbiology laboratories serving the National Health
972 Service (NHS) in England and Wales, and make recommendations to promote best
973 practice.

974 **Laboratory audit: methods**

975 In March 2019, an online survey was cascaded via email to senior biomedical
976 scientists (BMSs) or consultant microbiologists in England and Wales, via the Heads
977 of Laboratories for England and directly to operational microbiology leads in Wales.
978 The survey was designed and administered using Select Survey
979 (www.selectsurvey.net), although a Microsoft (MS) Word version was available on
980 request (Appendix 1). Questions were informed by a previous audit (Chalmers *et al.*,
981 2015) and covered selection criteria used for testing stools for *Cryptosporidium*,
982 diagnostic tests employed and any predicted changes to these, referral of positive
983 stools for species identification, and the mechanisms for reporting these results. The
984 laboratories received a reminder after one month and the survey remained open for
985 two months.

986 *Data analysis*

987 Following closure of the survey, data were exported, cleaned, and stored in MS Excel.
988 I managed all data and undertook the analyses and write up.

989 Diagnostic laboratories in England were aggregated into larger geographical areas
990 correspondent with Public Health England (PHE) regions (North, South, Midlands and
991 East, London). Response rate was calculated as the number of responses divided by
992 the number of laboratories receiving the survey. Proportions were reported, and 95%
993 confidence intervals (CI) calculated where appropriate using the Wilson Score method
994 for proportions (Wilson, 1927; Newcombe and Altman, 2000). Reported practices and
995 approaches to testing were assessed with the contemporaneous version of SMI B31.

996 **Laboratory audit: results**

997 The questionnaire was cascaded to 90 laboratories in England and Wales, using
998 email, via the senior BMS or head of the laboratory. In total, 45 usable responses
999 were received from laboratories that tested stool samples, a response rate of 50%.
1000 The North of England represented the majority (42%) of the responses, followed by
1001 the South (22%), Wales (16%), London (13%) and lastly the Midlands and East
1002 providing the smallest proportion (7%).

1003 *Routine testing for Cryptosporidium and selection criteria applied*

1004 Laboratories were asked if they routinely tested for *Cryptosporidium* under their
1005 current protocol. Of the 45 laboratories, 23 (51%; CI 37.0 - 65.0) reported routinely
1006 testing all stool samples (i.e. not applying any selection criteria). The remaining 22
1007 laboratories (49%; CI 35.0 - 63.0) reported that selection criteria were applied (**Error!**
1008 **Reference source not found.**), although in nine laboratories this was selecting stools
1009 from community cases of diarrhoea, i.e. excluding inpatients that developed diarrhoea
1010 more than three days after their admission. This is sometimes called the “three-day
1011 rule” and is generally applied on a local basis, taking positivity rates and submission
1012 numbers into account (Bauer, 2001; Public Health England, 2014). Thus, in total,
1013 32/45 (71%; CI 56.6 – 82.3) laboratories reported testing all stools from community
1014 cases of diarrhoea.

1015 Seven of the 45 laboratories (16%; CI 7.7 - 28.8) tested all non-formed stools.
1016 Additionally, laboratories reported various other criteria or combination of factors
1017 including; immune status of the case, travel and/or exposure history, age of the case,
1018 and at the specific request of a clinician or health practitioner (Table 4).

1019 Table 4: Selection criteria used for testing for *Cryptosporidium*, reported by audited
 1020 microbiology laboratories in England and Wales, 2019 (n=45)

Criteria for testing	Number of laboratories reporting criterion (%)
No criteria applied	
All stool samples	23 (51%)
Criteria applied (more than one may apply)	
All except inpatient for >3 days	9 (20%)
All non-formed stool samples	7 (16%)
Immunocompromised status	7 (16%)
Travel history	6 (13%)
Age group	6 (13%)
Exposure history	4 (9%)
At clinician's request	3 (7%)

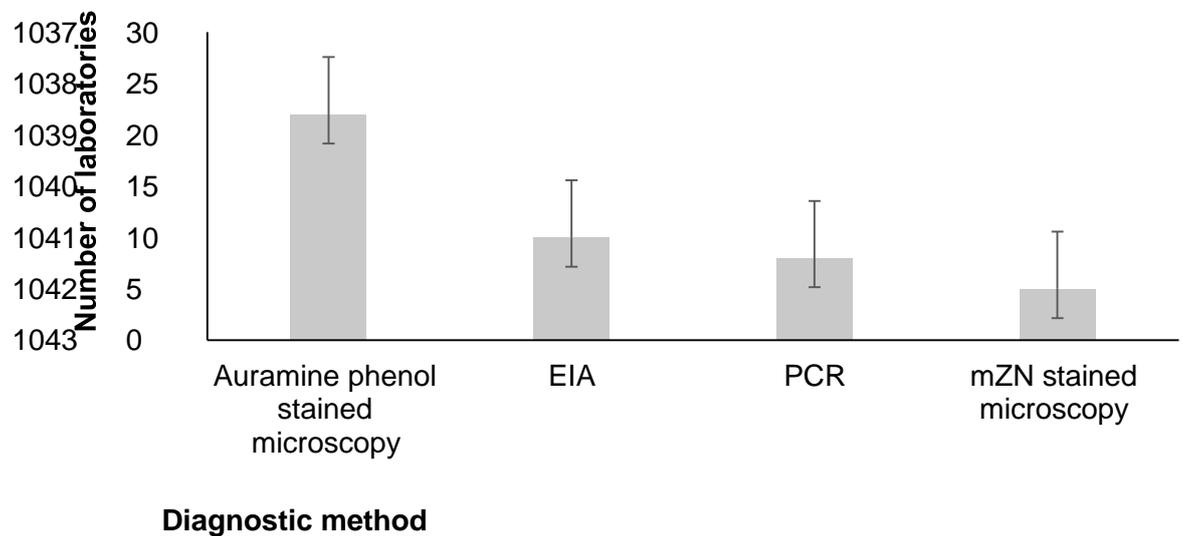
1021 In all six laboratories that selected specimens according to patient age, different
 1022 categories and cut-offs were reported: <5 years, 0-14 years, 017 years, any <16
 1023 years, >2 years, and 0-45 years.

1024 Routine testing of all stools for *Cryptosporidium* (i.e. not applying any selection criteria
 1025 as recommended) varied by region, but the differences were not significantly different.
 1026 The highest proportion was in the North of England reported by 14/19 laboratories
 1027 (74%; CI 51.2 - 88.2), followed by 4/7 (57%; CI 25.0 - 84.2) in Wales, 4/10 (40%; CI
 1028 16.8 - 68.7) in the South of England and 1/3 (33%; CI 6.1 - 79.2) in the Midlands and
 1029 East. However, all six responding laboratories from the London region reported
 1030 applying selection criteria.

1031 *Test methods used*

1032 Figure 9 shows the reported primary diagnostic test methods used for
 1033 *Cryptosporidium*. Auramine phenol (AP) stained microscopy was most commonly
 1034 used, by 22/45 (49%; CI 35.0 - 63.0) responding laboratories, followed by EIA (n=10;

1035 22%; CI 12.5 – 36.3), PCR (n=8; 18%; CI 9.3 – 31.3) and mZN stained microscopy
 1036 (n= 5; 11%; CI 4.8 – 23.5).



1044 Figure 9: Primary diagnostic methods for *Cryptosporidium* in stools, reported by
 1045 audited microbiology laboratories in England and Wales, 2019 (n=45)

1046 Of those 22 laboratories that reported using AP stained microscopy as the primary
 1047 test, five (23%) confirmed positive reactions using mZN, and one (5%) reported
 1048 confirmation using an immunochromatographic lateral flow test (RIDA®QUICK
 1049 *Cryptosporidium*, R-Biopharm). The most commonly used EIA kit was the microplate-
 1050 format GIARDIA/CRYPTOSPORIDIUM CHEK® (Techlab, Inc.) (7/10; 70%). The
 1051 remaining three laboratories reported either using the cartridge format
 1052 GIARDIA/CRYPTOSPORIDIUM QUIK CHEK™ kit (Techlab, Inc) or the microplate
 1053 format kits *Cryptosporidium* Stool Antigen EIA (IVD Research Inc.) or
 1054 CRYPTOSPORIDIUM II™ (Abbott). All ten laboratories using EIA reported confirming
 1055 positive reactions: five confirmed with the GIARDIA/CRYPTOSPORIDIUM QUIK
 1056 CHEK™ (Techlab, Inc) (50%), four (40%) with either mZN or AP stained microscopy,
 1057 and one (10%) requested a repeat specimen. All eight laboratories that reported using
 1058 PCR used the same assay, the EntericBio Gastro Panel 2 (SeroSep).

1059 *Referrals for species identification*

1060 Over three quarters of laboratories (n=36, 80%) reported referring *Cryptosporidium*-
1061 positive stools to the CRU for species identification, and of these 24 (67%) reported
1062 updating their own laboratory information management system (LIMS) with the result.
1063 Thirty-one (86%) sent the samples routinely, while the other five laboratories reported
1064 sending samples either on request or in an outbreak situation. I was unable to
1065 ascertain reasons for not sending samples for species identification, but most
1066 laboratories reported that they would be willing to do this in the future.

1067 *Changes to practice*

1068 Five of the 45 laboratories (11%) reported having made recent changes to practice,
1069 all of which were adoption of PCR within the preceding year. Thirteen laboratories
1070 reported plans for future changes, of which 12 (92%) included adoption of PCR. Two
1071 laboratories expected to reduce their testing of stools to exclude inpatient samples
1072 (3-day rule).

1073 **Discussion**

1074 *Routine testing for Cryptosporidium and selection criteria applied*

1075 Overall, 69% (95% CI 54.3 - 80.5) of laboratories routinely tested all stools from
1076 community cases of diarrhoea, higher than the 54% (95% CI 44.6 – 64.3) reported
1077 from the preceding audit in 2013-2014 (Chalmers *et al.*, 2015). However, selection
1078 criteria were often applied, and testing remains below the recommendation in SMI
1079 B31 (Public Health England, 2014), with regional differences in the proportion of
1080 laboratories selectively testing stools. Different criteria are used to determine
1081 selective testing, either alone or in conjunction with other criteria. Reassuringly, fewer
1082 laboratories reported using stool consistency as a criterion than previously, reduced
1083 from 19% (95% CI 11.9-28.4) (Chalmers *et al.*, 2015), to 16% (95% CI 7.7-28.8) in
1084 this study.

1085 A prior inconsistency in the guidance for *Cryptosporidium* testing, highlighted by
1086 Chalmers *et al.* in 2015 (Chalmers *et al.*, 2015), led to clarification that all faecal
1087 samples submitted for the investigation of diarrhoeal / gastrointestinal illness should
1088 be tested for *Cryptosporidium* and that stool consistency should not be used as a
1089 criterion for selective testing (Public Health England, 2014; UK Standards for
1090 Microbiology Investigations, 2019). Stool consistency has long been considered an
1091 unreliable predictor of *Cryptosporidium* positivity (D P Casemore, Armstrong and

1092 Sands, 1985; Jokipii and Jokipii, 1986), and asymptomatic infection has also been
1093 demonstrated (Newman *et al.*, 1999; Hunter, Hughes, Woodhouse, Syed, *et al.*, 2004;
1094 Davies *et al.*, 2009; Johansen *et al.*, 2014).

1095 Also importantly, cryptosporidiosis patients often exhibit a recrudescence of
1096 symptoms during acute episodes, and the spectrum of severity of symptoms may vary
1097 (Palmer and Biffin, 1990; Hunter, Hughes, Woodhouse, Raj, *et al.*, 2004). Thus, cases
1098 presenting to healthcare may have had symptoms for some time, or be in an interim
1099 asymptomatic period, and testing only liquid stools would certainly underestimate
1100 incidence. Additionally, variations in primary diagnostic test used might contribute to
1101 differences in detection in stools submitted after the acute clinical episode has
1102 passed. Relapsing and recuperating patients remain infectious and could present a
1103 transmission risk. A new syndromic UK SMI that supersedes SMI S7 (UK Standards
1104 for Microbiology Investigations, 2019) recommends inclusion of *Cryptosporidium* in
1105 the primary test set for the investigation of gastroenteritis in community settings,
1106 irrespective of whether the stool specimen presented takes the shape of container or
1107 not.

1108 Other selection criteria were inconsistent, with laboratories reporting differing age
1109 groups, health status of case, overseas travel destinations, and known exposures.
1110 Age selection in particular seemed inconsistent and without rationale, a finding
1111 reported previously in the literature (RM., 2008; Chalmers *et al.*, 2015; Alexander *et*
1112 *al.*, 2017). While a focus on the younger age groups is not without justification, the
1113 adult age group represents an important proportion of all cases (Palmer and Biffin,
1114 1990; KG. *et al.*, 2010) and an excess of adult cases might well help identify particular
1115 transmission vehicles, such as water supplies (KG. *et al.*, 2010) or food items (McKerr
1116 *et al.*, 2015). Six laboratories reported foreign travel as a selection criterion. Whilst
1117 this, in conjunction with other criteria, may broaden the samples selected for testing,
1118 used alone it may exclude a substantial proportion of otherwise identified cases
1119 (Public Health England, 2017c). Varied and inconsistent selective testing may
1120 contribute to underestimation of incidence, spurious distribution patterns and
1121 transmission being less well understood.

1122 *Test methods used*

1123 Choice of test may be influenced by several attributes including, automation, technical
1124 skill required/available, the ability for multiple pathogens to be tested for
1125 simultaneously, national guidelines, and costs. The majority of laboratories continued
1126 to use microscopy to detect *Cryptosporidium*. Auramine phenol stained microscopy

1127 remained the most frequently used method in England and Wales, used by 49% (CI
1128 35.0 - 63.0) laboratories. This was however lower than the 64% (52.9-73.0) reported
1129 in the preceding audit in 2013-2014 (Chalmers *et al.*, 2015) suggesting this is
1130 decreasing in popularity.

1131 There has been increasing use of EIAs over the past decade from 2.5% (CI 1.1 – 5.7)
1132 of laboratories in 2008 (Chalmers, 2008) to 22% (CI 12.5 – 36.3) in this study.
1133 Microplate format EIAs enable higher throughput than stained microscopy, which
1134 became important when diagnostic services in the UK were centralised into fewer
1135 laboratories testing more specimens.

1136 The biggest change highlighted by this audit was the more widespread use of PCR;
1137 18% (CI 9.3 – 31.3) of laboratories compared with just one laboratory in the 2013-
1138 2014 survey (Chalmers *et al.*, 2015). This is likely to be an accelerating development
1139 as 12 laboratories reported that they expect to introduce PCR testing in the near
1140 future. Similar to EIA, this is a high-throughput test with lower training requirements
1141 compared to microscopy. Whereas positive reactions generated by EIA should be
1142 confirmed for *Cryptosporidium*, and all laboratories reported doing so even though
1143 the methods used varied, there is no such requirement for PCR. Although all the PCR-
1144 adopted laboratories that answered this audit used the same test, we are aware that
1145 other tests are in use. Our results are perhaps biased by over-representation of
1146 responses from Wales where a Welsh Government initiative enabled harmonised
1147 adoption of the EntericBio Gastro Panel 2 in four of the seven laboratories in 2018.

1148 Approaches and changes in testing for *Cryptosporidium* may influence surveillance
1149 numbers, and tests with greater sensitivity may more closely reflect true incidence.
1150 Taking a multiplexed approach to pathogen testing, as offered by EIA
1151 (*Cryptosporidium* and *Giardia* are commonly combined in these assays) and the
1152 gastrointestinal diagnostic PCR panels, may accommodate loosening of any selection
1153 and testing criteria for individual pathogens. The effects of this have been indicated
1154 (Ellam *et al.*, 2008) and will become clearer over time. We know the detection ability
1155 of these tests differ, with immunofluorescence microscopy (IFM) sensitivity around
1156 97.4%, more than other standard tests applied (D P Casemore, Armstrong and
1157 Sands, 1985) and mZN or AP perhaps less able to detect parasite oocysts after
1158 clinical symptoms had ceased (R. Chalmers *et al.*, 2016) (24). Five out of the 46
1159 laboratories reported making recent changes to practice, four of which were moves
1160 to PCR from prior methods so increases in numbers may become apparent, as a
1161 move to PCR may allow criteria to be loosened.

1162 *Referrals for species identification*

1163 Over three quarters of responding laboratories referred samples to the CRU for
1164 species identification, and subtyping in outbreaks, and most of the remainder reported
1165 that they would be willing to do this in the future. However, there may be some bias
1166 in responses towards the most engaged laboratories, and important gaps in referrals
1167 (for example in the south west, Yorkshire and London) have been identified by the
1168 reference unit (unpublished data; personal communication Prof. R Chalmers). We will
1169 continue efforts to encourage referral, to coincide with the introduction of automated
1170 data capture from the reference unit that will better enable systematic national
1171 surveillance, ensure characterisation of outbreaks, and improve our understanding of
1172 *Cryptosporidium* epidemiology and transmission.

Recommendations

1173 Despite cautious interpretation of the results, we continue to reiterate the message
1174 supported by the SMI and recommend that stool consistency not be used as a
1175 selection criterion for testing for *Cryptosporidium* and that it is included in the primary
1176 test set for the investigation of gastroenteritis. Additionally, we encourage the revision
1177 of selection criteria especially where applied inconsistently, for example age groups.

1178 *Limitations*

1179 The overall response rate was 50%, perhaps reflecting survey overload, which had
1180 been reported anecdotally. Whilst the results generated important findings and allow
1181 recommendations for best practice to be made, a key limitation is the low and
1182 regionally variable response rate. Results may therefore not be applicable across the
1183 country. All seven microbiology laboratories in Wales responded and so a good rate
1184 of capture here is informative.

1185 *Acknowledgements*

1186 We wish to thank all the laboratory staff who provided the information.
1187 Special thanks to Andrew Fox, Public Health England, for support with
1188 dissemination and distribution of the survey, and engagement with
1189 laboratories.

1190 **Summary**

1191 The audit conducted across a sample of England and Wales laboratories highlights
1192 that the sensitivity of methods is increasing and that fewer laboratories are using stool
1193 consistency as a selection criterion. However, selection criteria were still applied, and
1194 testing was still below the expected and recommended amount, with regional
1195 differences in the proportion of laboratories selectively testing stools.

1196 In that sense, we can be fairly confident the data reported by the surveillance systems
1197 in these two countries is accurate for those cases presenting at healthcare.
1198 Nonetheless, some inconsistencies do still exist, and the audit does demonstrate that
1199 we are likely seeing some presentation and testing biases in specific groups
1200 (immunocompromised, younger children). Although this does help us to capture the
1201 most vulnerable of patients, and detect outbreaks, there are likely to be other
1202 unnoticed sporadic cases in the general population. Additionally, genotyping and
1203 characterisation of samples does not always take place, leading to biases in our
1204 understanding of the different contribution of transmission pathways to disease
1205 burden. Frequently stool consistency is used as a criterion, even though this is known
1206 to be an unreliable predictor of *Cryptosporidium* positivity (D. P. Casemore,
1207 Armstrong and Sands, 1985). Reassuringly, in our audit, we observed that a
1208 decreasing number (16%) of laboratories used stool consistency as a criterion.

1209 **Next steps**

1210 The previous chapters have allowed an exploration of the epidemiology of
1211 *Cryptosporidium*, including transmission pathways that we usually see in outbreaks
1212 in the UK, and testing practices that might mean we are missing cases.

1213 It is clear that the ascertainment of this pathogen is below 100% and it is likely that
1214 there are more community cases than we actually see. We know exposures are often
1215 only investigated in outbreak situations. A better understating of the sporadic cases
1216 that often go un-investigated might shed light on risks and exposures for infection.

1217 From these introductory chapters, I propose that:

- 1218 • There are likely to be uncaptured sporadic cases in the general population
- 1219 • Sporadic cases' exposures for infection might be different than the ones we
1220 usually consider
- 1221 • Exposures and transmission pathways may be different between *C. parvum*
1222 and *C. hominis*

1223 In the next chapter, Chapter 5, I go on to present a systematic review, which helps
1224 to highlight some of the main transmission pathways likely to drive infection. I use
1225 this chapter overall to consider remaining gaps in our knowledge and propose
1226 next steps for investigation. This helps to meet my first objective, to understand
1227 the distribution and burden of this infection in the UK, as well as considering
1228 detection and approaches to testing that underpin the surveillance data.

Chapter 5

Exposures associated with
sporadic infection with
Cryptosporidium in industrialised
countries: a systematic review

1229 **Introduction**

1230 We know from previous chapters that reported exposures for both *C. parvum* and *C.*
1231 *hominis* often overlap, and include consumption of contaminated drinking water and
1232 exposure to recreational waters, (Stafford *et al.*, 2000; Louie *et al.*, 2004; McCann *et*
1233 *al.*, 2014) and food-related outbreaks (Ethelberg *et al.*, 2009; Robertson and
1234 Chalmers, 2013; Åberg *et al.*, 2015; McKerr *et al.*, 2015). *C. parvum* is frequently
1235 associated with exposure to farm animals (Hoek *et al.*, 2008; Utsi *et al.*, 2016) and *C.*
1236 *hominis*, more anthroponotic, with person-to-person spread (Hannah and Riordan,
1237 1988; Newman *et al.*, 1994; Nichols *et al.*, 2006; Johansen *et al.*, 2014). However,
1238 exposures are often identified during the course of outbreak investigations, which may
1239 only represent a small proportion of cases (Chalmers and Giles, 2010). As a
1240 consequence, we cannot be certain that transmission pathways for sporadic disease
1241 are the same (Bouzid *et al.*, 2013). Despite case control studies which have
1242 investigated differences in risk for endemic and outbreak disease (P. R. Hunter and
1243 Thompson, 2005; Yoder, Harral and Beach, 2010), pathways to sporadic infection are
1244 still unclear and a substantial subset of reported cases remain unexplained.

1245 Given the absence of any systematic synthesis of reported evidence in the UK, and
1246 the few number of reviews in other industrialised countries, the aim of this work was
1247 to search the literature to describe exposures associated with sporadic infection with
1248 *Cryptosporidium* in industrialised countries.

1249 Research question: In industrialised populations, what exposures are associated with
1250 sporadic human *Cryptosporidium*?

1251 This work contributes to my second objective to examine exposures most associated
1252 with sporadic disease, and calculate how much these contribute to infection.

1253 **Methods**

1254 This review has been registered: PROSPERO number CRD42017056589

1255 Where relevant, methods followed recommendations made in the Cochrane
1256 Handbook for Systematic Reviews of Interventions (Higgins JPT and Collaboration,
1257 2011) and reporting followed guidance from “Preferred Reporting Items for
1258 Systematic Reviews and Meta-Analyses” (PRISMA) (Moher *et al.*, 2009; Shamseer
1259 *et al.*, 2015).

1260 **Search strategy**

1261 The search terms included the following MeSH terms/ keywords: (*Cryptosporidium*
1262 OR cryptosporidiosis) AND (epidemiolog* OR risk factors OR exposure OR
1263 transmission OR association) OR (cohort OR case-control OR “case control” OR
1264 case-crossover OR “disease outbreaks” OR meta-analysis OR longitudinal OR
1265 ecological). (Appendix 3)

1266 The last published search date was 15th May 2018, and grey literature on 21st May
1267 2019.

1268 Three steps were used to identify the literature including electronic database
1269 searching; reference list trawling from relevant papers; and an exploration of the grey
1270 literature. The choice of databases was following advice from a University of Liverpool
1271 Medicine and Dentistry Liaison Librarian, as those deemed to be most relevant to the
1272 re-search question and likely to yield the highest number of relevant papers.

1273 *Step one—peer-reviewed literature*

1274 One reviewer (CMCK) conducted electronic searches in the following databases of
1275 published literature considered most likely to yield the relevant papers:

- 1276 • PubMed
- 1277 • Web of Science
- 1278 • Scopus
- 1279 • Embase

1280 Search terms were sought within the title, abstract, and keywords of the documents
1281 contained in each database. Filters within the three databases were applied if
1282 required to restrict the results as appropriate according to inclusion criteria. The
1283 publications captured using the final agreed search terms were exported into
1284 reference managing software (Mendeley) and duplicates removed. The remaining
1285 publication titles will then be screened for relevance by two reviewers (CMCK and
1286 WS), using the inclusion and exclusion criteria.

1287 *Step two — hand searching in papers*

1288 Reviewers (CMCK and WS) will search reference lists to identify any further literature
1289 or relevant publications not previously captured in the other strategies. The abstracts
1290 of any references considered potentially relevant were sought and screened for
1291 inclusion using the inclusion and exclusion criteria.

1292 *Step three—accessing grey literature*

1293 One reviewer (CMCK) accessed grey literature relevant to the review question using
1294 published online resources which included bulletins and reports from relevant
1295 agencies, conference proceedings, and other relevant published outputs. A search of
1296 Google Scholar (and any other relevant agencies' sites, e.g. WHO) was undertaken
1297 by entering the term 'cryptosporidium' with 'risk factors', 'outbreak(s)', 'sporadic',
1298 'endemic', and/or 'transmission' into the application and reviewing the first 100 results
1299 for relevance. Using the same search terms and inclusion criteria, the same reviewer
1300 carried out an additional search for un-published theses work in the ProQuest
1301 database. Following agreement on inclusion, the work would be reviewed as per
1302 protocol. To refine and clarify the inclusion criteria and search terms and ensure that
1303 the criteria were applied consistently, the selection process was piloted by applying
1304 criteria to a sample of papers.

1305 **Abstract and paper selection**

1306 Following title selection, abstracts of the final included publications were screened
1307 independently by two members of the review team (CMCK and WS) to ensure
1308 consistency in the application of the inclusion and exclusion criteria. Any
1309 discrepancies were discussed and re-examined until we reached an agreement. A
1310 third reviewer (KP) was available for irreconcilable opinions on inclusion. The full texts
1311 for all included works were retrieved via the online library where possible and, if
1312 required, with the help of the University Liaison Librarian or by contacting authors. All
1313 full-text studies were screened independently by the same reviewers (CMCK and AW)
1314 to ensure that they conformed to the inclusion and exclusion criteria and
1315 discrepancies tackled as before. Full-text papers, which appeared in a language other
1316 than English, were shared with colleagues in the Health Protection Research Unit
1317 (HPRU) and wider university teams for assistance with translation. Searching ceased
1318 when no further relevant and/or not previously identified work was discovered

1319 **Eligibility and inclusion**

1320 Databases were initially searched with no restriction on year of publication. There
1321 were no restrictions on language, provided the abstract was available in English for
1322 the first round of screening.

1323 Studies conducted in industrialised countries (defined using OECD category of
1324 countries (*UNICEF National Committees, 2017*) and reporting on human subjects

1325 were included. All observational studies were included where they reported
1326 exposures and relevant quantitative results. Individual case reports were excluded.

1327 **Study selection process**

1328 Results were managed using the Covidence tool (Veritas Health Innovation, 2018).
1329 Screening of results and title screening was undertaken in duplicate by two reviewers,
1330 to ensure consistency in the application of the inclusion and exclusion criteria. There
1331 was a change in second reviewer during the abstract review however, the first and
1332 third reviewers did not change which maintained consistency. Discrepancies were
1333 discussed and re-examined, and a third reviewer was available for irreconcilable
1334 opinions on inclusion. Two papers were sent to the third reviewer for decision.

1335 **Data management**

1336 Data were extracted in duplicate using a standardised form developed in MS Access.
1337 The minimum data set for data extraction is available in Appendix 4. Data items were
1338 merged, and discrepancies discussed, prior to the agreement of which data were
1339 used for analysis.

1340 Studies were allocated a unique identifier (automatically generated) and categorised
1341 according to the following groups:

- 1342 • Included studies—studies that meet the eligibility criteria and are included in
1343 the review
- 1344 • Excluded studies—studies that do not meet the eligibility criteria and are
1345 excluded from the review
- 1346 • Studies awaiting classification—relevant studies that have been identified but
1347 cannot be assessed for inclusion until additional data or information are
1348 obtained
- 1349 • Ongoing studies—studies that are ongoing and meet(or appear to meet thus
1350 far) the eligibility criteria

1351 **Quality assessment**

1352 Papers were scored using the Newcastle-Ottawa Scale (NOS) (Wells *et al.*, 2000).
1353 This instrument is well piloted and is specific to non-randomised study types. The
1354 NOS was completed independently by both reviewers for all studies. Results were
1355 then amalgamated, and areas of discrepancy discussed prior to agreement on final
1356 scores. This instrument provides an overall judgement on quality using a scoring

1357 system by evaluating three parameters (selection, comparability, and outcome)
1358 across eight specific domains. The maximum score for each study is eight: four stars
1359 for selection, one star for comparability and three stars for exposure/outcome
1360 domains could be awarded if factors were unlikely to introduce bias. The studies were
1361 considered of high quality if NOS score was 6–8 stars (Bouzid, Kintz and Hunter,
1362 2018), moderate quality for a score of 5 stars and studies having fewer than five points
1363 considered at high risk of bias (Luchini *et al.*, 2017).

1364 **Data synthesis**

1365 Data were summarised presenting the papers' main findings including population
1366 under study, outcome(s) measured/case definition, effect measures and reported
1367 statistics, and exposures. Exposures, depending on how they were measured, were
1368 grouped into transmission pathways, following discussion and consensus with
1369 authors. Broadly the pathways considered included water, animals, food, and person-
1370 to-person spread. We further sub-categorised exposures following discussions with
1371 the study group, in order to better represent specific known transmission risks for
1372 *Cryptosporidium*, with possible differences between species highlighting the
1373 anthroponotic versus mostly zoonotic pathways.

1374 We did not proceed with a traditional meta-analysis as for each exposure, as fewer
1375 than five studies were included.

1376 We proceeded with a visual and narrative analysis of results for each exposure in the
1377 transmission pathways. Where appropriate, available data were pooled in a visual
1378 analysis combining the exposures across studies using GraphPad Prism 8.3.0
1379 (GraphPad Software, 2019). Where a usable effect measure was not reported in the
1380 study, but data were available, odds ratios or relative risks were calculated. Where
1381 available, the multivariable-level effect measure was used in preference to the crude
1382 to ensure where possible we were using adjusted variables which remained
1383 significant predictors of infection after controlling for covariates. All results whether
1384 statistically significant or not, and those either showing the exposure as increasing or
1385 reducing risk of outcome, were considered in the analysis. For those studies where
1386 we used multivariable analysis, all results will have remained in the final, most
1387 parsimonious model.

1388 **Changes to the published protocol**

1389 The full methods and approach have previously been reported and a copy of the
1390 published manuscript is provided in Appendix 2 (McKerr *et al.*, 2018).

1391 Two changes were made to the published protocol approach, which are described
1392 and justified below.

1393 *Additional exclusion criteria*

1394 Following full text review, additional restrictions on type of cases and time period were
1395 applied to refine the selection of papers taken forward for data extraction.

1396 It became apparent to the authors that many of the included papers described
1397 (mostly) outbreaks in detail, with very different, but specific, settings and widely
1398 variable metrics for exposures. This made it difficult to synthesise meaningfully the
1399 papers' outcomes within the scope of this review. Additionally, outbreak-focused
1400 papers were often specific to one event, exposure, or setting, and posed the
1401 additional problem of more granular exposures and led to difficulties with quantifying
1402 direct exposure. As the focus of the review was sporadic, community cases, we
1403 applied additional criteria after full-text screening of papers to remove those
1404 describing outbreak investigations.

1405 Additionally, the older studies were less relevant for describing contemporary risks,
1406 and often traversed changes in policy and practice (such as changes to municipal
1407 water supply regulations) which may contribute to changes in reported exposures for
1408 disease over time. Therefore, in order to focus the synthesis on the most relevant and
1409 contemporaneous data we restricted inclusion at the data extraction stage to reports
1410 within the last ten years (2008-18).

1411 *Change of ROB tool*

1412 In the original protocol, I intended to employ ROBINS-I as a tool to assess risk of bias
1413 (Sterne *et al.*, 2016). When I began to pilot this tool to the detail in the papers that I
1414 had extracted, I felt that it did not fit the scope, as I was not looking at interventions.
1415 Additionally, I had had a change of reviewers in the team, a new reviewer with no
1416 experience of the ROBINS-I tool, and less capacity meant that we had to rethink our
1417 resource. I considered amending the tool for exposures, but following changes in
1418 capacity of the review team, and a discussion with the systematic review team at
1419 LRiG at University of Liverpool, I decided to use the NOS tool. This is well-validated,
1420 straightforward to use, and did not require un-validated amending. A key reason for
1421 using the very structure quality assessment ROBINS-I tool was to support meta-

1422 analyses, but as we were not proceeding with these fairly early on, myself and the
1423 team were satisfied that NOS was a suitable fit for this work. I go on to consider
1424 limitations of the NOS later in the systematic review discussion section.

1425 **All changes were considered by the full review team, and all reached**
1426 **agreement.**

1427 **All protocol changes have been registered on Prospero, and are approved.**

1428 **Results**

1429 The combined initial database search retrieved 2,115 articles. An additional article
1430 was found by screening reference lists, and one pre-print was included following a
1431 routine refresh of the search terms on PubMed. This reduced to 1,797 after removal
1432 of duplicates. The inclusion of Embase did not yield any additional papers not
1433 captured by PubMed, Web of Science, and Scopus.

1434 Based on title and abstract screening, 338 full-text articles were procured and
1435 retained for potential inclusion (Figure 10: PRISMA diagram showing manuscript
1436 capture and inclusion).

1437 After full-text screening, 208 peer-reviewed papers were excluded and the remaining
1438 130 taken forward to data extraction. We excluded papers which were part of wider
1439 studies and which reported on the same aspects of the study to prevent inappropriate
1440 weighting to one study reported multiple times.

1441 The application of additional selection criteria (as previously described) at this stage
1442 were two-fold:

- 1443 • To restrict the inclusion of papers to the past 10 years
- 1444 • To restrict the review to studies of sporadic disease

1445 These further restrictions resulted in the inclusion of eight articles (comprising 11
1446 studies), of which seven (comprising 10 studies) were suitable for further synthesis.
1447 The single paper excluded from any quantitative analysis did not have enough
1448 information for further analysis or calculations, and no reply was received from
1449 authors to our request for additional data.

1450 None of the identified grey literature articles were included (searched between May
1451 and Sept 2019) as they all described outbreaks and/or were outside the time. Six

1452 relevant theses were captured but were out of area or outside the time scope or were
1453 part of published papers we had already captured.

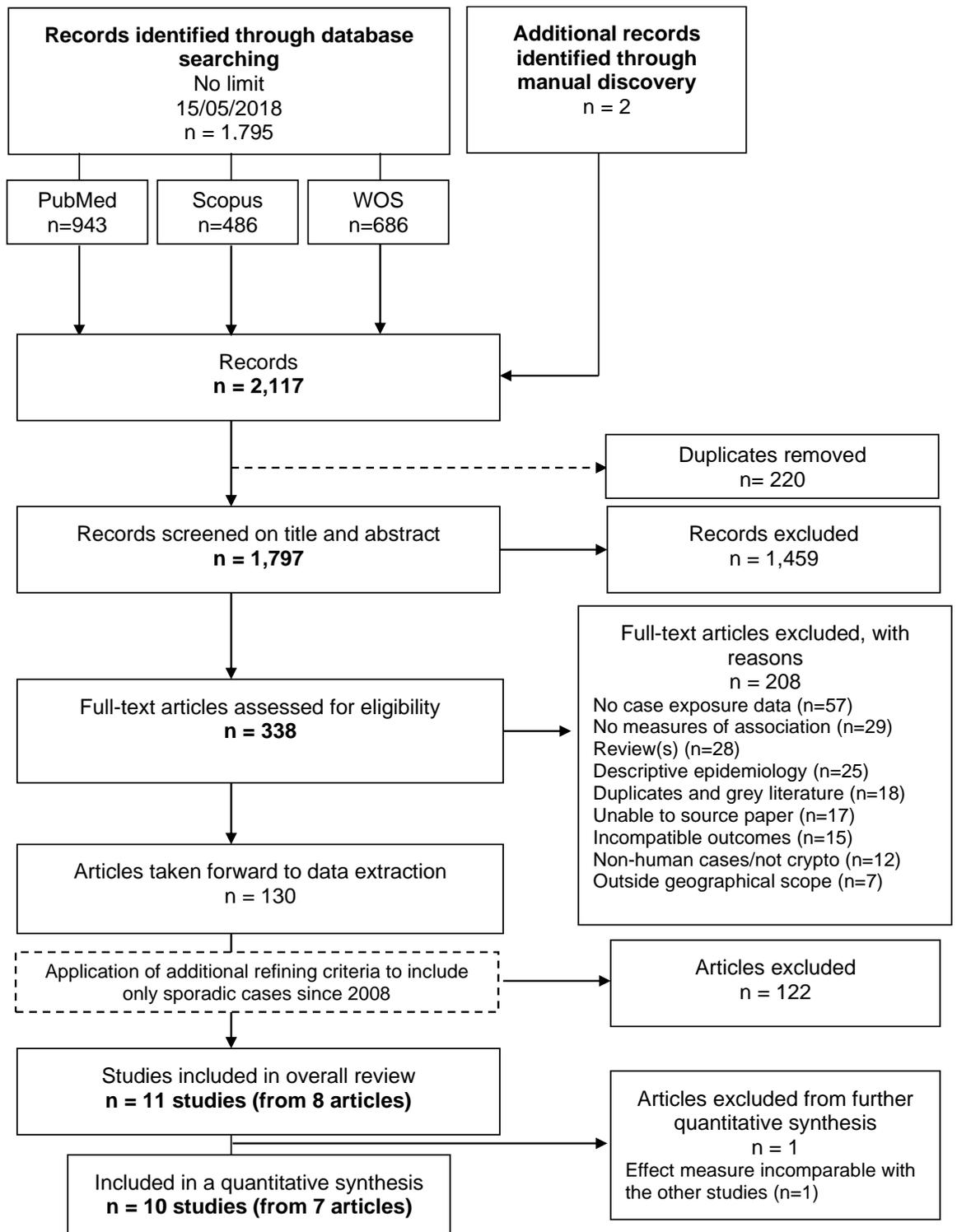


Figure 10: PRISMA diagram showing manuscript capture and inclusion

1454 **General characteristics of included studies**

1455 A total of eight papers describing 11 individual studies⁴ were included for review and
1456 potential analysis. Table 5 shows the NOS scores allocated to each of the articles,
1457 and reports on general strengths and weakness of the study designs. (For the
1458 Tollestrup cohort studies across three sites, (Tollestrup *et al.*, 2014) the overall article
1459 score was taken, as the study designs were the same and thus of identical quality).

1460 Almost all studies (n=7) were at least of moderate quality, although some specific
1461 biases were observed in the individual domains. Non-response rate in particular, and
1462 the description of and differences in responses in participants, was of some concern
1463 in almost all of the studies. A key quality aspect was risk of bias in the ascertainment
1464 of the exposure and its comparability to case exposure. In a couple of the articles, it
1465 was felt that case representativeness introduced a likely bias.

1466 Table 7 shows the main characteristics of the included studies.

1467 Four of the studies described case-control methods; two were case-case studies and
1468 one cross-sectional. Four studies (across two papers) reported outcomes based on
1469 serology and thus were classed as serological studies. All but one of the studies had
1470 over 100 participants⁵, with three comprising several thousand participants – these
1471 tended to be those that selected cases from large, nationwide surveillance databases.
1472 Study year ranged from 1999 to 2017 and the studies were conducted in five, large
1473 countries in three continents: Europe (UK and the Netherlands), North America (USA,
1474 Canada), and Australia. The European focus on the UK and the Netherlands might
1475 be explained by differences in the approach to detection and legislation (Putignani
1476 and Menichella, 2010).

⁴ The Fournet et al paper described three studies from the UK, Germany, and the Netherlands: we excluded the Germany study due to lack of available data and report this paper as two separate studies from the UK and the Netherlands.

The Nic Lochlainn et al paper includes only results for the overall study period, not individual years, to avoid duplicate counting.

The Tollestrup et al. paper described one large cohort study of 600 participants, but analysed data by residence of participants across three sites: we report results for the Tollestrup paper as three individual studies.

⁵ Study numbers for the smaller case-case study in the Fournet (2) UK paper were not reported

1477 Table 5: NOS scores allocated to each of the articles, and strengths and weakness of the study design

Study design	Author/ Year	Overall NOS Score (quality)	Strengths	Weaknesses
Cross-sectional	Ravel (2012)	6 (moderate)	<p>Surveillance data provided a rich data set which can be accessed with minimal resource burden</p> <p>Multiple exposures could be investigated</p> <p>Authors were able to clearly exclude known outbreak-related cases</p>	<p>Surveillance data can bias participation as only included captured cases</p> <p>Relevant to a specific area, which may decrease generalisability of results</p> <p>Compared cases to other enteric pathogens, so the exposure results are relative to other IID (with similar pathways)</p>
Case-control	NicLochlainn (2018)	5 (moderate)	<p>Timely administration of questionnaire so recall of past exposure(s) may be more accurate</p> <p>Laboratory diagnosed so less chance of misclassification of cases</p> <p>Large-scale and representative</p>	<p>Surveillance data can bias participation as only includes captured cases</p> <p>Exposures reported over 3 years, separately but lack of raw data means we could only use the final model</p> <p>Possibility of selection bias among those who completed and returned the exposure questionnaire</p>

Study design	Author/ Year	Overall NOS Score (quality)	Strengths	Weaknesses
Case-control	De Gooyer (2017)	5 (moderate)	<p>Frequency matching allows control for any confounding role of age</p> <p>Had a clear hypothesis for exposure and outcome</p> <p>Collect exposure data 14 days prior to illness, comparable with <i>Cryptosporidium</i> exposure window</p>	<p>Small numbers affects power and representativeness of results</p> <p>Followed an increase in cases that was not classed an outbreak, but may bias results towards a point source</p> <p>Controls were contacted in a different time period to cases which may introduce recall biases</p>
Case-control	Fournet (1) NL (2013)	7 (high)	<p>Controls randomly selected and representative</p> <p>Good control to case ratio</p> <p>Matching allows control for any confounding role of age and sex</p>	<p>Followed an increase in cases that was not officially classed an outbreak, but may bias results towards a point source</p>
Case-control	Valderrama (2009)	5 (moderate)	<p>Two controls per case</p> <p>Matching allows control for any confounding role of age and geography</p> <p>Controls randomly selected and representative</p>	<p>Low response rate/small numbers</p> <p>Followed an increase in cases that was not classed an outbreak, but may bias results towards a point source</p> <p>Controls were contacted in a different time period to cases which may introduce recall biases</p>

Study design	Author/ Year	Overall NOS Score (quality)	Strengths	Weaknesses
Case-case	Fournet (2) UK (2013)	4 (low)	<p>Surveillance data provides a rich data set which can be accessed with minimal resource burden</p> <p>Multiple exposures could be investigated</p>	<p>Followed an increase in cases that was not classed an outbreak, but may bias results towards a point source</p> <p>Compared current cases to case in previous years, so identified exposures may reflect an undetected outbreak or single source</p> <p>The case-case study was in a small area and the authors did not report numbers so results may not be generalisable</p>
Case-case	Pintar (2009)	5 (moderate)	<p>Looked at a reasonably long time period</p> <p>Authors were able to exclude travel and outbreak cases</p> <p>Laboratory diagnosed so less chance of misclassification of cases</p>	<p>Compared cases to other enteric pathogens, so the exposure results are relative to other IID (with similar pathways)</p> <p>Used smaller surrogate models due to low power</p>

Study design	Author/ Year	Overall NOS Score (quality)	Strengths	Weaknesses
Serological	Becker (2015)	6 (high)	<p>Used a well-established, representative and systematically collected dataset (NHANES)</p> <p>Case was defined as a positive IgG response to both markers, strengthening the case definition</p> <p>Large numbers</p>	<p>Restricted to ages 6-49 which might exclude key age groups</p> <p>This type of large-scale data collection might bias towards a certain characteristic(s) or responder</p> <p>General methodological issues with serology studies for <i>Cryptosporidium</i>, including;</p> <ul style="list-style-type: none"> - Increasing response with age - Hard to measure impact of non-transient exposures - Not clear when infection or disease occurred so difficult to establish accurate exposure window - Cannot distinguish species

Study design	Author/ Year	Overall NOS Score (quality)	Strengths	Weaknesses
Serological	Tollestrup (2014)	4 (low)	<p>At least one study ran for more than a year</p> <p>Good study size</p> <p>Able to collect environmental data and blood samples</p> <p>Multiple exposures could be investigated</p>	<p>Restricted to adults only which might exclude key groups</p> <p>General methodological issues with serology studies for <i>Cryptosporidium</i>, including;</p> <ul style="list-style-type: none"> - Increasing response with age - Hard to measure impact of non-transient exposures - Not clear when infection or disease occurred so difficult to establish accurate exposure window - Cannot distinguish species <p>Cannot establish temporal association between exposure and outcome - markers may reflect chronic, long-term exposure(s)</p> <p>Environmental samples collected after exposure</p> <p>Convenience sampling may introduce selection bias</p>

1478 Table 6: Hypothesis and main exposures investigated in each of the included studies

Study ref	Study period	Country	Study design	Hypothesis/ main exposures investigated
Ravel <i>et al</i> (Ravel <i>et al.</i> , 2013)	2005-2009	Canada	Cross-sectional	Clinical, demographic and exposure variables associated with sporadic, domestically acquired parasitic disease. Cases were assigned transmission routes based on: animal-person, environment, water, food, person-person routes
NicLochlainn <i>et al</i> (Nic Lochlainn <i>et al.</i> , 2019)	2013-2016	Netherlands	Case-control	General risk factors for sporadic cryptosporidiosis: demographics, symptoms, medical history, foreign travel, contact with animals, contact with ill persons, recreational activities, and food and drink consumption
de Gooyer <i>et al</i> (de Gooyer <i>et al.</i> , 2017)	February - March 2015	Australia	Case-control	To identify risk factors associated with region-wide increase in cryptosporidiosis with specific focus on recreational water activities as a hypothesis
Fournet <i>et al</i> (1) NL (Fournet <i>et al.</i> , 2013)	2012 (late summer)	The Netherlands	Case-control	To identify risk factors for an increase in sporadic cases compared to previous years, with a focus on accepted transmission routes: farm animals, recreational water, drinking water, including bottled, and travel history

Study ref	Study period	Country	Study design	Hypothesis/ main exposures investigated
Valderrama <i>et al</i> (Valderrama <i>et al.</i> , 2009)	August -September 2007	USA	Case-control	To identify risk factors for an increase in sporadic summer cases, specifically for community disease. Exposures included food and water consumption, recreational water, childcare, animal contact, person-to-person contact, and travel history
Fournet <i>et al</i> (2) UK (Fournet <i>et al.</i> , 2013)	weeks 32 to 42 of 2012	UK (North-East region)	Case-case	To identify risk factors for sporadic cases compared to previous years, with a focus on accepted transmission routes: farm animals, recreational water, drinking water, including bottled, and travel history
Pintar <i>et al</i> (Pintar <i>et al.</i> , 2009)	2005 - 2007	Canada	Case-case	Determining exposures for sporadic, endemic disease - addressed three routes of exposure based on a priori hypotheses: recreational water, environmental, and person-to-person transmission
Becker <i>et al</i> (Becker, Oloya and Ezeamama, 2015)	1999-2000	USA	Serological study (with cross-sectional approach)	Investigated correlates of social inequality (food adequacy, annual income, and the poverty income ratio) against the odds of positive serological response

Study ref	Study period	Country	Study design	Hypothesis/ main exposures investigated
<p>Tollestrup <i>et al</i> (a,b,c)</p> <p>(3 studies at separate sites)</p> <p>(Tollestrup <i>et al.</i>, 2014)</p>	<p>Study sites:</p> <p>a: 2004 - 2005 b: August 2006-February 2007 c: May - October 2006</p>	<p>USA</p>	<p>Serological study (with cohort approach)</p>	<p>Investigated if wastewater from onsite systems and private water supplies correlated to serological response:</p> <p>Hypothesis: that participants living in households with onsite wastewater systems and private wells would be more likely to have elevated serological responses compared to those living in households using municipal systems (because of increased exposure to <i>Cryptosporidium</i> oocysts)</p>

1479

1480 **Transmission pathways and exposures investigated**

1481 In discussion with the review team, recognising the faecal-oral transmission route for
1482 *Cryptosporidium* infection in humans, the following transmission pathways were
1483 established capturing the predominant exposures measured in the included studies:

- 1484 • **Animals** – pets and farm animal exposures
- 1485 • **Food** – eating spoiled foods, raw or fresh produce, unpasteurised beverages,
1486 and proxy exposures such as eating outside the home, killing/preparing own
1487 meat
- 1488 • **Outdoor activities/environmental exposures** – activities such as gardening
1489 and hiking/camping, and environments such as living on a farm
- 1490 • **Person-to-person spread** – contact with a case (close contact,
1491 caring/toileting capacity, and sexual contact) and general social
1492 contact/activities
- 1493 • **Water** – drinking water and recreational water contact/water sports
- 1494 • **Travel** - any travel away from usual area of domestic residence
- 1495 • **Season** – season of infection, usually extracted from surveillance data

1496

1497 Main findings are found in Table 7, which outlines the main exposures measured (as
1498 defined by the studies) and the main results reported with a relevant transmission
1499 pathway added.

1500 The included papers measured over 200 exposures across the 11 studies. Exposures
1501 measured were multiple and varied in detail and definition across each of the studies,
1502 and even within studies. Generally, studies were exploratory in nature. The 2015 de
1503 Gooyer *et al* study in Australia (de Gooyer *et al.*, 2017) was centred on a specific
1504 hypothesis about exposure to recreational water, but the rest of the studies tended to
1505 look at myriad exposures, following the main known routes to infection for
1506 *Cryptosporidium*. Exposure to animals was a popular pathway, with six studies
1507 investigating this and both livestock/farm animal exposure and pets and domestic
1508 animal contact were well investigated. Food items were investigated in five studies
1509 and personal contact (with a case or general social contact) in seven. All bar one
1510 study investigated some water exposures, the most commonly investigated pathway
1511 for *Cryptosporidium* cases.

1512

1513 The Becker (2015) (Becker, Oloya and Ezeamama, 2015), Pintar (2009) (Pintar *et*
1514 *al.*, 2009), and Tollestrup (2014) (Tollestrup *et al.*, 2014) studies also included risk

1515 factors relating to patient/case demographics and characteristics. We include a short,
1516 narrative overview of this content.

1517

1518 *Clinical variables, case demographics and social factors*

1519 Pintar's 2005-7 case-case study of the Canadian Integrated Enteric Disease
1520 Surveillance System (C-EnterNet) reported that the odds of a *Cryptosporidium* case
1521 being between six and twelve years old was five times greater than the other enteric
1522 pathogens. The non-*Cryptosporidium* controls had an older profile than the cases
1523 (mean:cases=21.7 years, controls=31.8 years, $p=0.01$). Among the serological
1524 studies, increased odds of seropositivity was observed with increasing age: in those
1525 over 60 years old compared to a wider baseline of under 40 years at two sites in the
1526 Tollestrup studies, and with increasing age in the Becker study ($p<0.001$). However,
1527 it is well known that cases exhibit a bi-modal age distribution pattern and serological
1528 studies can report increased serological positivity with increasing age (Pollock and
1529 Ramsey, 2011), which may reflect time and intensity of exposure or elevated
1530 serological responses increasing likelihood of detection (Chalmers *et al.*, 2013).

1531 In the Tollestrup (b) study, the authors reported a reduced odds of positive serological
1532 response to *Cryptosporidium* antigens in people reporting higher education levels. In
1533 the higher income countries, sporadic illness is more often observed among the less
1534 deprived areas and communities (Reeve, no date; Snel, Baker and Venugopal, 2009).
1535 In these countries, rurality is associated with increasing wealth while more deprived
1536 areas tend to be located in the city. Additionally, associated activities such as
1537 swimming and travel are likely to be less prevalent in the more deprived areas and
1538 as such the profile of *Cryptosporidium* in relation to deprivation is converse to that in
1539 the less industrialised areas (Lake *et al.*, 2009; Bouzid, Kintz and Hunter, 2018).
1540 Conversely, Becker *et al* analysed data extracted from the USA National Health and
1541 Nutritional Examination Survey (NHANES)⁶, between 1999 and 2000, and found that
1542 correlates to *C. parvum* included several poverty and inequality measures including
1543 country of birth other than USA ($p<0.001$) and ethnic groups other than non-Hispanic
1544 whites ($p<0.001$). These results are supported by similar work reporting that
1545 Hispanics, African Americans, and women, all had greater odds of reporting
1546 *Cryptosporidium* seropositivity (Frost *et al.*, 2004). Whilst relative poverty alone is

⁶ NHANES is series of surveys which examines about 5,000 persons each year in the USA and is considered to be a nationally representative sample. The NHANES interview includes demographic, socioeconomic, dietary, and health-related questions as well as medical, dental, and physiological measurements (Centers for Disease Control and Prevention (CDC), 2017).

1547 unlikely to directly cause infection with *Cryptosporidium*, it might steer exposure to
1548 particular pathways or risks, or indeed reduce the availability of resources required to
1549 avoid exposure, either by personal behaviour/engagement, general health status or
1550 access to a health infrastructure (Snel, Baker and Venugopal, 2009; Ellis *et al.*, 2017).
1551 However, assessing individual risk alongside population-level characteristics often
1552 introduces fallibility, especially when using surveillance data which may introduce a
1553 bias in participants based on their access to healthcare. Further work on specific
1554 population-level characteristics would be useful and may help contextualise some of
1555 the individual-level relationships between these characteristics and exposure to
1556 infection.

1557 *Exclusions*

1558 Whilst it is important to note these demographic-focused findings, these parameters
1559 are likely to be risk factors associated with exposures to *Cryptosporidium*: we only
1560 used metrics that could be categorised into *Cryptosporidium* transmission pathways
1561 in the further analysis, thus excluding case characteristics.

1562 Three studies looked at travel as a risk for disease (any travel away from usual
1563 domestic residence) but most often it was an exclusion criterion in order to ensure
1564 investigation of endemic indigenous cases. *Cryptosporidium* is often observed as a
1565 non-viral cause of gastrointestinal illness in returning travelers (Okhuysen, 2001) and
1566 travel might subject people to risk exposures. As such it is not an exposure for disease
1567 intrinsically, and susceptibility may be linked to immunity, or lack thereof, of travelers
1568 on short term holidays in endemic areas (Shlim *et al.*, 1999) as well as increased
1569 exposures in areas with lower hygiene or more frequent use of swimming pool in
1570 holiday resorts. We excluded travel from any further detailed analysis.

1571 Additionally, there were insufficient papers reporting on season or outdoor activities
1572 in enough detail to analyse these any further.

1573

1574

1575 Table 7: Characteristics and main exposures or factors measured and main results

Study type	Study	Country	Transmission pathway (or risk factor)	Exposure measured	Measure of association (reported) ⁷	95% CI	p	Level
Cross-sectional	Ravel, 2013	Canada	Animals	Contact with household pets	Proportion exposed = 49	34-64	-	
			Animals	Visited a farm, a petting zoo or fair	Proportion exposed = 18	9-32	-	
			Food	Ate food prepared outside home	Proportion exposed = 43	28-58	-	
			Food	Ate meat from any place other than the grocery store	Proportion exposed = 26	14-40	-	
			Food	Drank/ate any unpasteurised milk, juice, or dairy products	Proportion exposed = 8	2-19	-	
			Food	Shopped at a supermarket	Proportion exposed = 93	82-99	-	
			Food	Shopped at butcher shop	Proportion exposed = 20	9-34	-	
			Food	Shopped at farm	Proportion exposed = 7	1-18	-	
			Food	Shopped at farmer's market	Proportion exposed = 4	1-15	-	
			Outdoor activities/environmental exposure	Canoed, kayaked, hiked, or camped	Proportion exposed = 16	7-30	-	
			Outdoor activities/environmental exposure	Gardening	Proportion exposed = 13	5-26	-	
			Outdoor activities/environmental exposure	Lived on a farm or country property	Proportion exposed = 30	18-45	-	
			Person-to-person	Attended social gatherings	Proportion exposed = 18	9-32	-	
			Person-to-person	Hosted or attended a barbeque	Proportion exposed = 33	20-49	-	
			Person-to-person	Knew anyone outside the household with a diarrhoeal illness	Proportion exposed = 13	5-25	-	
			Travel	Domestic travel	Proportion exposed = 100	69-100	-	
Water	Bottled water	Proportion exposed = 53	39-67	-				

⁷ Where available, multivariable results have been used (OR=odds ratio, aOR=adjusted odds ratio)

Study type	Study	Country	Transmission pathway (or risk factor)	Exposure measured	Measure of association (reported) ⁷	95% CI	p	Level
			Water	City water	Proportion exposed = 49	35-63	-	
			Water	Drank untreated/raw water	Proportion exposed = 11	4-24	-	
			Water	Private well	Proportion exposed = 31	19-46	-	
			Water	Swam in/gone into ocean, lake, river, pool, hot tub	Proportion exposed = 46	31-61	-	
			Water	Used an in-home treatment system for drinking water	Proportion exposed = 24	13-38	-	
Case-control	NicLochlainn, 2018 ⁸	Netherlands	Food	Ate tomatoes	aOR=0.6	0.5-0.8	0.001	MVA
			Food	BBQ food	aOR=1.8	1.4-2.3	0.001	
			Person-to-person	Household person-to-person transmission	aOR=2.2	1.7-3.0	0.001	
Case-control	De Gooyer, 2017	Australia	Person-to-person	Household member with diarrhoea	aOR=12.6	2.13-75.1	0.006	MVA
			Water	Drank bottled water	aOR=6.31	1.39-28.7	0.017	MVA
			Water	Waterpark	aOR=36.9	3.12-435	0.004	MVA
			Water	Spa use	aOR=26.4	1.47-472	0.026	MVA
			Water	Recreational water	OR=3.18	1.15-8.79	0.023	UVA
			Water	Waterpark	OR=73.5	6.74-802	0.000	UVA
			Water	Public pool	OR=1.07	0.44-2.60	0.89	UVA
			Water	Private pool	OR=1.96	0.65-5.96	0.225	UVA
			Water	Natural bodies of water	OR=1	0.31-3.25	1	UVA

⁸ Reports the 3-year overall study results only

Study type	Study	Country	Transmission pathway (or risk factor)	Exposure measured	Measure of association (reported) ⁷	95% CI	p	Level
Case-control	Fournet, 2013 (1) (NL study)	Netherlands	Water	Drank bottled mineral water	aOR=2.72	1.10-6.76	0.03	MVA
			Animals	Contact with farm animals	Difference in Proportions (case v control) = 33% vs 40%	-	0.3	UVA
			Travel	Travel	Difference in Proportions (case v control) = 36% vs 22%	-	0.03	UVA
			Water	Drank tap water on daily basis	Difference in Proportions (case v control) = 67% vs 78%	-	0.07	UVA
			Water	Exposure to swimming pool, sea, river or lake	Difference in Proportions (case v control) = 70% vs 66%	-	0.56	UVA
Case-control	Valderrama, 2009	USA	Food	Consumption of produce from farm/farm stand	aOR=0.2	0.1-0.9	-	MVA
			Person-to-person	Attending social event	aOR=0.4	0.1-0.9	-	
			Person-to-person	Contact with child in child-care or in diapers	aOR=3.8	1.5-9.6	-	
			Water	Drinking untreated water from lake, river, or stream	aOR=8.0	1.3-48.1	-	
			Water	Exposure to any recreational water	aOR=4.6	1.4-14.6	-	
Case-case	Fournet, 2013 (2) (UK study)	UK	Animals	Dog ownership	Difference in Proportions (year 2012 vs 2009-11) = 46% vs 25%	-	0.02	UVA

Study type	Study	Country	Transmission pathway (or risk factor)	Exposure measured	Measure of association (reported) ⁷	95% CI	p	Level
			Food	Ate food prepared outside the home	Difference in Proportions (year 2012 vs 2009-11) = 32% vs 4%	-	0.001	
			Travel	Travel	Difference in Proportions (year 2012 vs 2009-11) = 54% vs not reported	-	0	
			Water	Bottled water	Difference in Proportions (year 2012 vs 2009-11) = 11% vs 10%	-	0.44	
			Water	Swimming pool use	Difference in Proportions (year 2012 vs 2009-11) = 18% vs 37%	-	0.02	
Case-case	Pintar, 2009	Canada	Animals	Visited a farm	OR=1.6	1-2.5	0.032	UVA
			Case characteristics	Age 0-5	OR=2.8	0.84-9.1	0	UVA
			Case characteristics	Age 6-12	OR=5.5	1.7-18	0	UVA
			Case characteristics	Age 13-17	--	0-0	0	UVA
			Case characteristics	Age 18-24	OR=1.7	0.38-7.9	0	UVA
			Case characteristics	Age 25-39	OR=2.2	0.65-7.4	0	UVA
			Case characteristics	Age 40-59	<i>Ref grp</i>	--	--	--
			Case characteristics	Age >60	OR=0.8	0.15-4.6	0	UVA
			Food	Ate a ready-to-eat product	OR=2.1	0.5-9.3	0.276	UVA
			Food	Ate at a fast food restaurant	OR=1.5	0.7-3.4	0.273	UVA
			Food	Ate at a food vendor	OR=2.8	0.6-13	0.186	UVA
			Food	Killed own food	OR=2.3	0.5-10	0.245	UVA
			Food	Meat from butchers	OR=0.8	0.2-3.5	0.516	UVA
			Outdoor activities/environmental exposure	Hiking, camping, or canoeing	OR=2.1	0.8-5.7	0.135	UVA
			Outdoor activities/environmental exposure	Lived on a farm	OR=2.5	1.1-5.6	0.023	UVA

Study type	Study	Country	Transmission pathway (or risk factor)	Exposure measured	Measure of association (reported) ⁷	95% CI	p	Level
			Person-to-Person	Attending social event	OR=0.23	0.05-0.99	0	MVA
			Person-to-Person	Family member with diarrhoea	OR=2.86	1.28-6.38	0	MVA
			Person-to-Person	Non-family member with diarrhoea	OR=2.1	0.64-6.87	0	MVA
			Person-to-Person	Attended a social gathering	OR=0.3	0.1-1.1	0.068	UVA
			Person-to-Person	Family member ill	OR=3	1.4-6.3	0.003	UVA
			Person-to-Person	Non-family member with diarrhoea	OR=1.9	0.7-5.3	0.184	UVA
			Season	Autumn vs spring as ref grp	OR=5.8	0.74-46	0	UVA
			Season	Summer vs spring as ref grp	OR=5.6	0.74-42	0	UVA
			Season	Winter vs spring as ref grp	OR=1.4	0.08-22	0	UVA
			Water	Municipal water supply	OR=2.43	1.05-5.65	0.04	MVA
			Water	Swimming in natural water	OR=2.91	1.14-7.38	0	MVA
			Water	Municipal water supply (vs private)	OR=2.2	1-4.8	0	UVA
			Water	Swimming in untreated water lake or river	OR=3.1	1.4-6.7	0.004	UVA
			Water	Swimming pool	OR=1.9	0.8-4.8	0.154	UVA
Water	Went swimming	OR=3.8	1.8-8.2	0.001	UVA			
Serological	Becker, 2015	USA	Case characteristics	Age (year increments from 6-49 years)	aOR=1.06	1.05-1.07	<0.001	MVA
			Case characteristics	Black ethnicity (vs White)	aOR=1.88	1.42-2.53	0.001	
			Case characteristics	Country of birth Mexico (vs USA)	aOR=2.96	1.99-4.25	0.001	
			Case characteristics	Country of birth Other (vs USA)	aOR=2.27	1.53-3.28	0.001	
			Case characteristics	Hispanic ethnicity (vs White)	aOR=1.76	1.38-2.28	0.001	
			Case characteristics	Other ethnicity (vs White)	aOR=2.13	1.1-4.07	0.04	
			Other	Annual income more than \$45,000 (vs <\$25,000)	aOR=0.61	0.41-0.9	0.03	
			Other	Food adequacy (not enough vs enough)	aOR=1.31	0.67-2.59	0.65	

Study type	Study	Country	Transmission pathway (or risk factor)	Exposure measured	Measure of association (reported) ⁷	95% CI	p	Level
Serological			Other	Poverty income ratio high (vs low)	aOR=0.75	0.51-1.1	0.14	
			Water	Untreated drinking water (vs treated)	aOR=1.19	0.97-1.45	0.13	
	Tollestrup, 2014 (a)	USA	Animals	Handled livestock	OR=0.53	0.29-0.97	--	MVA
			Water	Private wastewater system/well	OR=1.98	1.11-3.55	--	
	Tollestrup, 2014 (b)	USA	Water	Private wastewater system/well	OR=1.28	0.74-2.21	--	MVA
			Water	Plumbing work done in home	OR=2.11	1.10-4.03	--	
			Animals	Handled pets	OR=2.83	1.24-6.49	--	
			Case characteristics	Age 18-39	<i>Ref grp</i>	--	--	
			Case characteristics	Age 40-49	OR=0.89	0.40-2.01	--	
			Case characteristics	Age 50-59	OR=1.8	0.80-4.03	--	
			Case characteristics	Age 60+	OR=4.2	1.79-9.89	--	
	Case characteristics	College graduate (vs not)	OR=0.37	0.19-0.72	--			
	Tollestrup, 2014 (c)	USA	Water	Private wastewater system/well	OR=0.85	0.49-1.46	--	MVA
			Case characteristics	Age 18-39	<i>Ref grp</i>	--	--	
			Case characteristics	Age 40-49	OR=1.76	0.74-4.18		
Case characteristics			Age 50-59	OR=1.92	0.83-4.43			
Case characteristics			Age 60+	OR=3.69	1.61-8.46			

⁹ Used the response to the 27-kDa antigen as a 'strong' indicator of infection which remains elevated for longer than the 15/17-kDa antigen (Ong et al., 2005). Used final model only

1576 **Transmission pathways**

1577 We excluded the entire Ravel *et al* (2013) study from any further analysis as measures
1578 reported were proportion exposed rather than odds or risk, and the data were not
1579 available to enable calculation of an effect measure that was comparable with the other
1580 studies. Ravel *et al* (2013) used Canada's C-EnterNet¹⁰ surveillance system to link
1581 confirmed cases of sporadic, domestically acquired *Cryptosporidium*, Giardia and
1582 amoebiasis with exposures grouped by the main pathways: Water,
1583 animal/environment, person-to-person, and exposure to high-risk food, analysing each
1584 infection separately. For cryptosporidiosis cases, travel within Canada (100%), contact
1585 with household pets (49%), and swimming (46%) were the most frequently reported
1586 exposures. The animal/environment-to-person transmission pathway remained the
1587 most important factor in *Cryptosporidium* cases' exposure(s) (72%). This was followed
1588 by water-based transmission routes (52%) and exposure to risk foods (50%). We were
1589 unable to look at odds of *Cryptosporidium* using the other cases (amoebiasis and
1590 giardiasis) as 'controls', given the differences in the system characteristics and the lack
1591 of individual response data.

1592
1593 We further sub-categorised the transmission pathways following discussions with the
1594 study group, in order to better analyse the underlying exposures for *Cryptosporidium*
1595 (Figure 11). This level of granularity in exposures helps highlight differences between
1596 anthroponotic and zoonotic pathways, important in recognising differences between
1597 infecting species and when considering targeted public health messages. Although this
1598 made the numbers of studies within each category lower, the team felt that aggregating
1599 to top-level pathways lost important detail that is imperative in understanding
1600 *Cryptosporidium*.

1601 As no exposure included more than four studies, a traditional meta-analysis was
1602 considered inappropriate (Bruce, Pope and Stanistreet, 2008). However, a visual
1603 analysis allows us to easily see which studies reported on the main underlying
1604 exposures investigated in each pathway and discuss each of the results in light of the
1605 characteristics of the studies.

¹⁰ C-EnterNet is an integrated enteric pathogen surveillance system based on a sentinel site surveillance model collecting information on both cases of infectious gastrointestinal illness and sources of exposure within defined communities.

1606 Ten studies (across seven papers) were included in a further analysis allocating the
1607 exposures investigated in the studies into the relevant transmission pathway (where
1608 we could allocate a main route).

1609 All results whether statistically significant or not, and those showing the exposure as
1610 increasing or reducing risk of outcome, were considered in the analysis. We used
1611 multivariable results where we had them for the comparable exposure. Charts show
1612 the study name, study sample size, measure of association reported, and confidence
1613 intervals. Black dots represent statistically significant results, and red insignificant.
1614 (Figures 12-21)

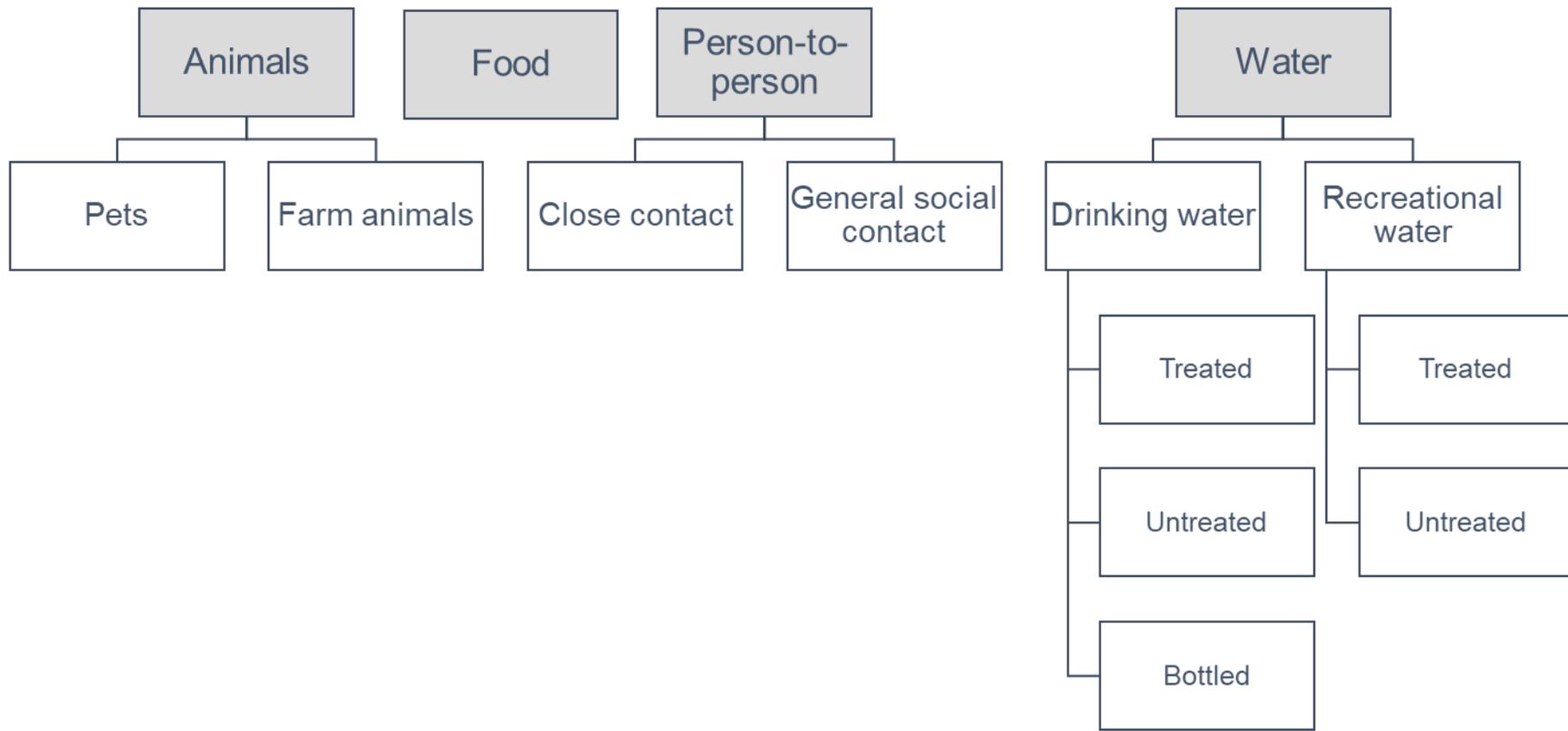


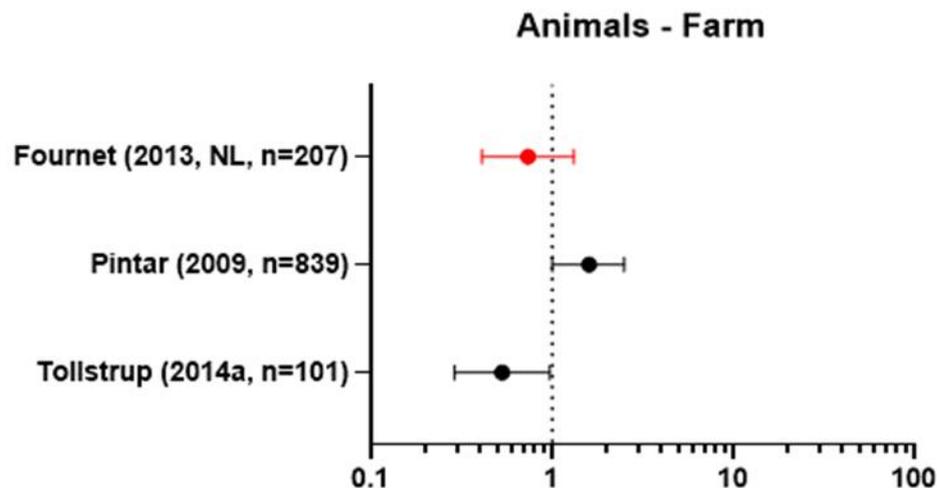
Figure 11: Transmission pathways and underlying exposures used to categorise variables measured in the included studies

1615 Results and discussion by transmission pathway

1616 Transmission pathway - Animal contact

1617 Animal contact exposure results were reported from five of the included studies. Animal
1618 exposures were categorised as livestock (n=3) and pets (n=2).

1619 Farm animals



1620 Figure 12: Exposure measured and results: Animal contact – Farm animals

1621 One study reported a statistically significant increased odds of disease (Pintar; OR=1.6;
1622 95% CI=1.1-2.5) and another reported reduced odds of seropositivity (Tollestrup (a),
1623 OR=0.53; 95% CI=0.29-0.97). The remaining study reported an insignificant result
1624 below 1.0 (Fournet (1), 2013; OR=0.74, 95% CI=0.41–1.32).

1625 The metric used in the Pintar study was “visited farm, petting zoo, or fair” which
1626 approximates animal contact but there was no detail about physical contact with
1627 animals. In their univariate analysis, they had an environmental exposure variable
1628 which was living on a farm. There is plausibility to this result, as direct contact with
1629 animals has been previously identified as an important risk factor for cryptosporidiosis
1630 (Kiang *et al.*, 2006; Grinberg *et al.*, 2011; Lange *et al.*, 2014), but this is often outbreaks
1631 and not sporadic disease.

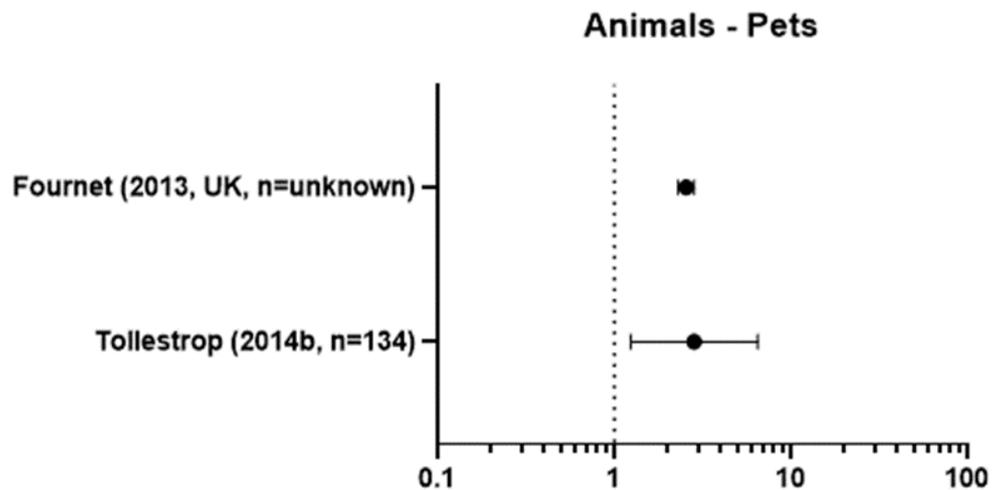
1632 The Tollestrup (study a) exposure measured direct handling of livestock and the study
1633 reported reduced odds. Although curious, this could be due to repeat or continuous

1634 exposure to the animals producing an elevated serological response in some
1635 participants. The use of a long-term marker, such as 27-kDa, as an outcome for
1636 demonstrating *Cryptosporidium* infection, has an impact on the interpretation of results.

1637 *Pets/Domestic animals*

1638

1639 Two studies¹¹ found significant positive associations with owning or handling pets.
1640 (Fournet (2), OR=2.56; 95% CI=2.3-2.84 and Tollestrup (b), OR=2.83; 95% CI=1.24-
1641 6.29).



1642 Figure 13: Exposure measured and results: Animal contact – Pets

¹¹ The Tollestrup (c) study did also investigate this exposure and it remained in the model; we have not reported it here with the other studies as it was related to the 15/17-kDa marker, and our results reflect positivity using 27-kDa.

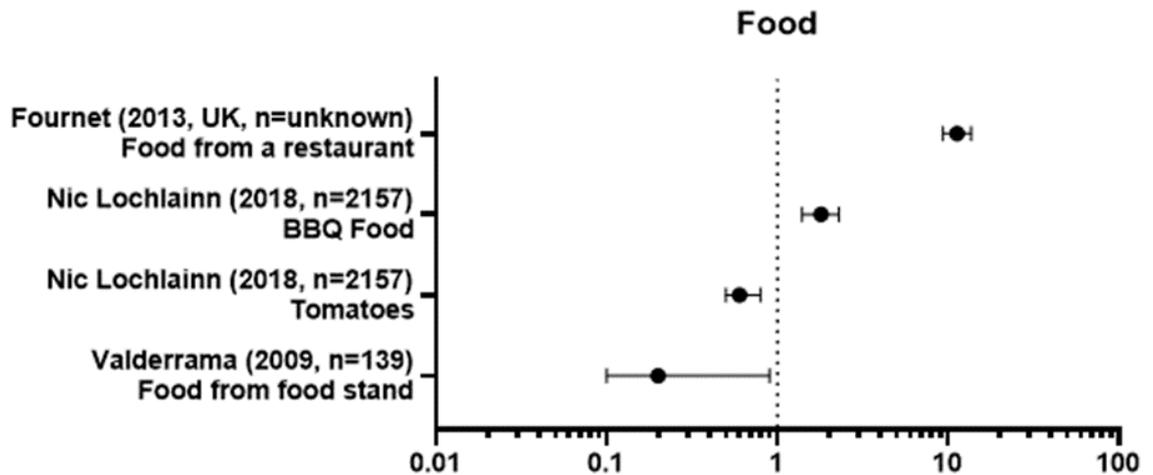
1643 Specifically, the Fournet (2) UK study considered dog ownership and as most of the
1644 cases were *C. hominis*, a more anthroponotic species, this is a peculiar result.
1645 However, we know that in this study exposures for cases were compared between
1646 years, and it may be a chance effect that dog ownership in the population changed, or
1647 indeed that it is a proxy exposure for an uncaptured variable.

1648 These results are unusual: little epidemiological evidence exists to suggest pets have
1649 any role to play in the transmission of disease (Robertson *et al.*, 2002b; Hunter,
1650 Hughes, Woodhouse, Syed, *et al.*, 2004; Pollock and Ramsey, 2011) especially for the
1651 two main species. The quality and robustness of these two studies do not allow
1652 complete confidence in the results: the Fournet (2) UK study reported on cases
1653 between time periods, and the Tollestrup (b) study used serological responses as a
1654 marker for infection and so must be interpreted differently to current disease. Yet, there
1655 is some evidence that oocyst shedding in cats and dogs can occur (Smith *et al.*, 2009;
1656 Chalmers and Giles, 2010) and species-specific infections have, albeit rarely, been
1657 detected in humans (Chalmers *et al.*, 2002; Chalmers *et al.*, 2009).

1658 This theory requires further investigation to be fully understood and described, and the
1659 addition of species identification in any study of this exposure may help better
1660 understand the true risks and pattern of infections in both domestic pets and their
1661 owners.

1662 **Transmission pathway - Food exposures**

1663 Results for food exposures were reported in three of the included studies (four
1664 variables) and this was the least investigated exposure route when considering all of
1665 the studies' upfront designs. All found significant results. Figure 14 shows results with
1666 the specific metric included: due to the variability of measures and the small number of
1667 results reported in the pathway, we did not separate exposures. Two variables (eating
1668 from a farm stand and eating tomatoes) were associated with reduced odds of illness
1669 (Valderrama; OR=0.2, 95% CI=0.1-0.9; and NicLochlainn; OR=0.6, 95% CI=0.5-0.8).



1670 Figure 14: Exposure measured and results: Food

1671 Two variables (eating BBQ food and eating outside the home) were associated with an
 1672 increased risk of illness (Nic Lochlainn; OR=1.8, 95% CI=1.4-2.3; and Fournet (2) UK
 1673 study; OR=11.32, 95% CI=9.36-13.7). The Pintar study did study several food variables
 1674 appropriate to eating outside the home, but none remained in the final models, likely
 1675 due to the limitations of using surveillance data (Pintar *et al.*, 2009).

1676 Previous work in Europe has increasingly demonstrated risks associated with food
 1677 (Casemore, 2001; Ethelberg *et al.*, 2005, 2009; McKerr *et al.*, 2015; EFSA, 2018). Our
 1678 results indicate a range of risks, but exposures measured were specific to each study
 1679 and its setting, making comparisons and assessment overall difficult. However, 'eating
 1680 tomatoes' is consistent with the literature where raw, salad food item appear to be
 1681 associated with reduced disease risk, such as carrots (Robertson *et al.*, 2002a) and
 1682 tomatoes (Hunter, Hughes, Woodhouse, Syed, *et al.*, 2004)

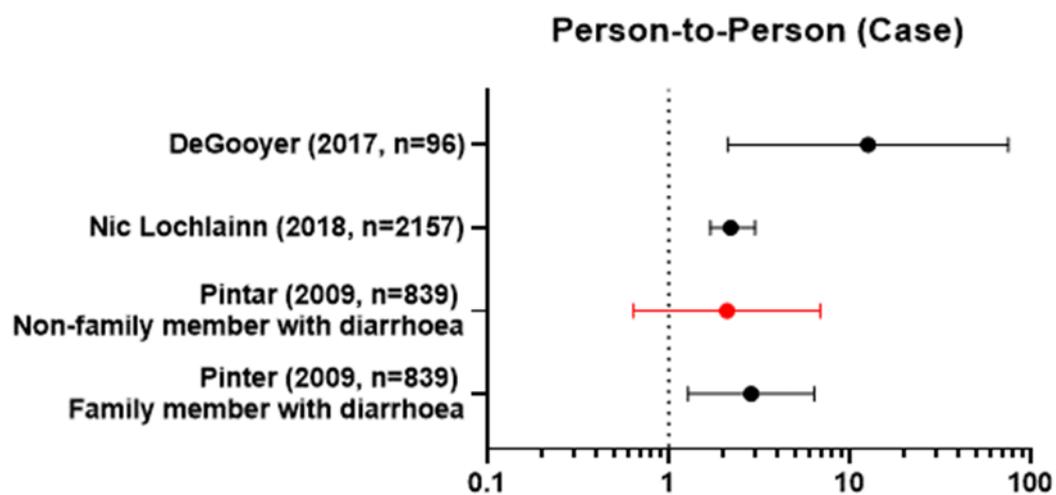
1683 Eating outside the home is often a risk factor for any infectious intestinal disease but in
 1684 the case of *Cryptosporidium* might be confounded by multiple factors. The profile of
 1685 food-borne disease is shifting, affected by globalisation and changes in food practices
 1686 (Waltner-Toews, 2019). Additionally, advances in diagnostic methods and surveillance
 1687 systems have extended the range of protozoa that may be linked to food (EFSA, 2018)
 1688 and this exposure should be further explored (Nichols, 2000; Robertson and Gjerde,
 1689 2001).

1690 **Transmission pathway - Person-to-person**

1691 Person-to-person, as a transmission pathway, was well investigated overall (five
1692 studies, four included) and represents the most consistent finding so far.

1693 *Contact with a case of diarrhoea*

1694 All three studies reporting on person-to-person contact with a symptomatic individual
1695 demonstrated correlations between exposure and disease (Pintar: OR=2.86; 95%
1696 CI=1.28-6.38 and OR=2.1, 95% CI=0.64-6.87; Nic Lochlainn: OR=2.2; 95% CI=1.7-3.0;
1697 and de Gooyer: OR=12.6; 95% CI=2.13-75.1).



1698 Figure 15: Exposure measured and results: Person-to-Person - Contact with a case
1699 of diarrhoea

1700 Variables included both home and outside-the-home contact, perhaps demonstrating
1701 the importance of differences between case contact in a household where caring and
1702 close contact is more likely and contact in a non-shared space. The de Gooyer and
1703 NicLochlainn studies, and one Pintar variable, all related to household transmission.
1704 The Pintar non-household contact variable reported lower odds and wider confidence
1705 intervals crossing 1.0, suggesting that transmission within the home is more important
1706 as a risk factor for sporadic disease. Additionally, this study excluded cases that initially
1707 had reported other illness in the home in order to accurately identify community index
1708 cases. This would suggest that any known cases came after the index illness, indicating

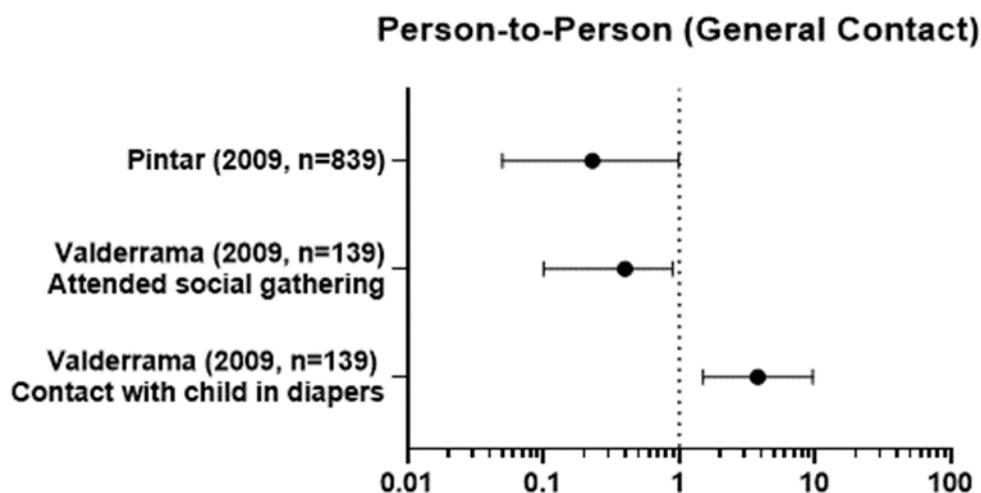
1709 that onward spread is more prevalent in *Cryptosporidium* than the other enteric
1710 illnesses.

1711 Contact with a case is a well-known, and plausible, transmission pathway to disease
1712 and exposures underlying this pathway are varied including childcare, and sexual
1713 transmission (Hannah and Riordan, 1988; Hellard *et al.*, 2003; Hunter *et al.*, 2004;
1714 Artieda *et al.*, 2012; Johansen *et al.*, 2014). This makes biological sense given the
1715 faecal-oral route of transmission and the high prevalence in younger children who may
1716 require help with toileting. The high odds ratios demonstrate the importance of this
1717 pathway to disease, particularly in the home environment. The included studies here
1718 have investigated symptomatic contact, but asymptomatic carriage has also been
1719 identified as a possible factor in transmission of sporadic disease in the home
1720 environment (Newman *et al.*, 1994; Hunter, Hughes, Woodhouse, Syed, *et al.*, 2004;
1721 Johansen *et al.*, 2014).

1722 *General contact*

1723 Variables that investigated social contact without the prerequisite of contact with a
1724 symptomatic individual were classed under general social contact, and ranged from
1725 quite specific, e.g. child-care, to broad, e.g. any social contact. All of the results were
1726 statistically significant.

1727 Two studies showed a decreased risk of disease associated with general person-to-
1728 person contact measured as attendance at 'any social gathering/event' (Pintar:
1729 OR=0.23; 95% CI=0.05-0.99) and (Valderrama: OR=0.4, 95% CI=0.1-0.9).



1730 Figure 16: Exposure measured and results: Person-to-person – General contact

1731 Both of these variables were investigating general social contact and might cover a
1732 range of activities and undoubtedly have a high exposure in all groups, thus the result
1733 is likely to be spurious.

1734 The Valderrama, 2009 study explored 'contact with a child in diapers' among
1735 community cases of disease and found an almost four-fold risk of disease (OR=3.8,
1736 95% CI=1.5-9.6). This could be driven by the higher prevalence observed in younger
1737 children, as well as the possible contribution of asymptomatic spread and is probably
1738 best considered separately to 'general social contact'. It has also been demonstrated
1739 that young children are drivers of secondary spread of disease, whether they are
1740 symptomatic or not (Hunter, Hughes, Woodhouse, Syed, *et al.*, 2004).

1741 Considering the person-to-person exposures together, it seems reasonable to suggest
1742 that the contribution of cases to onward spread warrants further investigation. This
1743 seems to apply particularly to the home environment, where we might identify easy and
1744 meaningful public health interventions to mitigate spread (Bloomfield *et al.*, 2012).
1745 Additionally, when symptoms are used to define a case, we might be losing vital
1746 information on asymptomatic disease, and how much that contributes to spread of
1747 infection.

1748 **Transmission pathway - Water**

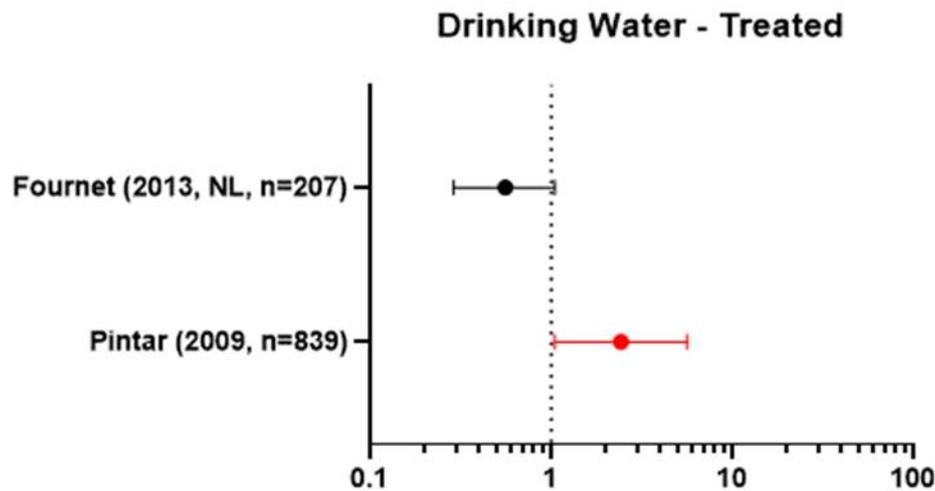
1749 Water exposures were investigated in nine of the ten included studies. This pathway
1750 was further disaggregated into drinking and recreational water exposures, with nine
1751 and five studies investigating these, respectively.

1752 *Drinking water*

1753 This was categorised as treated (n=2); untreated (n=5); and bottled (n=3).

1754 Treated

1755 One study (Pintar, 2009) found a significant result between consumption of municipal
1756 treated drinking water and an increased risk of disease (OR=2.43; 95% CI=1.0-5.7).



1757 Figure 17: Exposure measured and results: Drinking Water – Treated

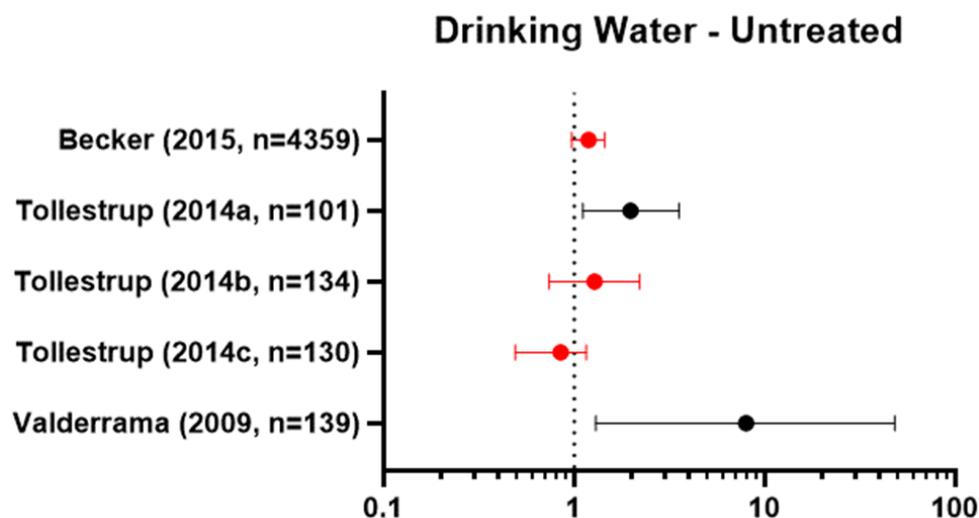
1758 There had been a sharp increase in cases of *Cryptosporidium* in this area just prior to
 1759 the study period. It is possible that the association between municipal water supplies was
 1760 due to a specific contamination and perhaps represents an outbreak, or was a proxy
 1761 for another association, such as rural versus urban residence. The other study
 1762 (Fournet, (1) 2013 shows an insignificant decreased risk of disease (OR=0.56; 95%
 1763 CI=0.29-1.05), with a smaller sample size. The metric was, “drank tap water on daily
 1764 basis” which is likely a prevalent and difficult to accurately measure metric. This study
 1765 was based on an observed increase in cases and comparisons were made between
 1766 years – it is feasible that drinking treated water may appear to have a protective effect
 1767 in comparison to drinking from an untreated or contaminated water source, particularly
 1768 if the increase was actually an undetected outbreak. Additionally, the odds ratios for
 1769 the Fournet, (1) NL study were calculated using presented data and are not those
 1770 reported in the manuscript. As such we are unable to comment on any confounders or
 1771 control for effects.

1772 An interesting finding here is that although water exposures were commonly
 1773 investigated, examinations were not often directed at drinking water, and more often
 1774 studies considered variables related specifically to drinking from untreated sources.
 1775 Although drinking water is often the cause of outbreaks and is considered to be the
 1776 main point for public health intervention only two of our studies reported final results
 1777 allocated to this pathway, and both studies were following an undetermined increase in
 1778 cases, which may not truly represent sporadic disease. It may be that following recent

1779 water treatment and regulatory requirements this is now considered a less burdensome
1780 transmission pathway for disease (I. R. Lake *et al.*, 2007).

1781 Untreated

1782 Two studies reported significant positive effects between the consumption of untreated
1783 drinking water and odds of infection with *Cryptosporidium*, ranging from almost two-fold
1784 (Tollestrup (a): OR=1.98; 95% CI =1.11-3.55) to an increased risk of eight times that of
1785 controls (Valderrama: OR=8.0; 95% CI=1.3-48.1).



1786 Figure 18: Exposure measured and results: Drinking Water – Untreated

1787 The 2009 Valderrama study had wide confidence intervals, the lower end of which was
1788 close to 1.0, despite quite a high odds ratio (8-fold). The data analysed were based on
1789 surveillance data extractions and so there are possible limitations on questions asked
1790 and answered. The authors grouped all and any untreated water consumption.
1791 Additionally, although not reported as an outbreak, this study was in response to an
1792 increase in cases across the state of Colorado, USA, and it may be possible that the
1793 elevated risks are a result of an outbreak driven by a specific untreated water source
1794 during the study period, although controls were matched by geography which should
1795 mitigate this to some extent.

1796 The Tollestrup (a) study included 200 of the 600 cohort participants, taking place in a
1797 semi-rural area, where there was a significantly higher percentage of participants using
1798 onsite wastewater systems or private wells than participants using municipal systems
1799 ($p=0.048$). It is also important to consider that the outcome measured in this study was

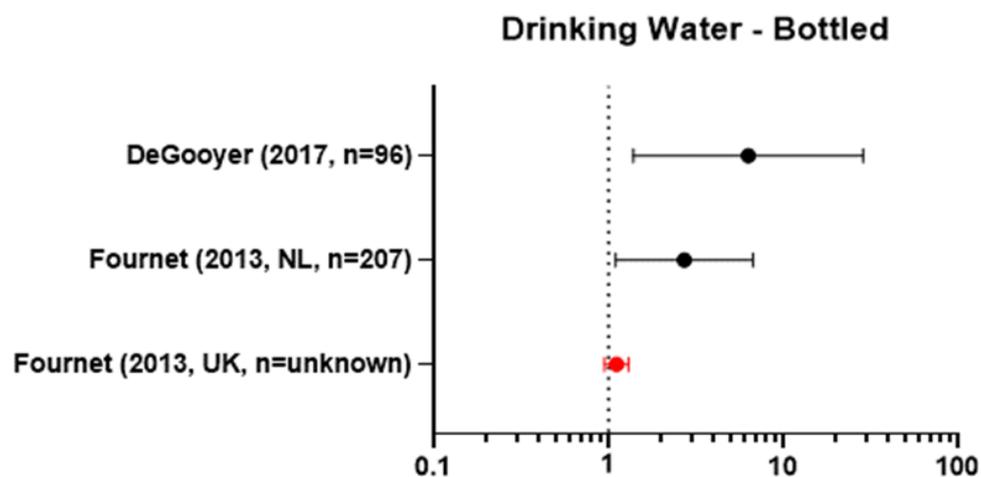
1800 serological response to 27kDa antigen rather than diarrhoeal illness and so our
1801 confidence in comparability may be reduced. Ongoing non-transient exposure to
1802 oocysts, perhaps in an untreated system, could result in a positive, and increasingly
1803 high, serological response in residents in this area. This may not, however, necessarily
1804 reflect current or prior disease.

1805 The remaining non-significant results were all fairly close to 1.0 with narrow confidence
1806 intervals. Results from the Becker (2015) study were not significant and close to no
1807 effect (aOR=1.19; 95% CI=0.97 - 1.45), despite being a large study of high quality. As
1808 with the Tollestrup studies, the outcome metric was serological response to a
1809 *Cryptosporidium* marker, which might indicate differences in exposure windows and
1810 actual infection cannot be accurately pinpointed.

1811 It is interesting to see that this is a well-investigated pathway, and it is biologically
1812 plausible that exposure to untreated drinking water could represent a source of
1813 *Cryptosporidium* oocysts. Nevertheless, even with the studies' limitations in mind, these
1814 results do not seem to indicate that this pathway is a major contributor to sporadic
1815 disease.

1816 Bottled

1817 All of the studies including results for this pathway reported positive associations with
1818 disease. Two of the three studies found a significant positive effect between drinking
1819 bottled water and increased odds of *Cryptosporidium* infection, ranging from almost
1820 three-fold (Fournet (1) NL study, 2013: OR=2.72, 95% CI=1.1-6.76) to more than five-
1821 fold (De Gooyer, 2017: OR=6.31, 95% CI=1.39-28.7).



1822 Figure 19: Exposure measured and results: Drinking Water - Bottled

1823 The odds ratio for the Fournet (1) study in the Netherlands was calculated using their
1824 reported data: They reported a 21% vs 11% exposure in cases and controls
1825 respectively. Most cases in the study were infected with *C. hominis* (GP60 subtype
1826 IbA10G2) which usually suggests either anthroponotic spread or perhaps sewage
1827 contamination.

1828 The de Gooyer study investigated sporadic cases in Australia, where the consumption
1829 of bottled water in the general proportion is fairly high (de Gooyer *et al.*, 2017) and, in
1830 this study, 86% of cases vs 54% of controls exposed was reported. Although exposure
1831 prevalence in the control group was lower, it still represents a reasonable amount of
1832 non-diseased participants that cannot be explained by that exposure.

1833 The Fournet (2) UK study was large and reported narrow confidence intervals yet did
1834 not demonstrated a difference in effect. However, the controls in this study were cases
1835 from different years: it is reasonable that drinking bottled water may have no effect on
1836 disease incidence in comparison to a period of time where cases were driven by an
1837 undetected outbreak with a single other source.

1838 If exposure to bottled water was a risk for sporadic disease (and not outbreaks following
1839 a specific contamination) we might expect to see exposure to tap water generally
1840 associated with a decreased risk of illness in those exposed, but two case-control
1841 studies in areas close to the de Gooyer investigation in Australia demonstrated no such
1842 effects of water associated with *Cryptosporidium* (Robertson *et al.*, 2002b). Also,
1843 paradoxically, water consumption consistently free from *Cryptosporidium* oocysts may
1844 lower ongoing exposure and thus immunity, opening people up to risk of disease from
1845 other infection pathways (Ramsay *et al.*, 2014) but this is still poorly understood (Frost
1846 *et al.*, 2005; Hunter and Thompson, 2005). However, *C. hominis* has been detected in
1847 finished mineral water samples following an outbreak in the UK (Nichols, Campbell and
1848 Smith, 2003) and Australia (Weinstein *et al.*, 1993) suggesting some plausibility in
1849 these results. In these studies, definitions of 'bottled water' varied and information was
1850 not specifically collected on amounts consumed to enable further examination of this
1851 association. Additionally, sociodemographic factors associated with bottled water use
1852 have previously been described (Hu, Morton and Mahler, 2011) and so there is a
1853 possibility that these results might be open to uncontrolled confounding.

1854 On balance, there is some evidence for bottled water as a risk for sporadic illness, but
1855 these studies offer insufficient quality and detail to make a resolute conclusion.

1856 *Recreational water*

1857 This was categorised as treated (n=4); and untreated (n=3).

1858 Treated

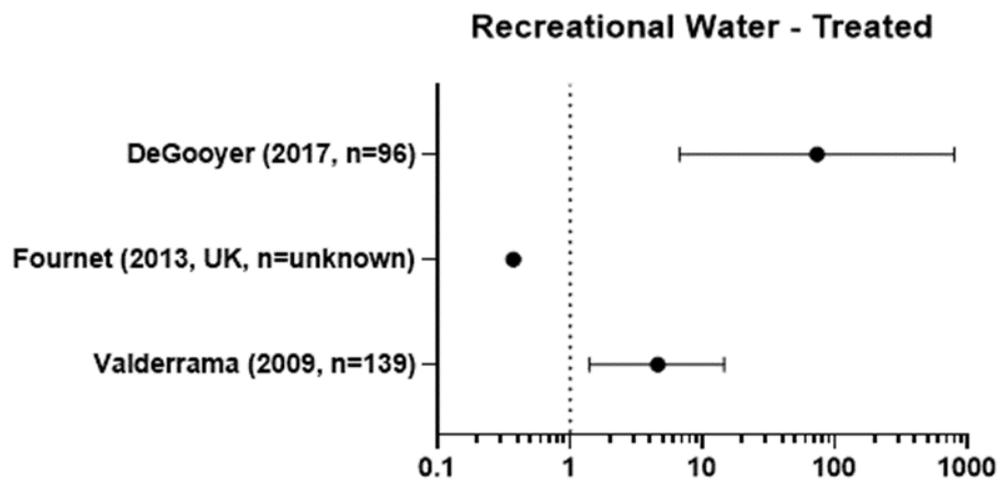


Figure 20: Exposure measured and results: Recreational water – Treated

1859 Three studies reported on four exposures that were categorised as treated recreational
1860 water, and all found significant results. Two studies reported an increased risk of
1861 disease associated with exposure to recreational water (Valderrama, 2009: OR=4.6,
1862 95% CI=1.4-4.6; de Gooyer, 2017: OR=26.4, 95% CI=1.47-472 for spa use, and
1863 OR=3.18, 95% CI=1.85-8.79 for general recreational water exposure).

1864 The de Gooyer study reported two significant variables related to recreational water
1865 exposure: using a spa and having any recreational water exposure. These were wide
1866 ranging definitions and captured any recreational water exposures (except those that
1867 might be specifically described as untreated). As such, we cannot be certain that
1868 specific exposures in this group are necessarily treated waters. This could misclassify
1869 the exposure and overestimate the effect. Using a spa was significantly associated with
1870 illness, although only 13% (4/30) of cases reported this exposure and the confidence
1871 intervals are wide. We also know that this work was examining an increase in cases
1872 that may have been linked to a particular recreational waterpark, although was not
1873 considered an outbreak. If these cases were linked to a particular water source, this
1874 could skew the results towards a positive effect.

1875 It is also worth noting that in Australia, recreational water exposures are a common
1876 cause of outbreaks and the prevalence of this recreational activity is high (Hellard *et*
1877 *al.*, 2000; Puech *et al.*, 2001). These activities represent a biologically plausible route
1878 to infection with *Cryptosporidium*, considering its faecal-oral transmission route and the
1879 likelihood of swallowing water, the chlorine resistant nature of oocysts, and the poorer
1880 hygiene habits of younger children (Stafford *et al.*, 2000).

1881 One of the studies reported decreased odds of disease (Fournet (1), 2013: OR=0.37,
1882 95% CI=0.33-0.42). This study originally reported proportions and we used these data
1883 to calculate odds ratios – thus these are not adjusted or controlled for any other factors,
1884 which may skew the effect measure. Also, the study is considering cases from one time
1885 period versus another, rather than comparing exposures between diseased and non-
1886 diseased participants. It is reasonable that the reported effect could be an artefact if
1887 recreational water exposure was the cause of the increase in the prior years. Whilst we
1888 may be able to confidently say that exposure to treated recreational water was not
1889 causing the increase in the study time period, we can be less confident that the results
1890 are generalisable and that they represent the true relationship between exposure and
1891 sporadic disease.

1892 It is likely that treated recreational water represents some risk for cryptosporidiosis, and
1893 this would be supported in other literature. However, these results suggest that it may
1894 well be more associated with outbreaks, or specific incidences of increases in disease.

1895 Untreated

1896 One of three studies reporting on untreated recreational water exposures found a
1897 significant result, demonstrating a three-fold increased risk of illness (Pintar, 2009;
1898 OR=2.91, 95% CI=1.14-7.38).

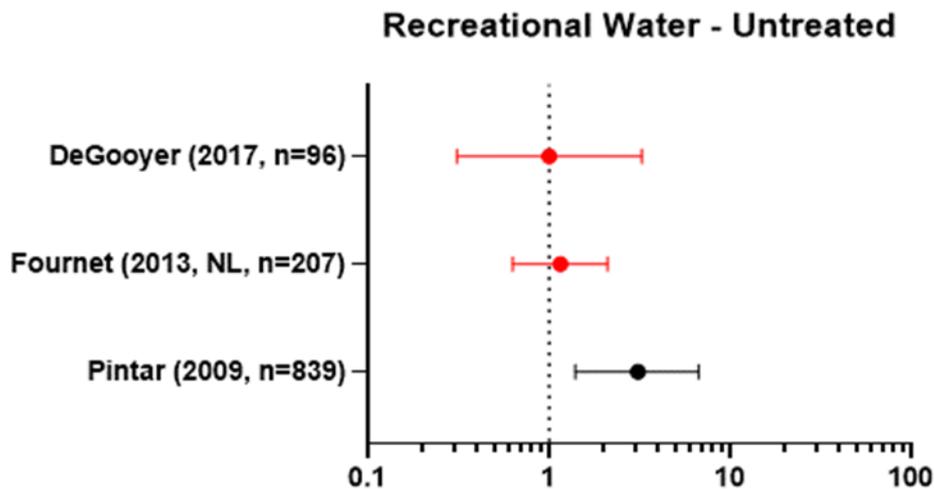


Figure 21: Exposure measured and results: Recreational water – Untreated

1899 After adjusting for age and season in the multivariable model, swimming in an untreated
 1900 water venue (river or lake) ($p=0.01$) was associated with increased odds of illness. The
 1901 proportion of cases exposed was, however, small at 25% (9/36) versus 10% (79/801)
 1902 for the controls (79/801). This study used other cases of enteric illness as controls,
 1903 including *Giardia*, which has similar exposure routes. This could make it more difficult
 1904 to detect small differences in risk between case and controls by over-representing
 1905 exposures and biasing towards the null, so in actual fact this effect measure could be
 1906 underestimated (Voetsch *et al.*, 2009). The data were however, taken from surveillance
 1907 records, and cases were only asked about exposures in the seven days preceding
 1908 illness which might not accurately capture the exposure window for *Cryptosporidium*.

1909 The remaining two studies were statistically insignificant, and both reported no effect
 1910 of this exposure on risk of disease in their study population (Fournet (1) (OR=1.15, 95%
 1911 CI=0.63-2.1) and de Gooyer (OR=1.0, 95% CI=0.31-3.25).

1912 Overall, the contribution of this exposure to disease in this review is not considerable.
 1913 This is interesting in light of the more positive effects we report for treated recreational
 1914 water. This may be due to untreated activities being associated with other
 1915 unmeasurable factors, such as adult, rather than child, populations (Dufour *et al.*,
 1916 2017).

1917 **Publication Bias**

1918 Due to the small number of studies against each exposure category (n<5), we did not
1919 consider a statistical analysis into heterogeneity and publication bias appropriate.

1920 **Conclusions**

1921 The case-characteristics collected from our included studies reflected the bimodal age-
1922 related pattern that *Cryptosporidium* follows in the US and the UK; a peak in young
1923 children, and a peak in adulthood (Dietz and Roberts, 2000b; Nichols *et al.*, 2006; Yoder
1924 and Beach, 2007).

1925 The animal transmission pathway was commonly investigated, although was not
1926 considered a main hypothesis for sporadic disease in any of the papers. We report a
1927 couple of unusual associations with disease and pet contact, specifically cats and dogs,
1928 although these should be considered in light of some of the methodological
1929 weaknesses of the studies concerned. Evidence for this route is conflicted and more
1930 research is needed to support or refute this pathway as a contributor to sporadic
1931 infection.

1932 Food exposures were not so frequently investigated, and metrics used were specific to
1933 the niche study population or hypothesis. Given that food items are increasingly
1934 identified in outbreak investigations (Casemore, 2001; Ethelberg *et al.*, 2005; McKerr
1935 *et al.*, 2015). this exposure, and protozoa that may be linked to food, should be further
1936 explored (Nichols, 2000; Robertson and Gjerde, 2001; EFSA, 2018).

1937 The person-to-person pathway was well investigated overall and represents the most
1938 consistent finding so far. Considering the person-to-person exposures, it seems
1939 reasonable to suggest that the contribution of cases to onward spread warrants further
1940 investigation. This seems to apply particularly to the home environment which is
1941 increasingly understood to be a significant setting for spread of *Cryptosporidium*
1942 infection (Newman *et al.*, 1994; Perry *et al.*, 2005; Bloomfield *et al.*, 2012; Johansen *et*
1943 *al.*, 2014) and would support public health messaging on preventing spread of disease
1944 at home (Public Health England, 2019).

1945 Our included papers investigated water exposures most frequently despite evidence
1946 that in industrialised countries in recent years, drinking treated mains water is unlikely
1947 to cause a significant amount of sporadic cryptosporidiosis (Sopwith *et al.*, 2005;
1948 Pollock *et al.*, 2014). However, despite improved legislation for drinking water quality
1949 (Lake *et al.*, 2007) deficiencies can persist and it is important to keep monitoring this

1950 pathway (Nichols *et al.*, 2009; Griffiths, 2017). We present some risks associated with
1951 bottled water, although this is likely to be a high prevalence exposure and may be
1952 confounded by socio-economic variables.

1953 Recreational water is more frequently associated with outbreaks and was not a major
1954 source of sporadic illness in this review, perhaps reflecting the episodic nature of pool
1955 water contamination events. Nonetheless, we know that standard treatment practices
1956 for recreational water, such as chlorination, are unsuccessful in eliminating
1957 *Cryptosporidium* oocysts (World Health Organization:, 2009). This does seem a well
1958 understood pathway (Chalmers, 2012; Cacciò and Putignani, 2014).

1959 When considering exposures for sporadic disease, it may be more pertinent for future
1960 studies to focus on food exposures, and research in this area is on the increase
1961 (Redmond and Curnin, 2018).

1962 Secondly, our results demonstrate that further detail is required on the person-to-
1963 person transmission pathway: although well investigated, exposures were variable, and
1964 no study hypothesised this as a risk for infection, with most results incidental to the
1965 study. We should seek to quantify and ascertain spread of infection in the home
1966 environment and build a profile of asymptomatic infections, through better
1967 observational studies and more routine sub-typing of isolates (Davies *et al.*, 2009;
1968 Chalmers and Katzer, 2013).

1969 **Limitations**

1970 As with any work that seeks to combine various different studies, significant limitations
1971 exist which should be considered in any synthesis.

1972 The main imitations to this work were the low number of papers included, which meant
1973 meta-analysis was not possible. Although we did not quantify heterogeneity,
1974 anecdotally, there were differences between the studies included in terms of study
1975 design, populations studied, data collected and variables measured, and outcomes,
1976 which may have made meta-analyses difficult regardless.

1977 In the studies, often several variables were measured which represented the same
1978 exposure, and participants may have been counted in either as they were not
1979 necessarily mutually exclusive. This is common to epidemiological studies, which are
1980 often undertaken in response to outbreaks, and defined by particular settings and
1981 putative exposures (Briggs *et al.*, 2014). The breadth of this makes the extrapolation of
1982 our results less robust, and context must be considered in assessing results. This might

1983 dilute any differences between exposures driving sporadic disease and outbreaks. A
1984 suggestion for further work would be to extend the time period to look specifically at the
1985 magnitude of changes in exposures and to review outbreaks in that context.
1986 Unfortunately, this was beyond the scope of this work.

1987 Additionally, there could be bias associated with the personal subjectivity of
1988 categorising exposures into pathways, especially without the granular detail of raw data
1989 or knowing the specifics of questions asked of participants. However, our robust
1990 methodological approach and commitment to duplicating all tasks in this review
1991 hopefully mitigates this as far as possible.

1992 Two included papers (Tollestrup *et al.*, 2014; Becker, Oloya and Ezeamama, 2015)
1993 measured their outcome using serological response to the 15/17kDa and 27kDa antigen
1994 groups, which can identify previous, as well as recent infection and cannot distinguish
1995 between species or genotypes (Leav, Mackay and Ward, 2003). Because we had two
1996 papers with this outcome, which judged it differently, we used response to the 27kDa
1997 to indicate a strong serological response and therefore count as infection. However, this
1998 has a longer positivity than the 15/17kDa, and could represent infection as long as nine
1999 months prior (Chalmers *et al.*, 2013). This might have meant we were correlating
2000 current or recent exposures to old infection and making inferences when in fact there
2001 was no link. However, as we were looking at sporadic infection as opposed to outbreak
2002 disease, it may be appropriate to consider more static exposures, in any case.
2003 Additionally, diarrhoeal disease from *Cryptosporidium* infection may well recur and has
2004 comparative longevity, that might well underpin some health seeking behaviour, so the
2005 other studies are also at some small risk of correlating exposures with disease in a
2006 different time frame. Furthermore, even those studies that do have accurate and recent
2007 onsets are often a) vulnerable to recall biases and b) have different windows of
2008 exposure for cases and controls (Food Standards Agency, 2000a).

2009 Often crucial detail was absent from the manuscript, and so allocation of measured
2010 variables to our exposures was occasionally arbitrary. However, we moderated this as
2011 far as possible by following a systematic approach and utilising our third reviewer for
2012 any discord. We would recommend that future research include large-scale
2013 observational studies with enough resource to achieve high study quality whilst
2014 maintaining resolution in exposures measured.

2015 In assessing study quality, we recognised that the NOS has significant limitations for
2016 use in this type of systematic review (Ioannidis, 2011). A number of studies we
2017 reviewed used surveillance data rather than more traditional study designs which

2018 meant that the NOS was not always directly applicable. Where this was the case, we
2019 have had to apply the tool as appropriately as possible and in discussion with reviewers.
2020 At present, there is no risk of bias tool that encompasses both case-control studies and
2021 surveillance data.

2022 Despite the limitations considered, this is the first systematic review considering routes
2023 of transmission in industrialised countries for sporadic *Cryptosporidium* and highlights
2024 that food routes are under investigated, and that person-person transmission, although
2025 recognised, is not thoroughly investigated.

2026 **Acknowledgements**

2027 Thanks to Ken Linkman at the University of Liverpool Harold Cohen library for his
2028 specialist aid with medical databases and search terms, Dan Pope at University of
2029 Liverpool Public Health Department for technical guidance for data synthesis, and to
2030 Peter Burrell at Public Health England for assistance with data extraction tools.

2031 Erica Kintz at University of East Anglia.

2032 Ruairaidh Hill at Liverpool Reviews and Implementation Group (LRiG), University of
2033 Liverpool.

Chapter 6

The epiCrypt study:
Investigating transmission of
Cryptosporidium in the home
environment

2034 **Introduction**

2035 **Case contact as an exposure for infection**

2036 In the previous chapter, I presented results from a systematic review that sought to
2037 outline the main exposures reported for sporadic cryptosporidiosis. All of the included
2038 studies that considered person-to-person spread as a transmission route
2039 demonstrated an increased risk of illness or infection associated with prior contact
2040 with a symptomatic individual (Pintar *et al.*, 2009; de Gooyer *et al.*, 2017; Nic
2041 Lochlainn *et al.*, 2019). Hence, onward spread of infection from exposure to a case
2042 might represent a risk for sporadic disease, but how much remains unclear. Metrics
2043 included in these studies encompassed contact both 'in the home' and 'outside-the-
2044 home', with the authors considering them to be different settings. Results in support
2045 of general contact, which included metrics such as attending any social event and
2046 any general contact with people were not as consistent, and reduced odds of illness
2047 were reported. This suggested transmission within the home as a particular setting in
2048 the pathway to infection, and that case contact is additionally risky in the household
2049 where close contact is more likely. This makes biological sense given the faecal-oral
2050 route of transmission and the higher prevalence of infection in younger children who
2051 may require help with toileting (Dietz and Roberts, 2000b; Hunter, Hughes,
2052 Woodhouse, Syed, *et al.*, 2004; Nichols *et al.*, 2006; Pintar *et al.*, 2009). This has
2053 been further buttressed by large-scale reports of spread in the home following
2054 outbreaks (Osewe *et al.*, 1996; Johansen *et al.*, 2014) which may well drive additional,
2055 sporadic cases.

2056 *Contribution of asymptomatic contact*

2057 Whilst the contribution of symptomatic case contact is plausible and measurable,
2058 identifying asymptomatic spread poses more difficulty: *Cryptosporidium* is not
2059 diagnosed based on symptoms alone and ordinarily only cases presenting with
2060 symptoms would be tested (Detection and diagnosis in humans). The burden of
2061 asymptomatic infection and its influence on spread is less well documented in
2062 *Cryptosporidium* research than for some other infections, particularly in countries like
2063 the UK. A study in the UK reported a point prevalence of 1.3% among asymptomatic
2064 pre-school children (Davies *et al.*, 2009) suggesting that a small amount of
2065 asymptomatic infection does occur. Furthermore, a Norwegian study looking at
2066 follow-on spread after two outbreaks observed asymptomatic secondary transmission
2067 (Johansen *et al.*, 2014). Additionally, although not representative of true

2068 asymptomatic infections, oocysts can be detected in stool after case symptoms cease
2069 and this period may present a risk of transmission (Chalmers *et al.*, 2016). The
2070 contribution of asymptomatic persons to the spread of infection warrants further
2071 investigation to better understand transmission risks and mitigate spread (Bloomfield
2072 *et al.*, 2012).

2073 *Risks specific to C. hominis and C. parvum*

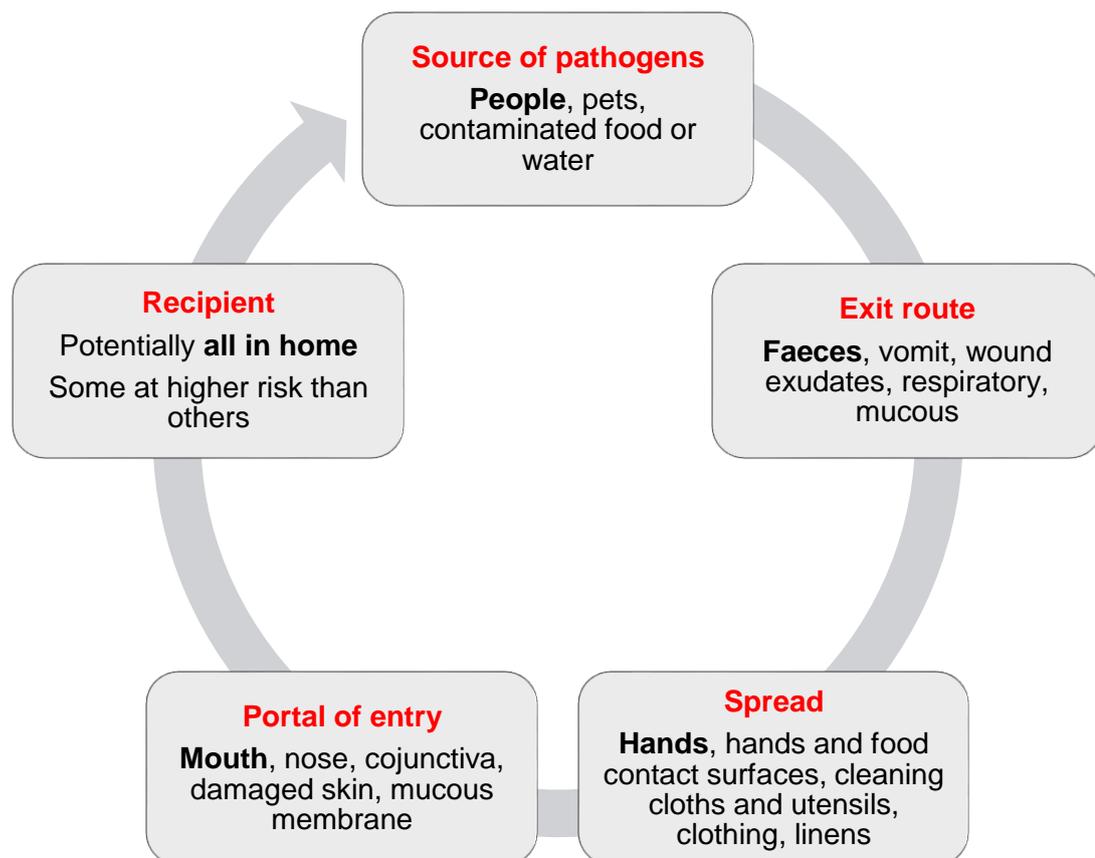
2074 Few papers included in the systematic review reported on infecting species and this
2075 information is still generally lacking despite developments in methodologies to
2076 determine this. In a study in the UK, Hunter *et al.* found that changing children's
2077 nappies was a risk factor specific to *C. hominis* (Hunter, Hughes, Woodhouse, Syed,
2078 *et al.*, 2004) and the Netherlands recently reported similar results (Nic Lochlainn *et*
2079 *al.*, 2019), specifying that *C. hominis* cases were more likely than controls to have
2080 been exposed to a case in the home. Additionally, the authors reported corroborating
2081 indicators supportive of this exposure, including living in smaller homes, and living
2082 with children.

2083 **The home as an infectious disease environment**

2084 With general cases of IID, there is an assumption that infections are mostly due to
2085 eating contaminated food or drinking contaminated water, but it is increasingly
2086 recognised that this is imprecise (Wheeler *et al.*, 1999; Food Standards Agency,
2087 2000b). *Cryptosporidium* especially suffers somewhat from perception as a
2088 waterborne pathogen, but outbreaks associated with this are far fewer now
2089 (Transmission pathways and the underlying exposures for infection). Humans as a
2090 source of enteric disease in the home have been reported for *Salmonella* (Evans *et*
2091 *al.*, 1998; Le Baigue *et al.*, 2000) and *Escherichia coli* (Le Baigue *et al.*, 2000; Werber
2092 *et al.*, 2008; Friedrich, 2011) infections. In Germany, a review of gastrointestinal
2093 outbreaks revealed over a third could be attributed to person-to-person spread and
2094 the household was the most common setting for this (Krause *et al.*, 2007).
2095 Surveillance is unlikely to pick this up in most systems, and in-home outbreaks or
2096 clusters probably represent an unidentified burden of disease (Day, 2001; Leder *et*
2097 *al.*, 2009). Work in Denmark on bacterial infections reported variation in the ability of
2098 organisms to cause household outbreaks, highlighting the contribution of heretofore
2099 under-recognised person-to-person spread (Ethelberg *et al.*, 2004). Differences in
2100 parameters of spread, such as serial interval, compared to other infections are likely
2101 for *Cryptosporidium*, reflecting specific differences in its infectivity and pathology

2102 (Biology and life cycle). An assessment of case and home attributes where onward
2103 spread and person-to-person transmission are suspected might help elucidate
2104 relevant higher risk characteristics such as crowding, age, immune status, of length
2105 of episode of illness (Perry *et al.*, 2005; Snedeker *et al.*, 2009).

2106 The risk of exposure to specific pathogens at home will depend on the extent to which
2107 they can be spread in that setting, via hands, contamination of fomites or food,
2108 exposure to pets, and close personal activities. Underlying exposures and risk factors
2109 may be multiple and it remains important to try to assess some of these in any
2110 analytical study (Bloomfield, 2001). A 2009 review by the International Scientific
2111 Forum on Home Hygiene described that outbreaks of IID in the home are largely
2112 preventable just by better general hygiene practices (Bloomfield *et al.*, 2012). The
2113 chain of infection and the features required for transmission of infection in the home
2114 can be simplified to that shown (Figure 22). These will differ according to organism,
2115 but considering characteristics of the *Cryptosporidium* parasite, the chain is likely to
2116 be mainly a direct faecal-oral route and spread based on a person-to-person pathway.



2117

2118 Figure 22: The chain of infection transmission in the home (bold are features likely
2119 key to *Cryptosporidium*) adapted from: Bloomfield, 2001

2120 **Rationale for the epiCrypt study**

2121 It seems reasonable to suggest that the contribution of cases to onward spread
2122 warrants further investigation. This might apply particularly to the home environment
2123 which is increasingly understood to be a significant setting for spread of
2124 *Cryptosporidium* infection (Newman *et al.*, 1994; Perry *et al.*, 2005; Bloomfield *et al.*,
2125 2012; Johansen *et al.*, 2014) and would help inform public health messaging on
2126 preventing spread of disease at home (Public Health England, 2019).

2127 An assessment of the previous work on secondary spread¹² in this particular setting,
2128 in conjunction with the systematic review, led to a clear justification for an analytical
2129 study in the UK population, which might be exploratory in nature, examining spread
2130 of *Cryptosporidium* infection in the home environment. I designed an observational
2131 study that recruited cases from across North West England and Wales. I aimed to
2132 identify index cases (the first case in a home identified in the surveillance system) of
2133 *Cryptosporidium*, and then determine if there were any other infections with
2134 *Cryptosporidium* in that home, either before or after the index case. Additionally, I
2135 sought to describe any home-level, case, or organism characteristics that might be
2136 associated with transmission in this environment.

2137 This study helps to meet my third objective to explore transmission in the home
2138 environment, and calculate the burden this might have on people in the home,
2139 considering longevity and severity of illness.

2140 **Methods**

2141 The protocol for this study has been published and a manuscript is attached in
2142 Appendix 5 (McKerr *et al.*, 2019).

2143 **Aims and objectives**

2144 The aims of this study were to:

- 2145 • estimate how much additional *Cryptosporidium* infection happens in the home
2146 where there is a case, and,
- 2147 • describe characteristics associated with transmission in the home.

¹² I use the term 'secondary spread' to mean any apparent onward transmission of disease originating from an index case, whilst recognising that this may be secondary or even tertiary level of spread.

2148 In order to meet these aims, I identified several measurable objectives, based on
2149 theories identified following the previous exploration of the literature:

2150 **Objectives:**

- 2151 • To estimate the number of additional cases in households with an index
2152 case
- 2153 • To calculate transmission prevalence in households with an index case
- 2154 • To estimate the prevalence of asymptomatic carriage in households with an
2155 index case
- 2156 • To identify specific household-level and case characteristics associated with
2157 homes that have additional cases and with homes that do not
- 2158 • To determine the number of cases by species and compare these in homes
2159 that have additional cases and in homes that do not

2160 **Study population**

2161 The study population comprised residents of North West England and of Wales.

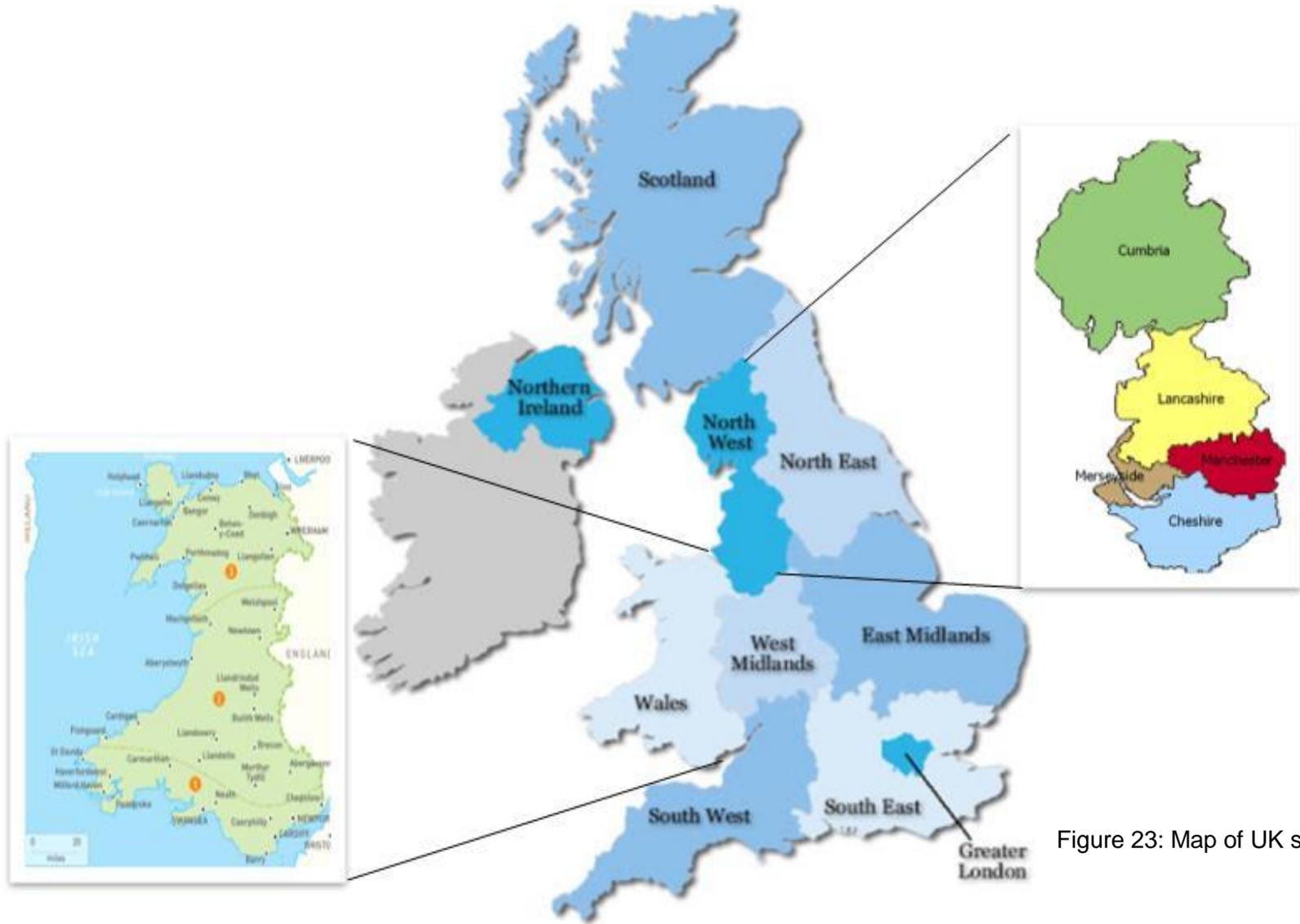


Figure 23: Map of UK showing NW England and Wales

2162 Figure 23 shows the position of these in the UK, with additional boxes to highlight
2163 local boundaries.

2164 The North West of England has a population of over 7 million people and is the third-
2165 most densely populated region in the United Kingdom (ONS, 2011). In 2016 over 600
2166 laboratory-confirmed *Cryptosporidium* cases were reported from the North West
2167 (8.4/100,000 population) (PHE, 2017).

2168 Wales has a population of over 3 million people (*Welsh Government | Census of*
2169 *population, 2015*). In 2016, over 400 laboratory-confirmed *Cryptosporidium* cases
2170 were reported from Wales, the highest rate of *Cryptosporidium* laboratory reports per
2171 100,000 population in England and Wales (15/100,000) that year (PHE, 2017).

2172 *Sampling frame*

2173 The sampling frame was derived from the two relevant surveillance systems
2174 previously described (Surveillance system descriptions), which capture laboratory
2175 confirmed reports of *Cryptosporidium* in England and Wales: The Second-Generation
2176 Surveillance System (SGSS) in Public Health England (PHE), and Tarian in Public
2177 Health Wales (PHW).

2178 All cases of laboratory confirmed *Cryptosporidium* reported from primary diagnostic
2179 microbiology laboratories in North West England and Wales, in the study year, were
2180 initially eligible. These were considered index cases.

2181 *Sample size*

2182 The North West & Wales report around 1,000 cases per year (PHE data, 2015). I
2183 assumed a participation rate of somewhere in the region of 40-60%, based on similar
2184 studies and approaches (Tam *et al.*, 2012; Waldram *et al.*, 2017)). I anticipated that I
2185 might initially enrol around 400 index cases. Using the 2011 Census indications of 2.4
2186 persons on average per household (ONS, 2011), I calculated a target overall
2187 recruitment number of 960 - 1,000 participants.

2188 Assuming that the rate of household transmission, defined as the proportion of
2189 households with more than one case, is anywhere between 0% and 20% (Newman
2190 *et al.*, 1994; MacKenzie *et al.*, 1995; Harrison *et al.*, 2002; Perry *et al.*, 2005; Johansen
2191 *et al.*, 2014; Waldram *et al.*, 2017), a range of required sample sizes was estimated
2192 (between 118-402). Aiming to recruit 400 households seemed feasible given time and
2193 resource and would allow confident demonstration of an odds ratio of 2.0, with type 1
2194 error of 0.05 and type 2 error at 0.20.

2195 **Study type**

2196 The identification of cases, and their subsequent recruitment, was cross-sectional,
2197 although the study also involved retrospective data collection and some prospective
2198 sampling. I based the design of the study on case ascertainment household cohort
2199 study approaches (Tsang *et al.*, 2016). This approach is often used in studying
2200 respiratory illnesses, where multiple-occupant households are recruited prospectively
2201 (usually from an entire community). Occupants are then followed up to identify
2202 infections. As well as being more tenable in terms of resource and follow-up,
2203 specifically these studies are useful in that the design allows for calculation of
2204 secondary infection risk and serial interval where the data allow. This allows us to
2205 capture heterogeneity in characteristics associated with secondary spread and
2206 transmission.

2207 **Study period**

2208 The study period was for 12 months, to account for seasonal variation in cases and
2209 *Cryptosporidium* species, and to allow maximum enrolment. The study began
2210 recruiting from England in October 2018 and from Wales in January 2019. Both study
2211 areas had an initial pilot phase of 1-2 months.

2212 **Ethical approval, governance, and registration information**

2213 The study design and approach to recruitment was approved by the North West –
2214 Liverpool East NHS Research Ethics Committee (Reference: 18/NW/0300). Access
2215 to surveillance data in order to identify cases and approach for recruitment was
2216 reviewed and approved by the Confidentiality and Advisory Group (CAG) under a
2217 specific support precedent, “Section 251: Accessing data prior to consent” (Reference
2218 18/CAG/0084).

2219 Contract study agreements and Data Sharing Agreements (DSA) between
2220 PHE/PHW/University and CRN were drawn up and approved by the individual
2221 organisations involved.

2222 The project is registered on the National Institute for Health Research (NIHR) portfolio
2223 (CPMS ID: 39458).

2224 The sponsor and supplier of indemnity for the study was The University of Liverpool
2225 (**UoL001340**). Additional internal governance was obtained from PHE and PHW in
2226 order to support cross-working relationships and information sharing.

2227 Welsh approvals were granted by Health and Care Research Wales (NHS Wales
2228 research permissions).

2229 A published research summary can be found here on the Health Research Authority
2230 (HRA) website:

2231 <https://www.hra.nhs.uk/planning-and-improving-research/application-summaries/research-summaries/the-epicrypt-study-household-transmission-of-cryptosporidiosis-v10/>

2234 Recruitment approach

2235 Cases of cryptosporidiosis were identified using the routine surveillance datasets for
2236 England and Wales (Surveillance system descriptions). Cases were recruited
2237 according to the flow chart outlined in Figure 24 : Sketch of process to enrolment.

2238 Eligible cases were contacted via post, by the relevant public health organisation, in
2239 the first instance. (Invite letter available in [Appendix 6](#))

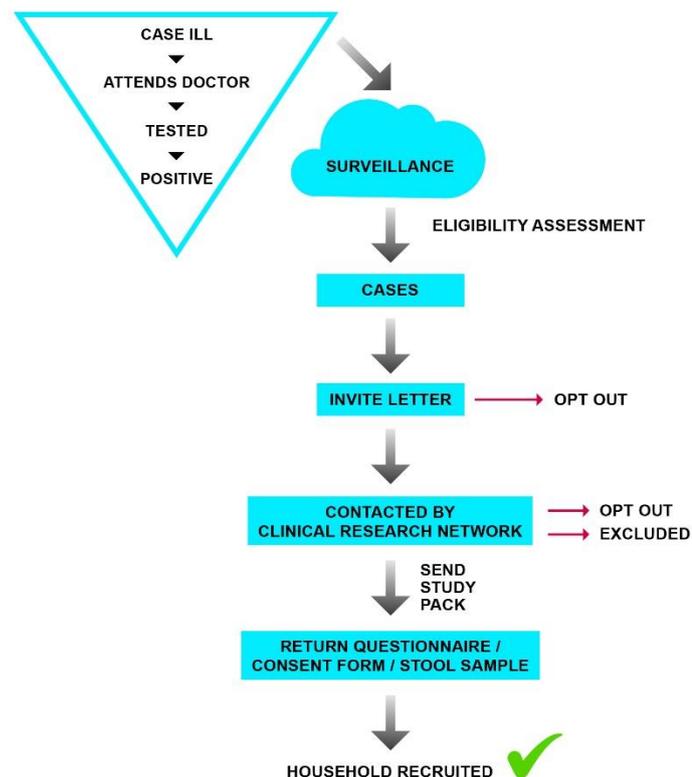


Figure 24 : Sketch of process to enrolment

2241 Following this, if cases did not opt-out, they were contacted via telephone by an NHS
2242 research nurse at the local Clinical Research Network (CRN) to chat about the study
2243 and determine if they would like to take part.

2244 This unusual opt-out approach to the recruitment process was driven by necessity
2245 and feasibility and I explored several options at the protocol drafting stage of the
2246 project, balancing data needs with possible patient burden. As the capture of cases
2247 in the surveillance systems is retrospective (i.e. the case has already been ill) and
2248 diagnosis of *Cryptosporidium* in the stool sample is undertaken by laboratory staff,
2249 there was no opportunity to consent individuals at the time of diagnosis. Thus, the
2250 recruitment process could not be achieved without initial access to patient information.
2251 I submitted a successful application to the CAG for specific Section 251 support. In
2252 this model, participants are given opt-out options at each contact and it is emphasised
2253 that they can withdraw at any time. Previous research demonstrates a good
2254 acceptability of this method¹³, recognising that an approach of ‘consent for each use’
2255 is burdensome for both researcher and participant (Willison *et al.*, 2008) (Taylor and
2256 Taylor, 2014).

2257 A note on public and patient involvement (PPI)

2258 Patients and the general public were not involved in the overall design of the study,
2259 but I did elicit some public opinion when finalising the approach to recruitment.
2260 Following valuable comments from the ethical review board, I undertook a short
2261 survey among specific Patient and Public Involvement (PPI) groups to gauge general
2262 attitudes towards accessing data prior to consent, specifically to support recruitment
2263 to research. I drafted a survey, which outlined the approach to recruitment and the
2264 framework of the study. I accessed a lay PPI group from the Infection and Global
2265 Health (IGH) panel at the University of Liverpool, and one from Health and Care
2266 Research Wales. The survey was sent directly to these groups for dissemination to
2267 members, and additionally the University of Liverpool IGH PPI group promoted the
2268 survey on Twitter.

2269 Participants were asked to think generally about the method of recruitment proposed
2270 and how they felt about this approach. In general, the feeling was that it is acceptable
2271 to access public health or clinical data for recruitment, especially to support much
2272 needed research. However, considerations and worries included the credentials and

¹³ Studies recruiting based on disease surveillance are common for IID, and many projects have taken this approach – the methodology for the epiCrypt Study has been influenced by design aspects of large-scale studies such as Enigma, IID2, and Integrate.

2273 affiliations of the person accessing the data, with NHS/public health staff generally
2274 viewed as more favourable than non-NHS (e.g. University). (Full report on PPI in
2275 [Appendix 7](#))

2276 **Enrolment**

2277 *Identification and first contact with the index case*

2278 Laboratory diagnosed reports of *Cryptosporidium*, and the corresponding patient
2279 contact details, were extracted from the relevant surveillance system by health
2280 protection staff. These were considered the potential 'index' cases – i.e. the first case
2281 in the home to be identified in the surveillance system.

2282 *Exclusion criteria applied*

- 2283 • Index case is in a single person household
- 2284 • Index case is a visitor to a household in the study area, but is registered with
2285 a GP outside the study area
- 2286 • Household is outside the study area
- 2287 • The index case is resident in an institution: retirement home, nursing home,
2288 prison, barracks, boarding school, or college/university halls of residence.

2289 All potentially eligible participants were issued a unique sequential study identifier.
2290 This unique number was used on all documentation from there on in to follow cases
2291 and households anonymously through the study. I was unable to access any patient
2292 identifiable data until the case (and household) had consented.

2293 Potentially eligible index cases were sent an invite letter (Appendix 6) through the
2294 post from the relevant organisation. The aim was to recruit the household.

2295 *Approaching to recruit*

2296 If a contacted index case did not opt-out within two weeks, their details were shared
2297 securely (using internally agreed practices) with named NHS research nurses at the
2298 NIHR Clinical Research Network North West Coast (CRN). The research nurses
2299 would then attempt to contact the index case (or parent/guardian of) via telephone
2300 (using internally agreed practices) to inform them about the study and offer them the
2301 opportunity to participate, if eligible. A maximum of three attempts were made, and
2302 nurses did not leave voicemails. If the approached index case was successfully
2303 contacted and interested in participating, or wanted more information, the research

2304 nurses prepared a study pack ([Appendix 8](#)) with the documentation and the required
2305 number of stool packs (1 per member of the home – the index case was not potted
2306 again). These were posted using a secure post process.

2307 Where a case could not be contacted by telephone, study packs were sent in the post.

2308 Index cases could be excluded at this stage where discussions with the case revealed
2309 that any of the previous exclusion criteria applied.

2310 *Participant consent*

2311 The study pack included consent forms and an explanation of the study. All outreach
2312 was specifically designed to allow potential participants to clearly understand the
2313 purpose of the study and to explain requirements of participation. A parent/guardian
2314 was required to sign the consent form on behalf of those <16 years old, in line with
2315 Gillick competence (House of Lords, 1985). The index case was asked to discuss the
2316 study with their household. Anyone wanting to take part was asked to fill in the consent
2317 for. The index case or appropriate adult should complete the questionnaire, and each
2318 additional consenting household member (not the index case) was asked to provide
2319 a stool sample for testing. At least one household member, as well as the index case,
2320 must have consented.

2321 *Study packs*

2322 Each index case identified, unless withdrawing, was sent a study pack in the post.
2323 The study packs were one per household. Study packs were populated with the
2324 required number of stool packs using Fe-Col[®] kits, which include a pre-addressed and
2325 secure postal bag (compliant with UN3373 regulations for mailing Cat B biological
2326 samples (*Category B - Medical Packaging, medical pouches and biological*
2327 *substances on un3373.com, no date*)).

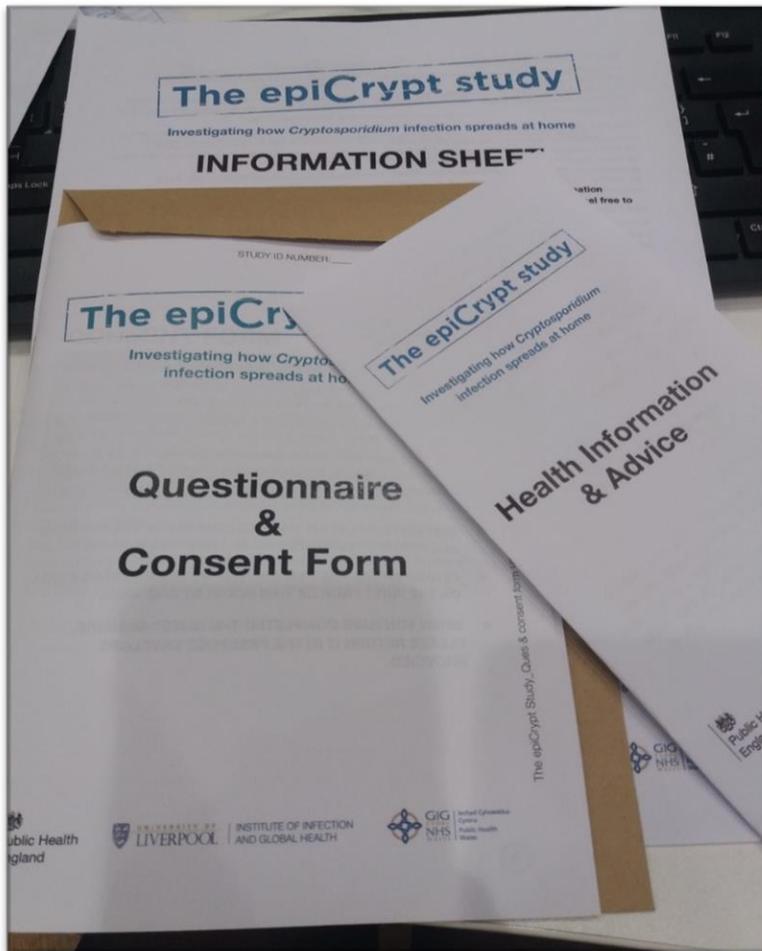


Figure 25: Photograph of study pack documents



Figure 26: Photograph of the Fe-Col stool collection kit

2330 Instructions for use were provided. For those index cases who were unable to be
2331 contacted and were sent packs in the post, three stool packs were included.

2332 The study packs contained:

- 2333 • A study information pamphlet
- 2334 • A questionnaire booklet for the index case or a suitable representative (e.g.
2335 parent, head of household) to complete, with a freepost envelope
- 2336 • A consent form for each participating household member to read, initial, and
2337 sign
- 2338 • A stool sampling pack (Fe-Col®) for each participating household member,
2339 with the required return postal envelope
- 2340 • An information leaflet on cryptosporidiosis and relevant health advice
- 2341 • An information sheet on General Data Protection Regulation (GDPR)
- 2342 • Participation, reminders, and disenrollment

2343

2344 If study materials were not received within 14 days of posting the pack, a reminder
2345 letter was dispatched by the research nurses at the CRN ([Appendix 9](#)). If study
2346 documentation was not returned within 14 days of posting the reminder letter, no
2347 further attempt at contact was made and the index case was removed from the study
2348 line list. (Figure 27: Flow-chart outlining steps to recruit a household and manage
2349 information up until either exclusion or enrolment)

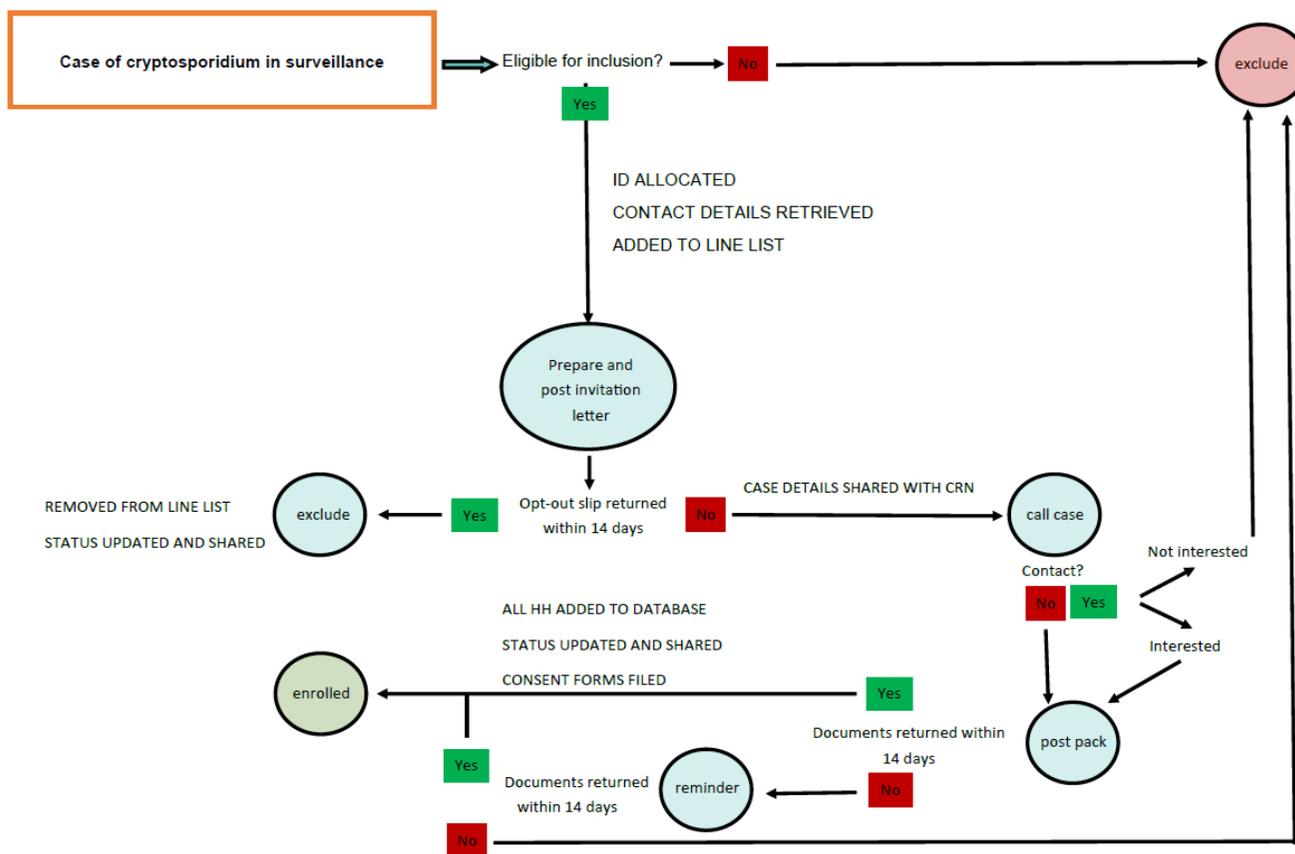


Figure 27: Flow-chart outlining steps to recruit a household and manage information up until either exclusion or enrolment

2351 **Data management and oversight**

2352 *Documentation*

2353 Questionnaires were returned directly to the University of Liverpool. Data were
2354 entered from the paper format to a corresponding MS Access database and held
2355 securely on a University of Liverpool drive in accordance with security protocols.

2356 *Household members' stool samples*

2357 Stool samples provided were sent directly to the Cryptosporidium Reference Unit. For
2358 purposes of data confidentiality and governance, stool samples returned to the CRU
2359 were pseudonymised with the unique study number. Participants were asked to add
2360 their sample date, age, and sex to their pots' stickers in order to facilitate identification
2361 of household members at analysis.

2362 *Identifying the index case samples*

2363 Original diagnostic laboratory numbers were retained with the index case information
2364 in the original line lists at PHE/PHW so that the diagnostic stool sample could later be
2365 identified at the CRU and grouped with the relevant household samples.

2366 Where a consenting family member had also been ill and had a positive diagnostic
2367 stool sample, these were recovered in the same way where possible.

2368 A full laboratory protocol is available in [Appendix 10](#).

2369 **Case definition(s)**

2370 Figures 28 & 29 outline case and household definitions used to categorise household
2371 members post-enrolment.

Individual case definitions	
Index case	The first case from a household identified in the surveillance system (person reported to a PHE/PHW surveillance system(s) following detection of <i>Cryptosporidium sp.</i> in a faecal sample, with a specimen date in the study year)
Additional household case	A person in a household of an index case, with self-reported similar symptoms (in questionnaire) that started within two weeks of the index case's onset
Asymptomatic carrier	A person in a household of an index case with: no reports of similar illness (in questionnaire); AND a <i>Cryptosporidium</i> positive stool sample

2372 Figure 28: Case definitions (individuals)

Household level definitions	
Household	Two or more people (not necessarily related) living at the same address in North West England or Wales who share cooking facilities and share a living room or sitting room or dining area [18].
Household member	A person who normally resides in the household and regularly shares food or toilet facilities (Public Health England, 2017b)
Household contact	A household member in a home where an index case has been identified
Household with transmission	A household that has at least one additional household case
Household without transmission	A household that has one case (the index case)

2373 Figure 29: Case definitions (households)

2374 **Data collection: Outcomes and measurements**

2375 *Questionnaire data*

2376 Full questionnaire available as part of the study pack (Appendix 8)

2377 The questionnaire was divided into four sections.

2378 Section A asked questions to help determine the composition of the household, the
2379 clinical details of the index case, and capture any other symptomatic household
2380 members. A table was used to collect information on any other symptomatic
2381 diarrhoeal illness in the house and capture relationships to the index case.

2382 Section B recorded activities of the index case, and others in the home, in the two
2383 weeks prior to the index case's onset, based on known exposures for
2384 *Cryptosporidium*.

2385 Sections C and D collected household variables, including the number of bedrooms
2386 and bathrooms, capturing those who share beds or baths, and asking about outside
2387 space and animals. We also asked about nappy changing and toilet training in the
2388 home, and about general hand-washing behaviour.

2389 *Stool collection and genotyping*

2390 All consenting household members of the index case were asked to provide a stool
2391 sample using the Fe-Col® kit provided in the study pack.

2392 Screening, confirmation, and species identification

2393 Samples returned from household members were processed by the laboratory and
2394 scored against the Bristol stool scale (BSS). They were then tested and quantified,
2395 only for *Cryptosporidium*, using immunofluorescence (IF) microscopy (CryptoCel,
2396 TCS BioSciences). Samples were then screened using an in-house real-time PCR
2397 targeting the 18S gene ("CRU18S" assay) (Hadfield *et al.*, 2011). Samples testing
2398 negative by both methods were discarded¹⁴.

2399 Positive samples were taken forward to undergo *Cryptosporidium* species
2400 identification using an in-house, duplex real-time PCR designed to identify *C. parvum*
2401 and *C. hominis*, and also enables identification of *C. cuniculus* and the horse genotype
2402 ("RT2" assay) (Robinson, Elwin and Chalmers, 2020). Organism DNA from any RT2

¹⁴ The original index case sample, and any original additional household samples able to be located underwent a slightly different approach, as these were already confirmed and screened. These samples were submitted at the RT2 PCR stage and sequenced as per the others if needed.

2403 negative samples was then amplified using another 18S PCR and sequenced to
2404 identify any other species (or identify a false-positive screen) (Robinson, Elwin and
2405 Chalmers, 2020). Ct values from the real-time PCRs, indicating amount of DNA, were
2406 recorded.

2407 Genotyping

2408 *C. parvum* and *C. hominis* samples were further investigated by sequencing part of
2409 the gp60 gene(Strong, Gut and Nelson, 2000).

2410 *C. parvum* samples were also investigated using a newly validated multilocus variable
2411 number of tandem repeats analysis (MLVA) scheme based on fragment sizing at
2412 seven loci (Pérez-Cordón *et al.*, 2020).

2413 Data items recorded for each stool sample were as follows:

- 2414 • Unique study household identifier (HH ID)
- 2415 • Age
- 2416 • Sex
- 2417 • Specimen date
- 2418 • Bristol stool scale 1-7
- 2419 • IFM result – pos/neg and oocyst count
- 2420 • CRU18S result - pos/neg, plus if positive Ct value
- 2421 • RT2 result – pos/neg, plus if positive Ct value
- 2422 • Species
- 2423 • GP60 subtype
- 2424 • MLVA profile

2425

2426 **Analyses approach**

2427 I wanted to ascertain if, in a home with a case of *Cryptosporidium*, there were any
2428 other cases; my primary research aim was to quantify this and describe any
2429 associated characteristics. This was established both by testing stool samples of
2430 household members living with a case of *Cryptosporidium* and reporting the numbers
2431 of additional cases (according to our pre-determined definitions) and also asking
2432 about self-reported illness in the home. The presence of other cases in the home was

2433 used to infer transmission for the analytical work, and later I go on to present some
2434 descriptive narrative and discussions around the certainty of this.

2435 *Descriptive analyses*

2436 I have presented the numbers of households and participants enrolled in the study,
2437 describing their characteristics, and elements of recruitment such as uptake.

2438 Categorical variables were compared using chi square tests and continuous data
2439 using Wilcoxon rank sums, where appropriate. Additionally, I undertook some
2440 separate descriptive analyses of households which had confirmed cases, including
2441 an examination of clinical symptoms, time between cases' onset of symptoms,
2442 relationships to the index case, and total disease burden on the home.

2443 *Households with and without transmission*

2444 A household with transmission was defined as one with at least one additional report
2445 of compatible illness within two weeks of the index case's onset.

2446 A household without transmission had no reports of compatible illness within two
2447 weeks of the index case's onset.

2448 Households with and without additional cases were compared. I compared
2449 household-level and case-level characteristics of households using univariable
2450 analyses to calculate odds ratios (OR) and p values (Wilson's/Fisher's test). All risk
2451 factors that had a p value less than 0.2 in a univariable analysis were considered in a
2452 multivariable logistic regression to identify independent risk factors for household
2453 transmission.

2454 The following primary outcomes were calculated:

2455 • The transmission rate/prevalence within households

2456 = number of cases in the home/numbers in the home (minus index case)

2457 = number of households with additional cases /number of households

2458 • The amount of asymptomatic carriage among those exposed to symptomatic
2459 case(s)

2460 = number of asymptomatic cases

2461 = number of asymptomatic cases per household/number in home (minus index case)

2462 • Odds of additional illness according to case/household characteristics and
2463 genotype

2464 = odds ratio (odds of a household reporting additional cases by organism species,
2465 case age and sex, size of the home, presence of pets, and any other relevant
2466 characteristics of interest from the descriptive analysis)

2467 *Missing data*

2468 Missing data points were excluded where appropriate, but the household record
2469 remained. Where I was able to, I supplemented missing information from one
2470 component, e.g. the questionnaire, with data from the other, e.g. laboratory data.
2471 Where a questionnaire was not returned, I used the information provided on the stool
2472 pots to estimate the number of people in the home. This is merely a proxy for total
2473 number in the household, as not each household member will necessarily have
2474 returned a stool sample.

2475 *Laboratory stool sample data and confirming infection*

2476 Each household's questionnaire dataset was supplemented with the corresponding
2477 individuals' stool sample results, using the unique identifiers. I considered for each
2478 result the clinical scores (aggregated Bristol stool scale (BSS) 1-7) and
2479 microbiological results (positive/negative, Ct values, species, and genotype result). A
2480 BSS of six or seven was considered a diarrhoeic indicator. An individual was
2481 considered to have a confirmed *Cryptosporidium* infection if the IFM and/or
2482 confirmatory PCR tests (i.e. RT2 or sequencing) were positive.

2483 I describe those homes with confirmed additional cases of *Cryptosporidium*. I examine
2484 the characteristics of these homes in more depth, including describing any likely co-
2485 primary cases, time to additional infections, and an examination of who in the home
2486 gets ill.

2487 *Data analyses*

2488 I undertook all data management, input, and analyses. Data were held in MS Access
2489 and MS Excel, with analyses undertaken in Stata v14 (StataCorp., 2015).

2490 **End of study**

2491 The study ended and closed to recruitment after one year of enrolment from each
2492 area.

2493 **Results**

2494 **Recruitment and uptake**

2495 The study year ran from October 2018-October 2019 for England, and January 2019-
 2496 January 2020 in Wales. Recruitment issues led to a possible dip in enrolment in two
 2497 periods: January, due to staffing and holidays, and an inability to reach index cases
 2498 on the telephone, and again in June when a postal licencing error led to undelivered
 2499 study packs over a 2-week period.

2500 Over 1,000 cases were reported to both surveillance systems over the study year
 2501 (n=1,030). Over half (57%) were from the North West of England, and the remainder
 2502 from Wales (43%), in line with initial estimations in the sample size calculation.

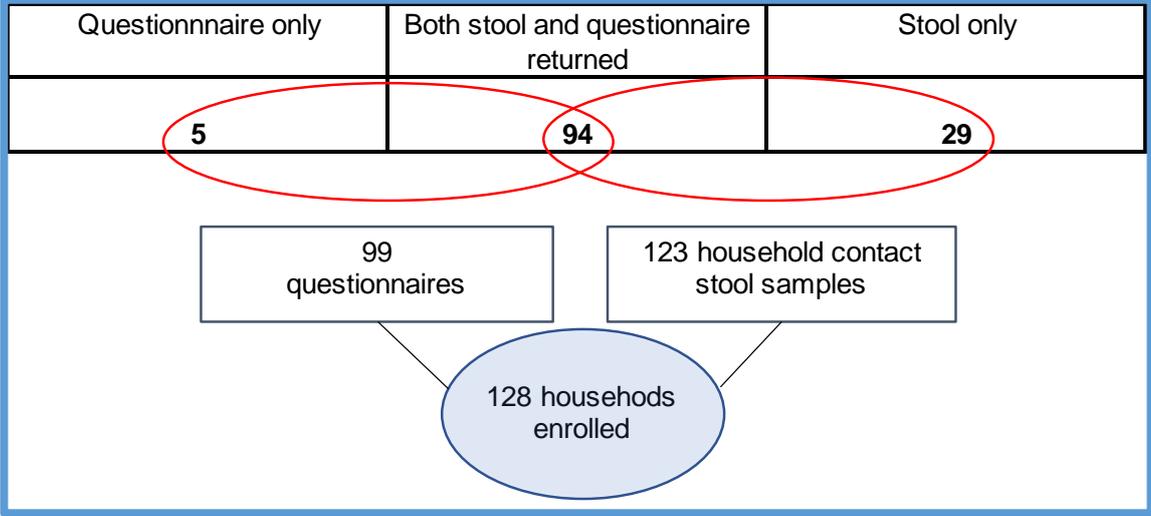
2503 Table 8: Recruitment figures for the epiCrypt study

	North West England	Wales	Total
Cases in study period	585	445	1030
Cases initially eligible	581	435	1016
Invite letters sent	578	405	983
Opt-outs returned	42	35	77
Index cases sent to CRN for contact to recruit	534	370	904
Cases CRN contacted by 'phone	287	154	441
Cases that opted out at 'phone contact stage	90 (31%)	51 (33%)	141 (32%)
HH study packs sent out in total	401	301	702
HH packs returned (questionnaire or stool, or both)	76	52	128
Percentage capture of all incident cases	13%	12%	12%
Percentage uptake (packs returned/packs sent)	18.9%	17.3%	18.2%

2504 *Households enrolled*

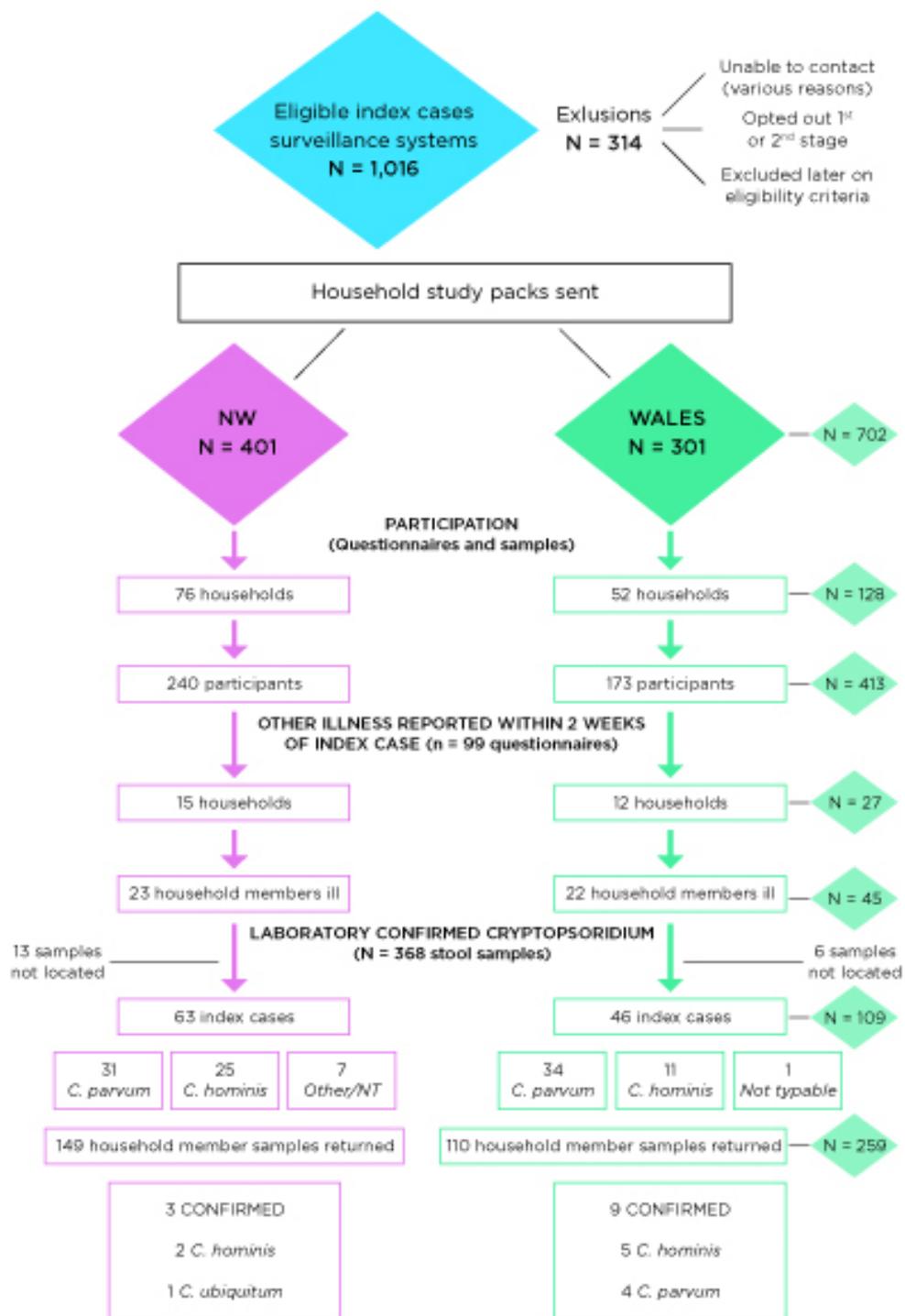
2505 We enrolled 128 index cases and their households into the epiCrypt study in the one-
 2506 year period of recruitment. Over half of these were resident in the North West of
 2507 England (n=76; 59%) and 41% were recruited from Wales (n=52). (Figure 30: and
 2508 Box 2)

2509 This amounted to 413 participants overall, of which 285 were HH contacts of an index
2510 case¹⁵.



2511 Box 2: Study elements returned

¹⁵ This uses a combination of the reported number of persons in the home from the questionnaire, and, where this was not returned, the number of household samples returned per index case. Numbers may not tally as not all household contacts who returned stool were included in the questionnaire data, and vice-versa.



2512 Figure 30: Flow chart of enrolment – questionnaires and stool samples

2513 Of these 128 households, 99 (77%) returned questionnaires and 123 (96%) supplied
2514 at least one stool sample from someone other than the index case. Seventy-four
2515 percent (n=94) of recruited homes supplied both elements.

2516 We were able to locate the corresponding index case diagnostic sample in 109/128
2517 (85%) cases.

2518 Overall, 259 household member stool samples were returned to the reference
2519 laboratory, which, along with the 109 index cases, yielded a total stool sample count
2520 of 368. Figure 30

2521 **Descriptive characteristics**

2522 *Speciation and further typing results*

2523 Of the 128 participating households, 109 index case samples were retrieved, along
2524 with 259 household member samples (n=368). The number of samples returned per
2525 participating household, excluding the index cases, ranged from zero to six (mean=2).

2526 Fifty-five percent (137) of household samples received were from female participants,
2527 and 45% (n=112) were from male. This information was missing for ten households.
2528 The age of household participants ranged from 8 weeks to 77 years old (n=250), with
2529 a median of 34 years (mean = 29.9).

2530 Speciation of typable index cases (n=106) revealed that most cases were *C. parvum*
2531 (n=65; 59.6%) and a third were typed as *C. hominis* (n=36; 33%). The remainder were
2532 *C. cuniculus* (n=3; 3%), *C. ubiquitum* (n=2; 2%), and three were untypable. Further
2533 gp60 typing results of these index case isolates can be found in Table 9.

2534 Eleven household contact samples (11/259; 4%) were positively identified and
2535 confirmed as *Cryptosporidium*. This is further examined in Speciation and further
2536 typing results.

2537 *Timing of specimens*

2538 The average time between the index cases' specimen date and the first household
2539 member specimen was 43 days (median = 41) meaning that on average we were
2540 mostly enrolling participants within six weeks or so.

2541 An examination of onset and specimen dates of all participants (where usable data
2542 were available) revealed that the average time from onset of illness to specimen date
2543 was 22 days (median=11). Among index cases only (n=99; returned a questionnaire,
2544 located sample, and were positive), the mean time from onset of illness to specimen
2545 date was 10 days (median=8). This is most likely because this group would present
2546 in a symptomatic period, or soon after. The difference in median time to sample might
2547 suggest that the lag time from illness to receiving household samples played some
2548 part in an under detection of infection.

2549 **A note:**

2550 Fifteen of the index case specimens were still to be sequenced at the point of writing
2551 this thesis. Unfortunately, due to unforeseen restrictions on laboratory capability due
2552 to COVID-19, these were unfinished. As it was unlikely that they would be completed

2553 before submission, I have proceeded without them. These have been recorded as
 2554 unknown.

2555 Table 9: Speciation and gp60 subtyping results for included index cases

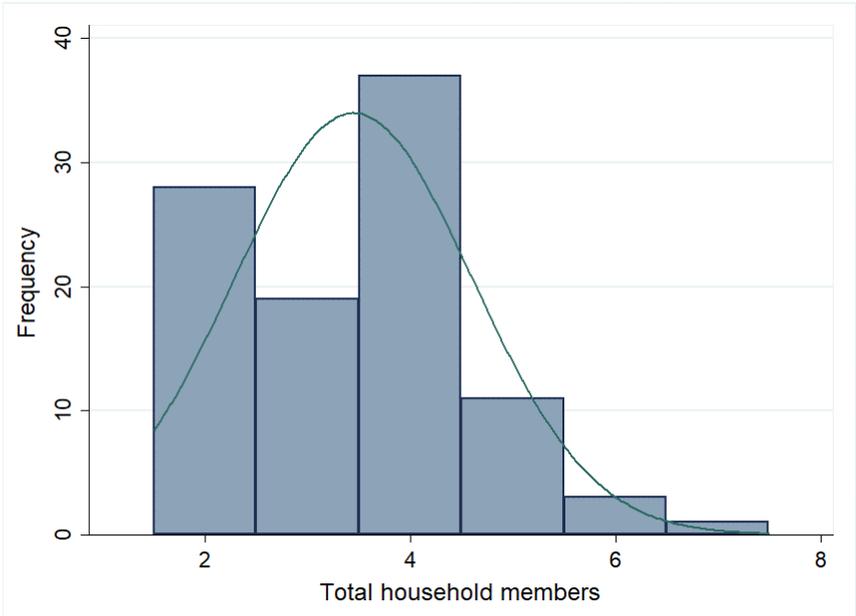
Species (total speciated isolates = 106)	gp60 subtype	n	(%)
<i>C. parvum</i> (n=65)	IlaA15G2R1	15	23.1%
	IlaA17G1R1	11	16.9%
	IlaA19G2R1	2	3.1%
	IlcA5G3J	2	3.1%
	IldA16G1	2	3.1%
	IldA17G1	2	3.1%
	IldA21G1	2	3.1%
	IldA22G1	2	3.1%
	IldA24G1	2	3.1%
	IlaA11G1R1	1	1.5%
	IlaA16R1	1	1.5%
	IlaA17R1	1	1.5%
	IlaA18G1R1	1	1.5%
	IlaA18G3R1	1	1.5%
	IlaA19G1R1	1	1.5%
	IlaA19G3R1	1	1.5%
	IldA20G1	1	1.5%
	Unknown	17	26.2%
<i>C. hominis</i> (n=36)	IbA10G2	23	63.9%
	IbA12G3	6	16.7%
	Unknown	7	19.4%
<i>C. cuniculus</i> (n=3)	VaA13	1	33.3%
	VbA35	1	33.3%
	Unknown	1	33.3%
<i>C. ubiquitum</i> (n=2)	Unknown	2	100.0%

2556 *Household size and composition*

2557 All participating households were families rather than friends or housemates.

2558 I was unable to ascertain any differences between households that participated and
 2559 those that did not, due to the study design: no data or identifiers are available for those
 2560 who did not participate.

2561 Household size
 2562 Household size ranged between two and seven, with a median of four people per
 2563 household (mean = 3.4) (Figure 31). Forty-seven percent of households enrolled
 2564 reported three or fewer people currently living in the home.



2565
 2566 Figure 31: Frequency histogram of total number of people per household across all
 2567 homes enrolled in the study

2568
 2569 Household members
 2570 Data were derived from 99 questionnaires and 29 laboratory samples for all 128
 2571 households. Seven participants (household contacts) were missing age data.
 2572 Table 10 shows the make-up of participating households according to age group of
 2573 all participants.

2574 The age range of participants in the 128 households ranged right across the age
 2575 profiles, with a high proportion of households represented by adults living with
 2576 younger children in parental capacity. Almost half of households (n=61; 48%) had at
 2577 least one child under five living in the home and eight (6%) had an infant under one
 2578 year old. Eleven (9%) households had at least one person over 65. In total, the 413
 2579 participants were mostly comprised of 25 to 44-year olds (37%), with a decent
 2580 proportion of young children represented (21% under 5 years).

2581 Table 10: Make-up of participating households according to age group of all
 2582 participants

Age band	Households with a member in this category (n=128)		Participants in this category (n=413)	
	Number	%	Number	%
<1	8	6.3%	8	1.9%
1-3	44	34.4%	51	12.3%
4-5	26	20.3%	29	7.0%
6-14	50	39.1%	68	16.5%
15-24	22	17.2%	27	6.5%
25-34	52	40.6%	78	18.9%
35-44	53	41.4%	76	18.4%
45-64	9	7.0%	56	13.6%
65+	2	1.6%	20	4.8%

2583 Rooms and crowding

2584 Information on rooms in the home was populated in all but two of the returned
 2585 questionnaires (n=97; 97%). Number of bedrooms ranged from one to six, with a
 2586 median value of three. I calculated a bed-to-person ratio as a proxy variable for
 2587 overcrowding, including all household members reported in the questionnaire¹⁶.
 2588 Ratios ranged from 0.4 to 2.5. If we consider anything less than 1 to indicate some a
 2589 level of overcrowding, 37 (38%) households fell into that category. No difference was
 2590 discernible in this by rural/urban residence (p=0.973).

2591 *Geography of households*

2592 Seventy-six households were enrolled from North West England (59%) and 52 were
 2593 recruited from Wales (41%). Cases originated from various local authorities. I have
 2594 not included an analysis at any lower geographical level due to the possibility of
 2595 deductive disclosure.

2596 The IMD score data were determined using the LSOA derived from postcode. Due to
 2597 ethical restrictions, I was unable to retrospectively access the postcode of the index
 2598 case using laboratory data, and as such geographical analysis only includes those
 2599 households that returned questionnaires (n=99).

¹⁶ Measures of over-crowding ordinarily do not include those under 1 in the home, but my calculations include all members of the household

2600 A corresponding LSOA and subsequent IMD score and quintile was available for
 2601 82/99 households (83%). The remainder (n=17) had partial or incorrect postcodes
 2602 reported on the questionnaire. There was no difference between participating
 2603 household's deprivation quintile by the country of residence (Prob > |z| = 0.156).

2604 Over a quarter of participating households fell into the lower five deciles, representing
 2605 the higher deprivation areas (28/82; 34%) while 66% of homes (n=54/82) were in
 2606 those in the more affluent areas. Of these 82 households, I was able to allocate
 2607 aggregated Welsh and English official rurality indicators: 30/82 (37%) were homes
 2608 considered to be in a rural area (6 NW England, 24 Wales), and 52 (63%) as urban
 2609 (37 NW England, 15 Wales).

2610 Sixty-three of those 82 were either *C. parvum* (n=43) or *C. hominis* (20) index cases
 2611 (five other species, 14 not located) (Table 11). Among the participating cases that
 2612 resided in rural areas (and were speciated), most were *C. parvum*, while most *C.*
 2613 *hominis* cases were reported from urban areas (p=0.029). Eleven households (11%)
 2614 reported living on a farm and six (6%) reported using a private water supply (of which
 2615 five were the farm households). All six homes on a private water supply had a *C.*
 2616 *parvum* index case (p= 0.074) as did 9/10 (one not speciated) cases who reported
 2617 living on a farm.

2618 Table 11: Index case species *C. parvum* or *C. hominis* among homes in either urban
 2619 or rural areas

Index case species	Home in a rural area (n, %) N=25	Home in an urban area (n, %) N=38	Total (n, %) N=63
<i>C. parvum</i>	21 (84.0%)	22 (57.9%)	43 (68.3%)
<i>C. hominis</i>	4 (16.0%)	16 (42.1%)	20 (31.8)

2620 *Animals in the home*

2621 Over half of the homes recruited that returned a questionnaire reported having pets
 2622 (55/99) (Table 12). There was a slight difference in the distribution of *Cryptosporidium*
 2623 species of index case, with more *C. parvum* among those who had pets. However,
 2624 *C. parvum* cases were more frequently reported across the study overall, and this
 2625 result was not statistically significant (p=0.291). Additionally, 21/99 (21%) households
 2626 reported keeping some non-companion animals – mostly chickens, and cattle.

2627 Table 12: Reports of domestic/companion animals

Domestic/pet animal	Number of households reporting this in/at the home
Cats	21
Dogs	36
Birds	3
Horses	2
Reptiles	3
Fish	12
Other	9

2628

2629 Index case characteristics

2630 We enrolled 128 index cases into the study, 109 of which had corresponding stool
2631 samples located at the reference unit (85%). Of the 109 index case samples tested,
2632 most were *C. parvum* (50.8%) followed by *C. hominis* (28.1%).

2633 Table 13: Frequency of species detected among index cases

Index case species	Frequency	%
<i>C. parvum</i>	65	50.8
<i>C. hominis</i>	36	28.2
<i>C. cuniculus</i>	3	2.4
<i>C. ubiquitum</i>	2	1.6
Index case sample not located	19	14.8
Not confirmed	2	1.5
Not typable	1	0.8
Total	128	100

2634 Sex and age of index case

2635 Data on sex were missing for one case; all age data were complete.

2636 Age of the index case ranged from 9 months to 78 years old with a mean age of 22
2637 (21.8;95% CI 17.8-23.3) and a median value of 12 years, suggesting a preponderance
2638 of cases among the younger age groups. Females represented 49.6% (n=63) of index
2639 cases, and males 50.4% (n=64). There was a difference in distribution of ages among
2640 the sex categories, with male cases tending to be younger (n=127; p=0.030). There
2641 was no significant difference in the age distribution among cases of *C. hominis* versus
2642 *C. parvum* (p= 0.256). However, despite similar ranges among the sexes in *C. parvum*
2643 index cases, a wider range is observed in female *C. hominis* cases. (Figs 32 & 33)

2644 The index case was a child under five years old in almost 30% of the 128 recruited
2645 households (n=38; 29.7%) and two-thirds of those were male (n=25; 65.8%; p=0.023).
2646 Children under five years old represented over a quarter of the *C. hominis* index cases
2647 (27.8%) and more than a third of the *C. parvum* (33.9%)

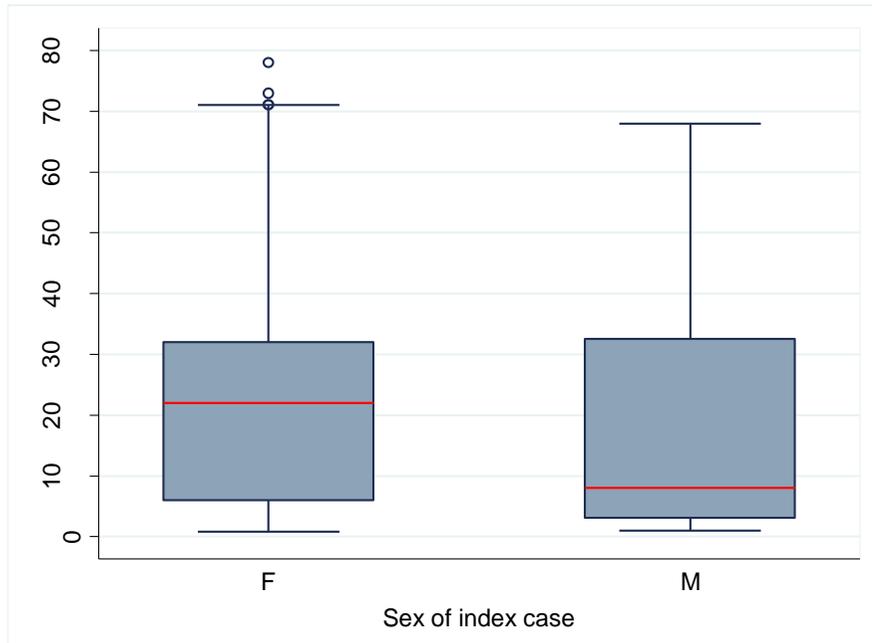


Figure 32: Boxplot: Age and sex of index case

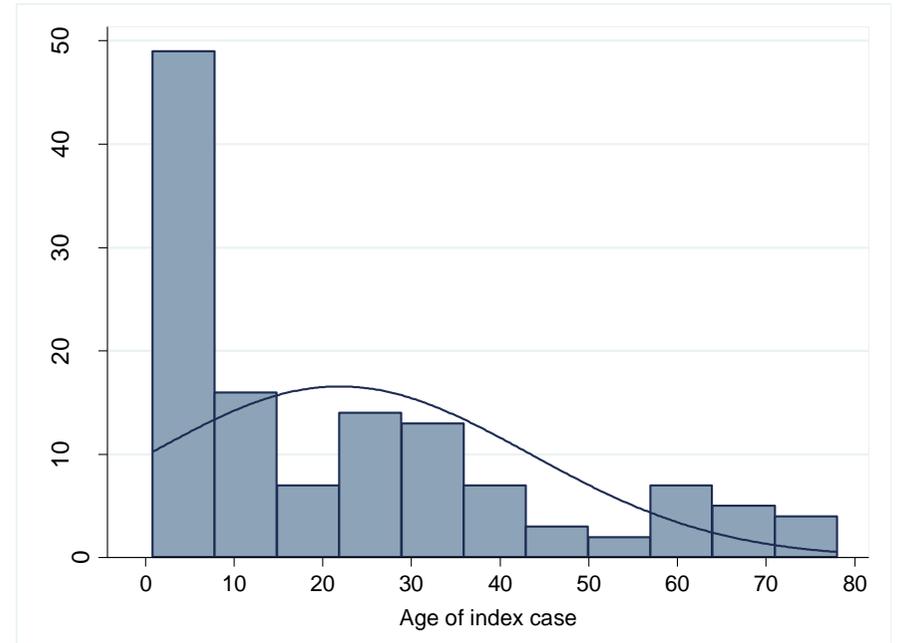


Figure 33: Frequency histogram: age of index case

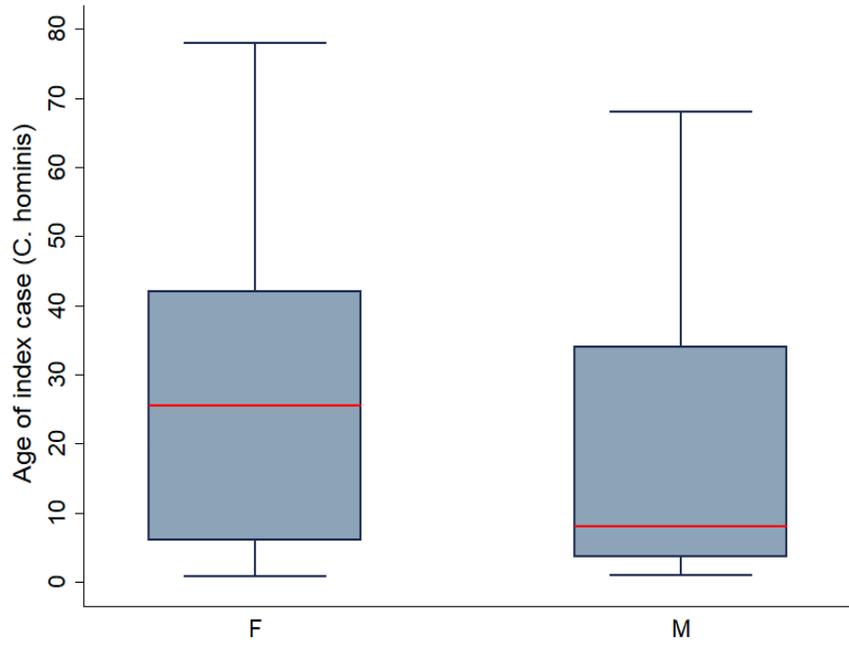


Figure 34: Boxplot: Age and sex of *C. hominis* index cases

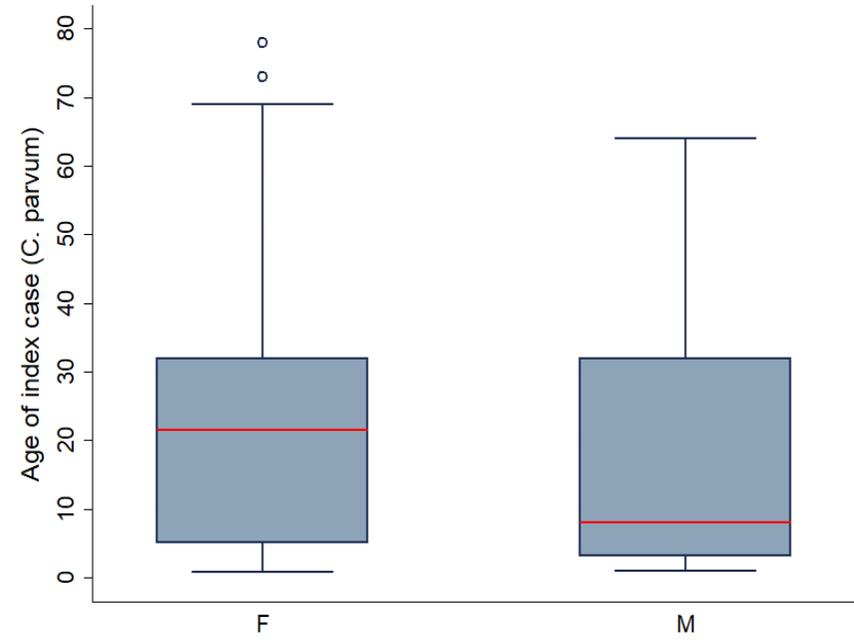


Figure 35: Age and sex of *C. parvum* index cases

2650

2651 *Clinical presentation and symptoms in the index cases*

2652 Clinical information was only available for those index cases who returned a
2653 questionnaire (n=99).

2654 The most frequently reported symptom was diarrhoea (95%) followed by stomach
2655 pain (78%). Less than half of cases reported both diarrhoea and vomiting (49%).

2656 More than a quarter (27.3%) of cases reported some other symptom(s). These most
2657 frequently included foul-smelling stool, sleep disturbances, lethargy and exhaustion,
2658 loss of appetite, and joint pain. In addition, several cases reported ongoing, long-term
2659 and recurring illness and two people reported being admitted to hospital.

2660 *Age and sex*

2661 Table 14 shows reported symptoms in the index case by sex and selected age band.

2662 Nausea, headache, and stomach pain were more frequently reported among older
2663 cases ($p=0.007$, $p=0.002$ and 0.002 respectively). Median age was lower in those
2664 reporting vomiting, although not significantly so ($p=0.137$). Overall, fewer females
2665 than males reported vomiting, (45.7% vs 52.8%), and in particular the proportion of
2666 those under ten years old reporting vomiting was higher in males than in females.

2667 *Infecting species*

2668 In the main there were few differences between index case infecting species and
2669 symptoms reported, although among *C. parvum* cases there were more reports of
2670 high temperature (57% versus 24% in *C. hominis* cases; $p=0.007$). (Table 15)

2671 *Length of illness*

2672 The number of days an index case was symptomatic was reported in 89/99 (89%)
2673 returned questionnaires. Time ill ranged from one to 90 days, with a median of 14
2674 days (mean=18 days). In 96% of cases (n=85) symptoms persisted for 7 days or
2675 more, and 60% of index cases reporting persisting symptoms for at least two weeks
2676 (n=53). There was no relationship between symptoms reported and length of illness.

2677 Table 14: Symptoms reported by index cases, by age and sex

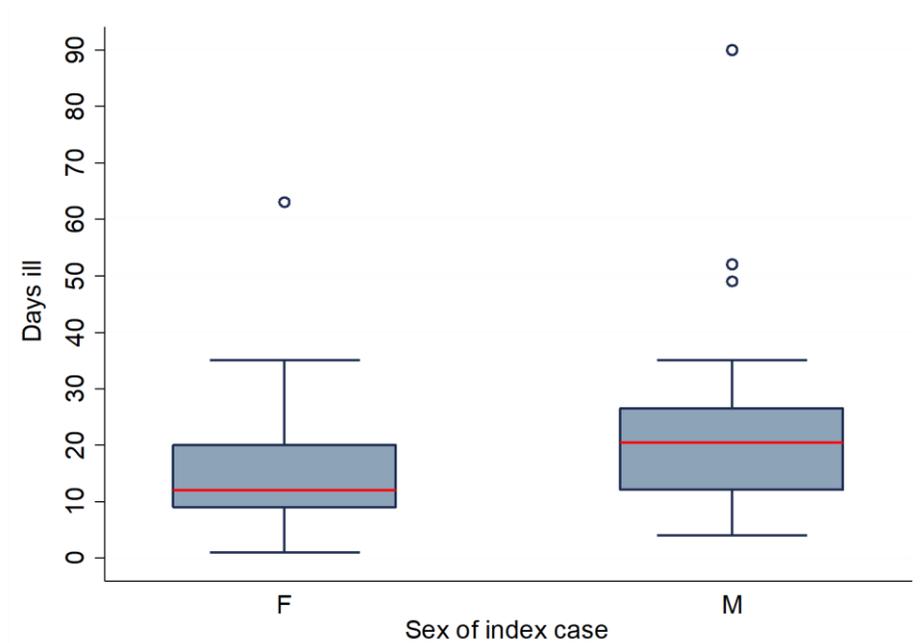
	N	Diarrhoea		Vomiting		Nausea		Pain		Fever		Headache	
Male	53	50	94.3%	28	52.8%	25	47.2%	42	79.2%	22	41.5%	15	28.3%
Under 5	20	20	100.0%	11	55.0%	5	25.0%	14	70.0%	11	55.0%	1	5.0%
5-9	10	9	90.0%	8	80.0%	7	70.0%	8	80.0%	3	30.0%	3	30.0%
10-19	6	6	100.0%	3	50.0%	3	50.0%	6	100.0%	1	16.7%	2	33.3%
20-34	5	4	80.0%	4	80.0%	3	60.0%	4	80.0%	2	40.0%	2	40.0%
35-49	7	6	85.7%	2	28.6%	4	57.1%	7	100.0%	3	42.9%	5	71.4%
50-64	4	4	100.0%	0	0.0%	3	75.0%	3	75.0%	2	50.0%	2	50.0%
65+	1	1	100.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Female	46	44	95.7%	21	45.7%	26	56.5%	35	76.1%	22	47.8%	13	28.3%
Under 5	11	10	90.9%	4	36.4%	3	27.3%	2	18.2%	3	27.3%	0	0.0%
5-9	3	3	100.0%	1	33.3%	1	33.3%	3	100.0%	0	0.0%	0	0.0%
10-19	5	5	100.0%	4	80.0%	5	100.0%	5	100.0%	4	80.0%	2	40.0%
20-34	15	14	93.3%	6	40.0%	9	60.0%	15	100.0%	11	73.3%	8	53.3%
35-49	2	2	100.0%	1	50.0%	2	100.0%	2	100.0%	1	50.0%	1	50.0%
50-64	4	4	100.0%	2	50.0%	4	100.0%	4	100.0%	2	50.0%	2	50.0%
65+	6	6	100.0%	3	50.0%	2	33.3%	4	66.7%	1	16.7%	0	0.0%
All sexes	99	94	94.9%	49	49.5%	51	51.5%	77	77.8%	44	44.4%	28	28.3%
Under 5	31	30	96.8%	15	48.4%	8	25.8%	16	51.6%	14	45.2%	1	3.2%
5-9	13	12	92.3%	9	69.2%	8	61.5%	11	84.6%	3	23.1%	3	23.1%
10-19	11	11	100.0%	7	63.6%	8	72.7%	11	100.0%	5	45.5%	4	36.4%
20-34	20	18	90.0%	10	50.0%	12	60.0%	19	95.0%	13	65.0%	10	50.0%
35-49	9	8	88.9%	3	33.3%	6	66.7%	9	100.0%	4	44.4%	6	66.7%
50-64	8	8	100.0%	2	25.0%	7	87.5%	7	87.5%	4	50.0%	4	50.0%
65+	7	7	100.0%	3	42.9%	2	28.6%	4	57.1%	1	14.3%	0	0.0%

2678

2679 Table 15: Symptoms reported by index cases, by species (or other result)

Species/result	N	Diarrhoea		Vomiting		Nausea		Pain		Fever		Headache	
<i>C. cuniculus</i>	3	2	66.7%	1	33.3%	1	33.3%	3	100.0%	2	66.7%	2	66.7%
<i>C. hominis</i>	25	25	100.0%	11	44.0%	13	52.0%	19	76.0%	6	24.0%	7	28.0%
<i>C. parvum</i>	51	48	94.1%	28	54.9%	26	51.0%	41	80.4%	29	56.9%	13	25.5%
<i>C. ubiquitum</i>	1	1	100.0%	0	0.0%	0	0.0%	1	100.0%	0	0.0%	0	0.0%
<i>Cryptosporidium</i> not confirmed	1	1	100.0%	0	0.0%	1	100.0%	1	100.0%	0	0.0%	0	0.0%
<i>Cryptosporidium</i> sp., not typable	1	1	100.0%	1	100.0%	1	100.0%	1	100.0%	0	0.0%	0	0.0%
Index specimen not located	17	16	94.1%	8	47.1%	9	52.9%	11	64.7%	7	41.2%	6	35.3%
Total	99	94	94.9%	49	49.5%	51	51.5%	77	77.8%	44	44.4%	28	28.3%

2680

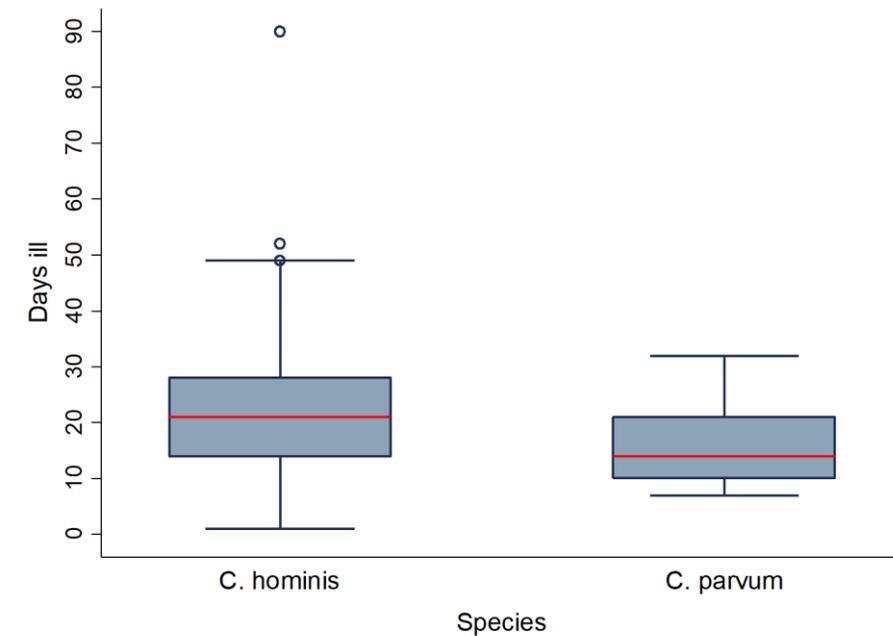


2681 Figure 36: Boxplot: Length of illness (days) in index case, by sex ¹⁷

2682 There were some differences in length of illness by sex of case: males were more
 2683 likely to report a longer illness with a median symptom time of 21 days, versus 15
 2684 days among female cases (p=0.003). Figure 36

2685 Of these 89 cases, 69 (77.5%) were either *C. parvum* or *C. hominis*, with *C. hominis*
 2686 cases reporting longer illnesses (p= 0.004).

¹⁷ The ends of the whiskers represent 1.5*IQR due to consideration of outlier values



2687 Figure 37: Boxplot: Length of illness (days) in index case, by infecting species¹⁸

2688 *Season of reporting*

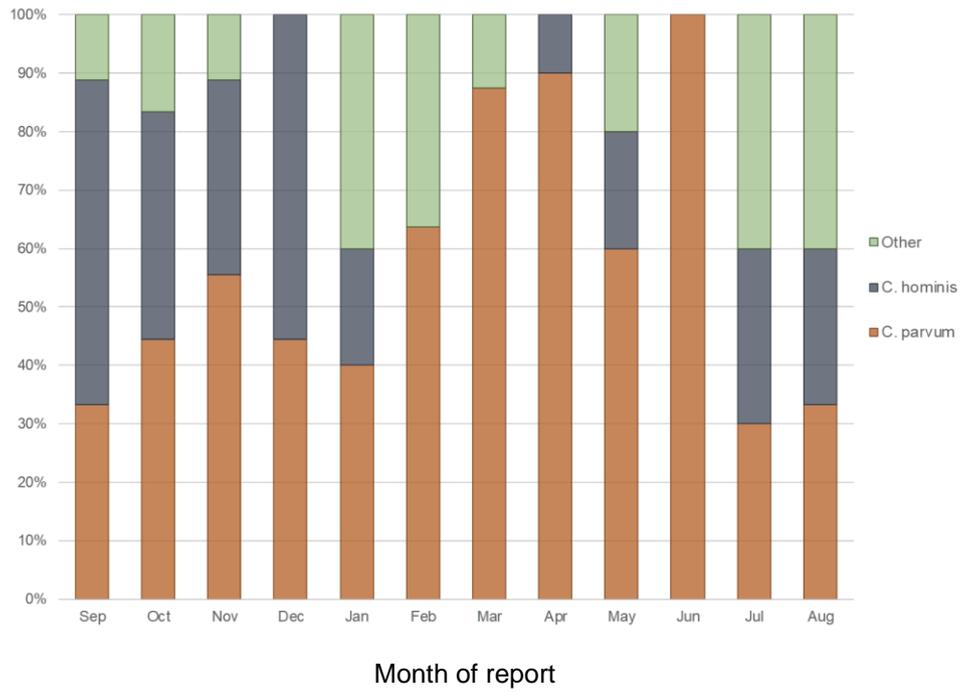
2689 I used the onset date provided in the questionnaire where available; otherwise, month
 2690 of onset was estimated using the specimen date provided with the laboratory data.
 2691 Data on onset/specimen date were missing for five cases.

Index cases were recruited in every month of the study year. Most of the cases reported onsets in August, September, and October. January and June had the lowest number of index cases, but this could be due to recruitment anomalies described earlier.

2692 Figure 38 shows cases the proportion of index case species received by month
 2693 (where the index case was genotyped and had a usable date) (n=101)).

2694 *C. parvum* represented most cases overall, particularly from February to June,
 2695 inclusive. *C. hominis* cases increased later in the year, from September to December.

¹⁸ The ends of the whiskers represent 1.5*IQR due to consideration of outlier values



2696 Figure 38: Proportion of index cases species reported, by month reported to
 2697 surveillance

2698 **Other illness in the home**

2699 For this analysis, I have used a combination of questions from the questionnaire: the
2700 'A8_Else' question asked "*Has anyone else in the home been ill with similar symptoms*
2701 *within two weeks of the index case?*" and 'Table A' allowed participants to add clinical
2702 detail on those that had been ill in the home. Table A relates only to consenting
2703 individuals, whereas 'A8_Else' can report on anyone in the home without divulging
2704 detail. Additionally, 'A8_Else' identifies households where anyone was ill with similar
2705 symptoms to the index case, within two weeks of the index case. This could be before
2706 or after the index case's onset. Table A reports on illness at any time, although I did
2707 request onset dates. I have tried to combine where possible these questions.
2708 Additional similar illness in the home within two weeks of the index case is used here
2709 as a proxy measure for households with transmission.

2710

2711 *Burden of illness*

2712 Transmission prevalence

2713 – within 2 weeks of the index

2714 Twenty-seven out of 99 households (27%) indicated that there had been at least one
2715 case of additional illness in the home within two weeks of the index case. Of these,
2716 10 (37%) were prior to the index case. This amounted to 45 household members out
2717 of 423 participants (using questionnaire data) giving rise to an attack rate of 10.6%.

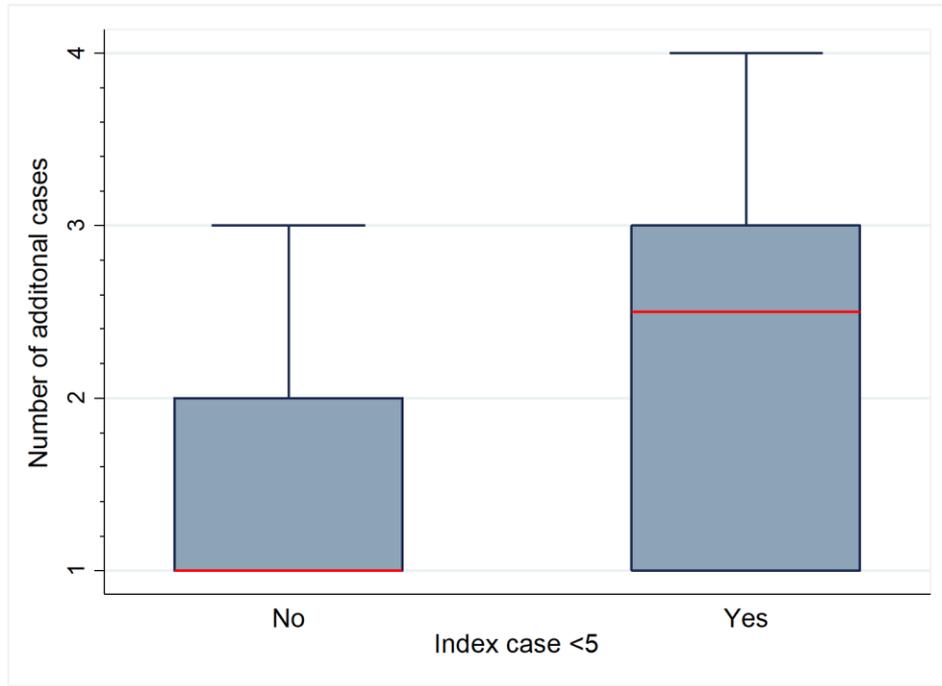
2718 – at any time

2719 Forty-four homes reported in Table A that there had been some compatible illness in
2720 the home (at any other time), with 25 of these in the last two weeks.

2721 Excluding the index cases, 76 additional cases of illness at any time in the home were
2722 reported, out of 242 at risk household contacts, giving rise to an attack rate of 31.4%.

2723 Number of additional cases

2724 Among the 99 households, the number of additional people in the home with any
2725 illness reported within 2 weeks (i.e. excluding the index) per home ranged from one
2726 to four (n=25; I excluded two records where they had replied YES to QA8 and NO to
2727 "Any other illness in the home"). On average, 1.8 additional cases were reported per
2728 household. This was generally higher in homes where the index was less than five
2729 years old (Figure 39 & Table 16)



2730

2731 Figure 39: Boxplot: Additional cases per household, by age of index case in the
2732 home

2733 Table 16: Number of additional cases within 2 weeks, per household, plus range
2734 data, by age of index case

Number of additional cases	Index >5	Index <5	Total
1	9	4	13
	69.23	33.33	52
2	3	2	5
	23.08	16.67	20
3	1	5	6
	7.69	41.67	24
4	0	1	1
	0	8.33	4
Total additional cases	13	12	25
Range	1-3	1-4	
Median	1	2.5	
Mean	1.38	2.25	

2735

2736 *Index case characteristics*

2737 Sex

2738 In 16 (59%) of these homes reporting transmission the index case was male ($p=0.484$)
2739 and in 44% ($n=12$) the index was less than five years old ($p=0.084$).

2740 Length of illness

2741 There was no statistically significant difference in the length of index case illness
2742 ($p=0.838$) in households that did and did not report other illness. There was no
2743 relationship between the number of days of illness reported by the index case and
2744 number of additional cases in the home (Spearman's rank correlation $p=0.543$). On
2745 average, 13 days elapsed between onset in the index case and the next case (median
2746 = 10). The shortest of these was zero days, possibly a co-primary case; these data
2747 did not allow for further examination of this.

2748 Potty/Toilet training

2749 Of the 38 index cases under five, 17 were in nappies, and seven were currently
2750 undergoing potty/toilet training. Where the index was under five and the home
2751 reported additional cases, 40% reported using nappies, versus 68% in homes where
2752 there was no transmission ($p=0.149$) There was no difference between species of
2753 index and whether or not the index was in nappies ($p=0.973$) or potty training
2754 ($p=0.131$).

2755 Burden of additional cases by index case infecting species

2756 The number of additional cases was greater in households where the index case was
2757 infected with *C. hominis* species (rank sum p value= 0.03). Less than 20% of the *C.*
2758 *parvum* index cases reported additional illness in their household (19.6%), compared
2759 to 48% of the *C. hominis* indexes ($p=0.010$).

2760 Table 17 shows households with and without additional illness, by species of index
2761 case.

2762 Table 17: Households with and without additional illness, by species of index case

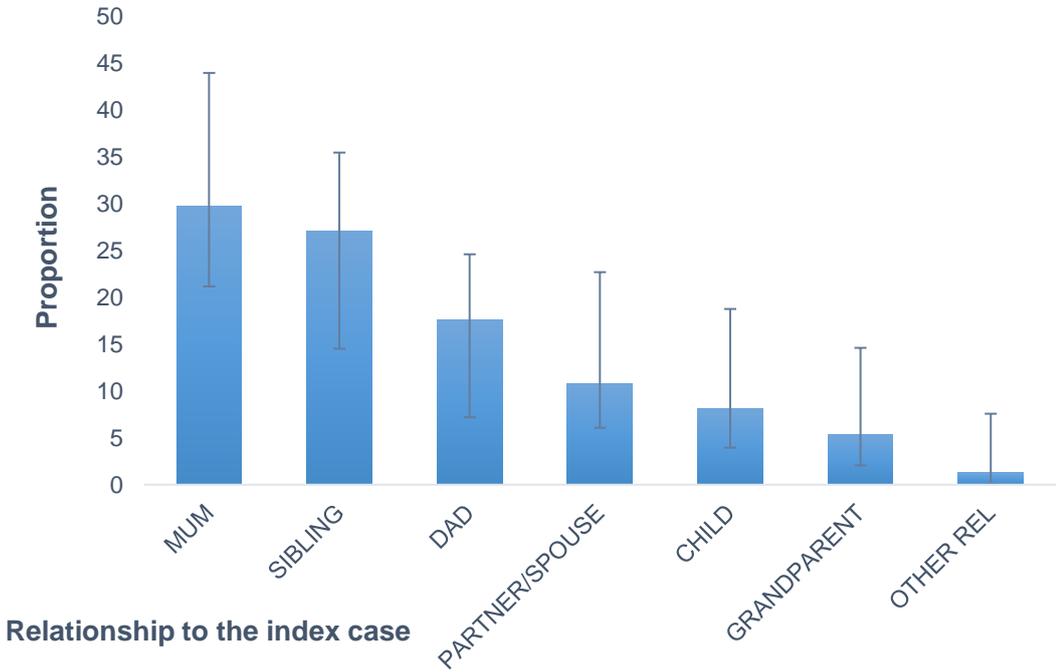
	Species/speciation result				Index not located	Not confirmed	Total
	<i>C. hominis</i>	<i>C. parvum</i>	<i>C. ubiquitum</i>	<i>C. cuniculus</i>			
Anyone else ill in the home within 2 weeks of the index case							
Yes	12 44.4%	10 37.0%	1 3.7%	0 -	4 14.8%	0 -	27 100%
No	13 18.1%	41 56.9%	0 -	3 4.2%	13 18.1%	2 2.0%	72 100%
Total	25 25.3%	51 51.5%	1 1.0%	3 3.0%	17 17.2%	2 2.0%	99 100.0%

2763

2764 *Who is at risk in the home?*

2765 Relationships

2766 Table A in the questionnaire collated self-reported illness for individuals in the home.
2767 This information is used for this analysis. Overall, 74 people reported having additional
2768 illness, across the 99 households who completed the questionnaire. The most
2769 affected person in the family was mothers, who represented 30% of this additional
2770 illness (n=22; 95% CI: 21.2 - 43.9). This was followed by siblings who represented
2771 27% of illness (n=20; 95% CI: 14.52 - 35.46). (Figure 40)



2772 Figure 40: Proportion of additional illness in households by relationship to the index
2773 case

2774 *Accessing a clinician/GP*

2775 Thirteen of the 74 participants reporting illness said they visited a doctor (17.5%).
2776 Largely these were children, siblings of the index case (5; 38.5%).

2777 **Characteristics associated with transmission in the home**

2778 *Univariable analysis*

2779 Table 18 shows the number of households reporting selected exposures and
2780 characteristics, by whether or not participants reported other compatible illness within
2781 two weeks of the index case.

2782 **Infecting species**

2783 The variable most strongly associated with transmission was the infecting species of
2784 the index case. Among homes that reported transmission, there was a preponderance
2785 of *C. hominis* cases versus *C. parvum* cases and this exposure was three times more
2786 likely in homes with additional cases (OR = 3.78).

2787 **Children in the home**

2788 Homes with additional cases were twice as likely to report the index case being a child
2789 less than five years old (OR = 2.23) with 44% of households with transmission
2790 reporting this exposure, compared to just over a quarter of homes without additional
2791 cases (26.4%). Additionally, this relationship remained when examining any children
2792 under five in the home. Having an index case who attended a nursery was more than
2793 twice as likely in homes with transmission (OR = 2.5).

2794 **Household size and crowding**

2795 Although not statistically significant, being in a home with three or fewer people was
2796 reported in a greater proportion of those homes without additional cases (OR = 0.56).
2797 This is supported by the other crowding indicators; less than one toilet per person (OR
2798 = 3.29) and less than one bedroom per person (OR = 1.96) were both more prevalent
2799 in homes where there was transmission.

2800 **Deprivation, sex of case, symptoms**

2801 There was no statistically significant difference in IMD Score (0.6982) in households
2802 that did and did not report other illness. Sex, deprivation decile, and illness symptoms
2803 were not associated with differences in transmission.

Table 18: Households reporting selected exposures and characteristics, by whether or not participants reported other compatible illness within two weeks of the index case, with odds ratios and 95% CI

Characteristic of home and case (exposure)	Households (N=99) reporting that exposure		Households with additional reported illness (A8_Else=1, n=27)		Households without additional reported illness (A8_Else=0, n=72)		Odds ratio	95% CI	P value
	n	%	n	%	n	%			
Index case is <i>C. hominis</i> species (vs <i>C. parvum</i> (n= 76))	25	32.9%	12	54.5%	13	24.1%	3.78	1.171, 12.236	0.01
Fewer than 1 toilet per person (n= 96, excl. 3 values)	72	75.0%	23	88.5%	49	70.0%	3.29	0.839, 18.729	0.06
Index case attends nursery	21	21.2%	9	33.3%	12	16.7%	2.50	0.786, 7.651	0.07
Children (5 years old or under) in household	48	48.5%	17	63.0%	31	43.1%	2.25	0.898, 5.728	0.08
Index case under 5 years old	31	31.3%	12	44.4%	19	26.4%	2.23	0.792, 6.158	0.08
Crowded (fewer than 1 bedroom per person) (n=97, excl. 2 missing values)	37	38.1%	13	50.0%	24	33.8%	1.96	0.708, 5.378	0.15
Nappies/potty training anyone in home	32	32.3%	11	40.7%	21	29.1%	1.67	0.591, 4.582	0.27
Pets in household	55	55.6%	17	63.0%	38	52.8%	1.52	0.563, 4.246	0.36
Index case in nappies or toilet training	23	23.2%	7	25.9%	16	22.2%	1.23	0.369, 3.735	0.70
Index shares bed	39	39.4%	11	40.7%	28	38.9%	1.08	0.392, 2.906	0.87
Length of illness > 14 days (n=89, 10 missing values)	41	46.1%	12	46.2%	29	46.0%	1.00	0.361, 2.767	0.99
Both diarrhoea and vomiting in the index case	48	48.5%	13	48.1%	35	48.6%	0.98	0.398, 2.411	0.99
Length of index case's illness >7 days (n=89, 10 missing values)	79	88.8%	23	88.5%	56	88.9%	0.96	0.197, 6.242	0.95
Most deprived deciles (lowest 5 IMD vs top 5) n=82	28	34.1%	7	33.3%	21	34.4%	0.95	0.280, 3.011	0.93
Index cooks regularly for home	34	34.3%	9	33.3%	25	34.7%	0.94	0.322, 2.604	0.90
Female index case	46	47.9%	11	40.7%	35	48.6%	0.73	0.266, 1.943	0.48
Total household members <=3	47	47.5%	10	37.0%	37	51.4%	0.56	0.225, 1.379	0.21

2805 *Logistic regression*

2806 Associations with transmission in the home were features of the index case: being
2807 infected with *C. hominis* versus *C. parvum*, and attending nursery, with a reduced
2808 odds ratio among females. Only having an index case of *C. hominis* was
2809 independently associated with transmission in the home in the multivariable model.

2810 Table 19: Logistic regression model of variables (retaining age<5 and sex in the
2811 model)

Variable (index case characteristics)	Odds ratio	Std. error	z	P> z 	95% CI	
<i>C. hominis</i>	4.46	2.68	2.48	0.013	1.37	14.53
Attends nursery	4.21	5.14	1.18	0.239	0.38	46.14
Less than 5	0.91	1.07	-	0.931	0.09	9.23
Sex - Female	0.64	0.37	-	0.44	0.20	1.99
-cons		0.11	-	0.00	0.06	0.57

2812

2813 **Description of homes with confirmed additional infections**

2814 *Positivity of samples and confirmation in household contacts*

2815 We were only able to confirm infection in a small proportion of household samples
2816 (4%; n=12/259). Six out of the 88 samples amplified using the CRU18s screening
2817 PCR were subsequently negative and were not *Cryptosporidium*.

2818 From 137 confirmed positive samples, there were 85 gp60 and 58 MLVA results.

2819 *Description of households and results by species*

2820 ***C. cuniculus***

2821 Testing identified three *C. cuniculus* cases among the index samples. These cases
2822 arose from three households, and all were adult cases. All returned questionnaires

2823 None of these reported any other compatible illness in the home and corresponding
2824 household member samples were negative.

2825 ***C. ubiquitum***

2826 Three *C. ubiquitum* cases were identified across two households; two index cases
2827 and one additional confirmed household infection. All were in children. The samples
2828 have yet to be sequenced at the gp60 gene.

2829 The index case from household (HH) 1 was nine years old. This household returned
2830 a questionnaire and did report compatible illness in the home, but no additional detail
2831 was recorded in the questionnaire. The onset was in July and the case reported being
2832 ill (with diarrhoea and abdominal pain) for 30 days. No household contact samples
2833 were received from this home.

2834 The index case from HH 2 was a two-year-old female. The household member who
2835 was positive was 10 months old, likely a sibling, with a BSS of five. One additional
2836 stool was provided from this home (adult female) which was negative, and the stool
2837 was formed (BSS = 3). The participants did not return a questionnaire; consequently,
2838 I was unable to ascertain reports of additional illness.

2839 ***C. hominis***

2840 Forty-three *C. hominis* positive samples were reported overall: 36 index cases and
2841 seven household samples. Of the 33 that had corresponding GP60 results, there were
2842 two subtypes identified: IbA10G2 (n=27; 81.8%) and IbA12G3 (n=6; 18.2%).

2843 The **IbA12G3** cases were all index cases – no confirmed household samples were
2844 identified with this subtype. However, of the six cases, five returned questionnaires
2845 and all of them reported additional illness in the home. These were mostly male (n=5;
2846 83.3%) and in a range of children and adults. Of those records for which data were
2847 available, time from onset to specimen date was 11 days and the mean duration of
2848 illness was 21.6 days (median = 20).

2849 The **IbA10G2** cases were from a range of index and (n=230 household (n=4)
2850 samples. One case occurred in January and the remainder had specimen dates
2851 between June and December. They occurred in a range of ages, from 1-71 years,
2852 with 14 females (51.8%) and 13 (48.1%) males. Of those records for which data were
2853 available, average time from onset to specimen date was 11 days and the mean
2854 length of time ill was 26.5 days (median = 21).

2855 ***C. parvum***

2856 Sixty-nine samples were positive for *C. parvum*: 65 index cases and four household
2857 member samples (across three households). Of these, 50 had GP60 results, and 17
2858 subtypes were identified. These were predominantly IlaA15G2R1 (15; 30%) and
2859 IlaA17G1R1 (11; 22%).

2860 The **IlaA15G2R1** cases were all index cases – no confirmed household samples were
2861 identified with this subtype. They occurred in all ages although tended to be in young
2862 children (range 1-68; mean 11, median 3). Males and females were equally
2863 represented, and cases occurred across most of the year. Fourteen households had
2864 returned a questionnaire and six of these reported other illness in the home. Of those
2865 records for which data were available, average time from onset to specimen date was
2866 6 days and the mean length of time ill was 11.8 days (median = 11).

2867 The **IlaA17G1R1** cases were also all index case samples. Five of the 11 returned
2868 their questionnaire and two of these households reported other illness. These
2869 subtypes occurred in both adults and children, but all cases were in under 40-year
2870 olds (range 1-38; mean 21). Of those records for which data were available, the
2871 average time from onset to specimen date was 6 days and the mean length of time ill
2872 was 18.5 days (median = 21).

2873 *Other clinical indicators in household contact samples*

2874 The BSS was used as an additional clinical indicator, with a score of 6/7 considered
2875 indicative of diarrhoea. (This indicator was only applied to household samples

2876 returned via post, as the original index case sample material was not available for
2877 assessment and they are presumed symptomatic). Of all specimens submitted, 88
2878 (33.9%) were considered diarrhoeic. Nonetheless, 28 (31.8%) of these said they had
2879 no symptoms of illness. All of these specimens were subsequently not confirmed as
2880 *Cryptosporidium*. Conversely, all those household member samples that were
2881 confirmed had BSS scores of under six (i.e. formed).

2882 *Asymptomatic infection*

2883 There were two positive samples identified in participants (1.6% of households) who
2884 did not report illness: both were from adult males, both *C. hominis*. One of these was
2885 gp60 subtype IbA10G2, the other subtype was not identified. Both were parents of an
2886 index case (both *C. hominis* IbA10G2). In both cases, other illness was reported in
2887 the home. One of these samples was retrieved from an originally submitted diagnostic
2888 specimen, which may question the validity of the case reporting no symptoms.

2889 *Confirmed infections and additional illness in the home*

2890 Of 128 households, 82 had both an index case located and returned a questionnaire,
2891 allowing me to merge these data to complete a narrative description of homes where
2892 confirmed additional infections were identified. Using responses from Table A in the
2893 questionnaire, 39/82 (47.6 %) reported some additional illness at any other time in
2894 the home (not necessarily within 2 weeks).

2895 Table 20 shows the number of households that had any member samples confirmed
2896 as *Cryptosporidium*, by the index case result. Overall, ten households in this study
2897 had confirmed infection in at least one other person (7.8%). These were all in homes
2898 where we were able to identify a corresponding original index case sample. These
2899 ten homes with confirmed additional infection yielded 28 household member samples
2900 in total. Of these 28 samples, 12 (42.9%) were confirmed: this comprised one
2901 household member in each of eight homes, and two household members apiece in
2902 the remaining two homes.

2903 Table 20: Number of households that had any member samples confirmed as
 2904 *Cryptosporidium*, by the index case result

		Confirmation of infection in any HH member sample	
Index case species	All	Yes	%
<i>C. cuniculus</i>	3	0	0%
<i>C. hominis</i>	36	6	17%
<i>C. parvum</i>	65	3	5%
<i>C. ubiquitum</i>	2	1	50%
<i>Cryptosporidium</i> not confirmed	2	0	0%
Not typable	1	0	0%
Total	109	10	9%

2905 In 6/10 (60%) of the households in which additional infection was identified, the
 2906 samples were identified from original previously submitted samples, rather than newly
 2907 collected ones. In this way, we know that in all of these cases it is quite likely that
 2908 symptoms were present, as this is what would drive the person to seek clinical
 2909 assessment and have a sample taken. This may be because of severity of symptoms,
 2910 or shorter time from onset to sample than those who submitted samples in the usual
 2911 way. I go on to discuss the possible impact of this in the discussion.

2912 In those samples where we retrieved a subtyping result, all corresponding household
 2913 contact samples had the same gp60 as the index case and no multiple infections
 2914 were identified.

2915 We were able to confirm infection in two individuals' samples who reported no
 2916 symptoms. However, we did observe that 28/81 (34.6%) individuals who did not report
 2917 symptoms submitted stool samples that were scored a BSS of 6 or 7, indicating
 2918 diarrhoeic consistency.

2919 **An overview of the burden of disease in homes with confirmed additional**
2920 **cases**

2921

2922 **Household A**

Index: <i>C. ubiquitum</i>	No typing
HH sample 1: <i>C. ubiquitum</i>	No typing

2923 One household had an index case of *C. ubiquitum* with one additional stool sample,
2924 which was also positive. No further subtyping has been available.

2925 The index case was a two-year-old female, and the positive household contact was
2926 a 10-month-old female, likely a sibling. There were 39 days between the specimen
2927 dates of these two cases, making spread a possible explanation. However, the
2928 household did not return a questionnaire and no further examination of this home was
2929 possible.

2930

2931 **Household B**

Index: <i>C. parvum</i>	gp60 IIdA21G1
HH sample 1: <i>C. parvum</i>	gp60 IIdA21G1

2932 This household had an index case of *C. parvum* and returned one household sample,
2933 which was also positive. An identical gp60 subtype was identified in both samples.
2934 The index case was a child and the additional confirmed infection was in Mum, who
2935 did report compatible illness. Their onsets were 17 days apart, and the index case
2936 had been ill for three weeks in total, making this a likely case of transmission. Overall,
2937 illness in the home extended over 24 days.

2938 Mum's sample was a previously submitted one, and time from onset to specimen
2939 collection was 13 days, although symptoms had reportedly ceased by that time.

2940 The questionnaire revealed that this index case also lived with Dad and two older
2941 siblings, none of whom reported illness and whose samples were not submitted for
2942 testing. (Figure 41)

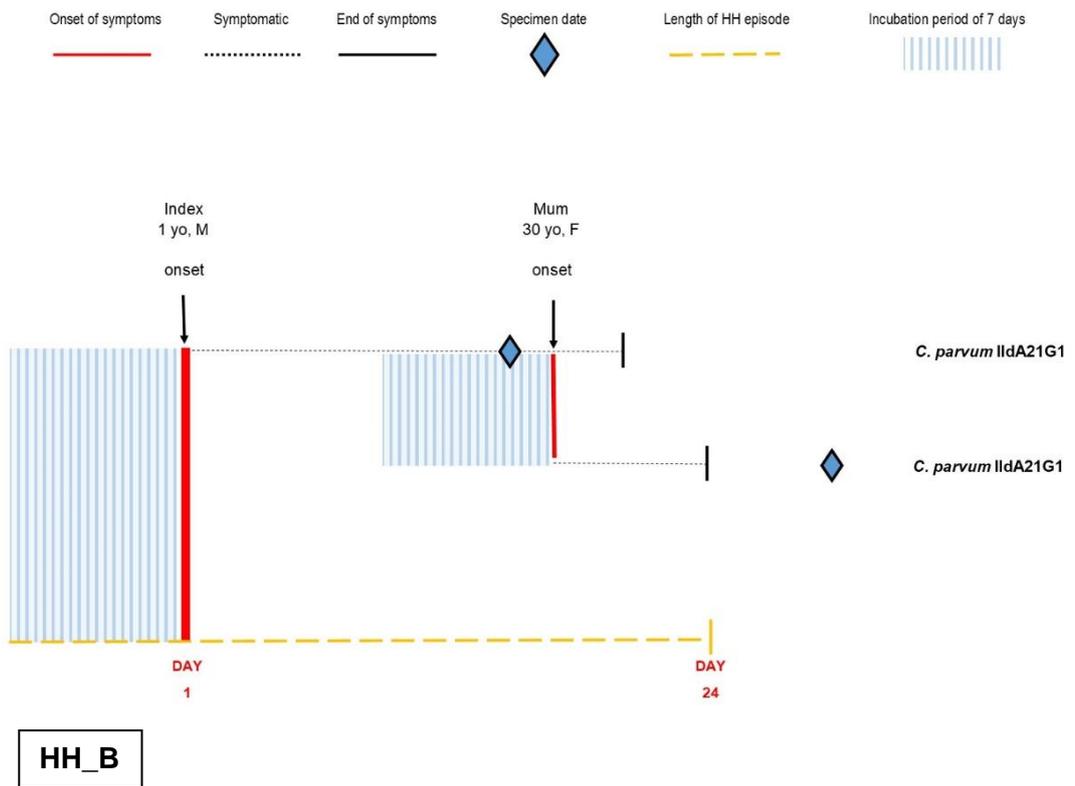


Figure 41: Depiction of course of illness in the home, with laboratory results_Household B

2943

2944 **Household C**

2945

Index: <i>C. parvum</i>	gp60 IIdA16G1
HH sample 1: <i>C. parvum</i>	gp60 IIdA16G1
HH sample 2: <i>Cryptosporidium</i> not detected	--
HH sample 3 <i>Cryptosporidium</i> not detected	--

2946 The household C index case was *C. parvum*, with gp60 subtype IIdA16G1. Three¹⁹
2947 additional household contact samples were received by the laboratory, of which one
2948 was positive for *C. parvum* IIdA16G1.

2949 The index case was ten years old, and the additional confirmed infection was in a
2950 seven-year-old sibling. Samples were also submitted for Mum and Dad;
2951 *Cryptosporidium* was not detected in either. The positive sibling did reportedly have
2952 symptomatic illness, as well as Dad, who reported a short, two-day illness. Both
2953 children reported illness of eight days and overall the home reported a duration of
2954 illness of 15 days. (Mum was not ill and is not on the chart)

2955 The time from index onset to the first (sibling) illness was five days, and a further eight
2956 days elapsed until Dad began to get ill. This could represent co-primary infections in
2957 the children with spread to the parent, or secondary or even tertiary levels of spread
2958 from the index case. The family did report common exposures to the index, including
2959 water sports, contact with pets, and travel.

2960 The time between onset and specimen collection for the index and positive household
2961 contact was 7 and 2 days, respectively. For Dad, who could not be confirmed, this
2962 was 62 days. Although Mum did not report symptoms, the submitted stool sample
2963 was considered diarrhoeic (BSS = 6). (Figure 42)

¹⁹ This family returned four additional samples, but one of those was suspected to be the sibling again, and so I have excluded.

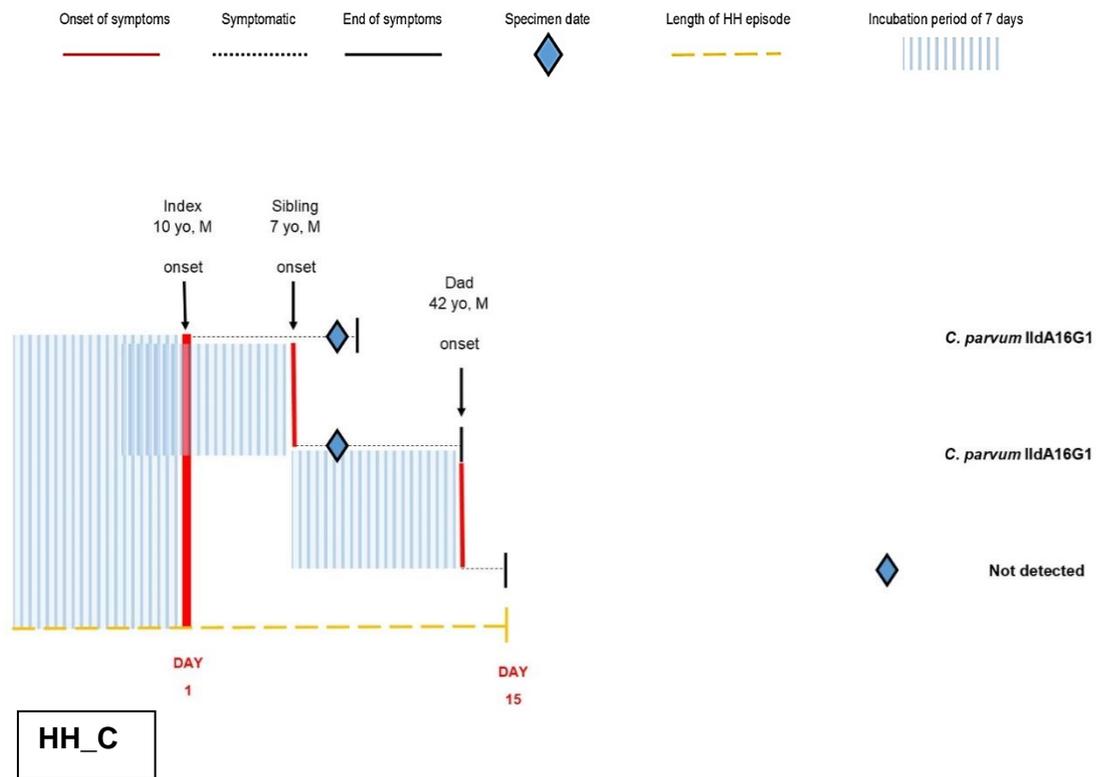


Figure 42: Depiction of course of illness in the home, with laboratory results_Household C

2964 **Household D**

Index: <i>C. parvum</i>	MLVA sequence 4-14-5-8-27-28-16
HH sample 1: <i>C. parvum</i>	MLVA sequence 4-14-5-8-27-28-16
HH sample 2: <i>C. parvum</i>	MLVA sequence 4-14-5-8-27-28-16

2965 The household D index case was *C. parvum*. Two additional household contact
2966 samples were linked to this household, and both were positive for *C. parvum*.

2967 The gp60 subtype analysis could not be confirmed for these samples. However, the
2968 laboratory was able to complete the MLVA and all three samples were prolife 4-14-5-
2969 8-27-28-16, and indistinguishable.

2970 The index case was a two-year-old girl, and both household contact samples were
2971 taken from siblings (aged two and four). All children reported symptoms: nine days
2972 duration for the index case, and eight and ten days for the siblings. (Accurate onset
2973 data were not available for one of the siblings). Mum and dad were also reported as
2974 living in the home, but no samples were submitted. The household contact cases both
2975 had samples submitted on the same day. This suggests that the index may have
2976 infected both siblings at around the same time.

2977 The time between onset and specimen collection for the index and one of the positive
2978 siblings was five and four days, respectively. No BSS data were available, but each
2979 sample would have been taken during a symptomatic period. (Figure 43)

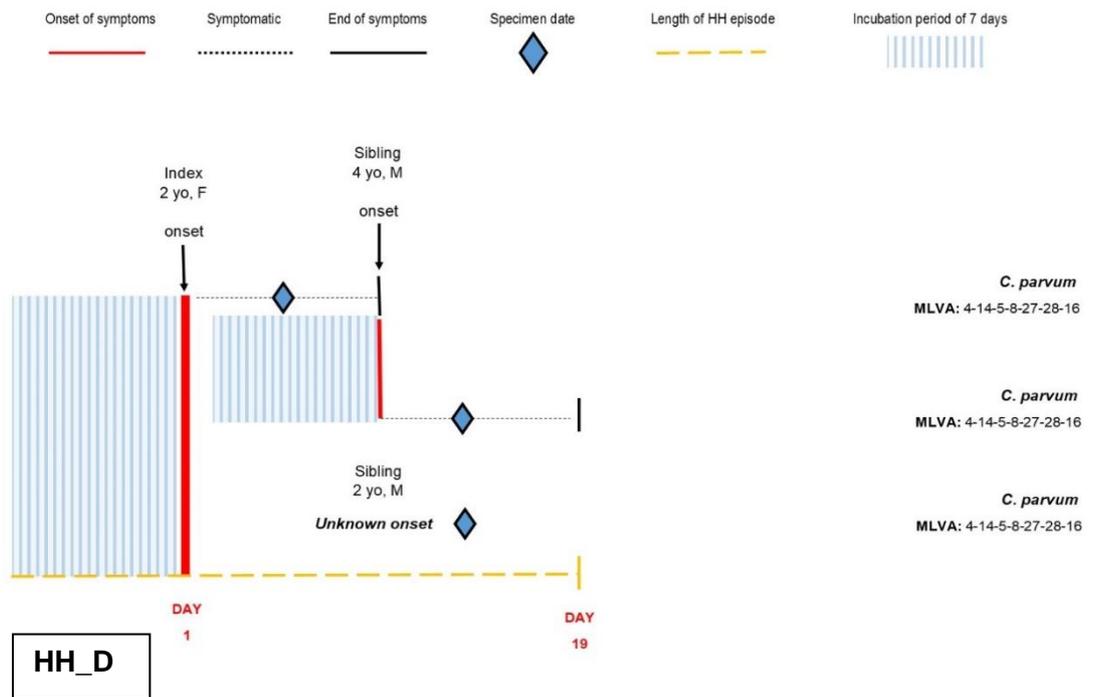


Figure 43: Depiction of course of illness in the home, with laboratory results_Household D

2980 **Household E**

Index: <i>C. hominis</i>	gp60 IbA10G2
HH sample 1: <i>C. hominis</i>	No typing

2981 The household E index case was five-year-old male with confirmed *C. hominis*
 2982 IbA10G2. One household sample was returned to the laboratory and confirmed as *C.*
 2983 *hominis*. The positive household contact was the child’s mum, a 34-year-old female.
 2984 The questionnaire revealed four in the household altogether: another sibling with no
 2985 illness, and dad, who did report symptoms. No samples were received for those two
 2986 participants.

2987 The index case reported a long illness (35 days) and mum was symptomatic for a
 2988 week. The time from index onset to the next possible infection (dad) was 24 days,
 2989 and it was 28 days until mum’s symptoms began. This could represent spread to one
 2990 or both parents directly from the index, or tertiary levels of spread from the index case
 2991 to dad, and then from dad to mum. The family did report recent travel.

2992 The time between onset and specimen collection for the index and positive household
 2993 contact was 9 and 14 days, respectively. Mum’s sample was taken seven days post-
 2994 symptoms, with a BSS of two (formed stool), and *Cryptosporidium* was detectable.
 2995 The overall burden of illness on this home was considerable with 75% of occupants
 2996 ill spanning a period of 35 days. (Figure 44)

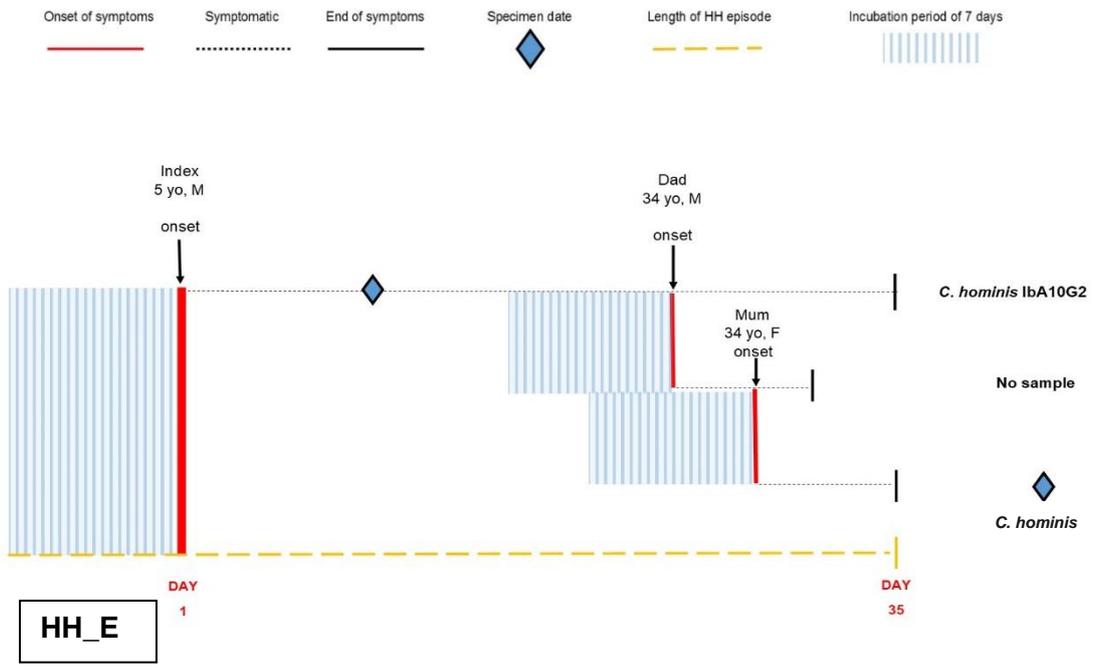


Figure 44: Depiction of course of illness in the home, with laboratory results_Household E

2997

2998 **Household F**

Index: <i>C. hominis</i>	gp60 lbA10G2
HH sample 1: <i>C. hominis</i>	No typing

2999 The household F index case was 42-year-old female with confirmed *C. hominis*
3000 lbA10G2. One household sample was returned to the laboratory, was speciated, and
3001 confirmed as *C. hominis* (without typing). The positive household contact was a 42-
3002 year-old male.

3003 There were 46 days between the two specimen dates, suggesting that this was
3004 transmission. The household contact's BSS was five, so he may have had some
3005 symptomatic illness. The household did not return a questionnaire and no further
3006 examination of this home was possible.

3007

3008 **Household G**

Index: <i>C. hominis</i>	No typing
HH sample 1: <i>C. hominis</i>	gp60 lbA10G2

3009 The household G index case was one-year-old male with confirmed *C. hominis*.
3010 Subtyping was not available from the original sample. One household sample was
3011 returned to the laboratory, was speciated, and confirmed as *C. hominis*, with gp60
3012 lbA10G2. The positive household contact was the child's mum, a 31-year-old female.
3013 The questionnaire revealed three people in the household altogether: no sample or
3014 information was received for the additional participant.

3015 The index case was reported as having a long illness (21 days) and mum was
3016 symptomatic for two weeks. The time from index onset to mum's infection was 14
3017 days. This could represent spread from the index case to parent. However, the family
3018 did report other exposures such as travel, swimming, and farm visits.

3019 The time between onset and specimen collection for the index and positive household
3020 contact was 18 and 10 days, respectively. Both samples were taken during a
3021 symptomatic period. No BSS was recorded as both samples were retrieved from
3022 original specimens.

3023 The overall burden of illness on this home was considerable with two thirds of
3024 occupants reporting illness over a period of 28 days. (Figure 45)

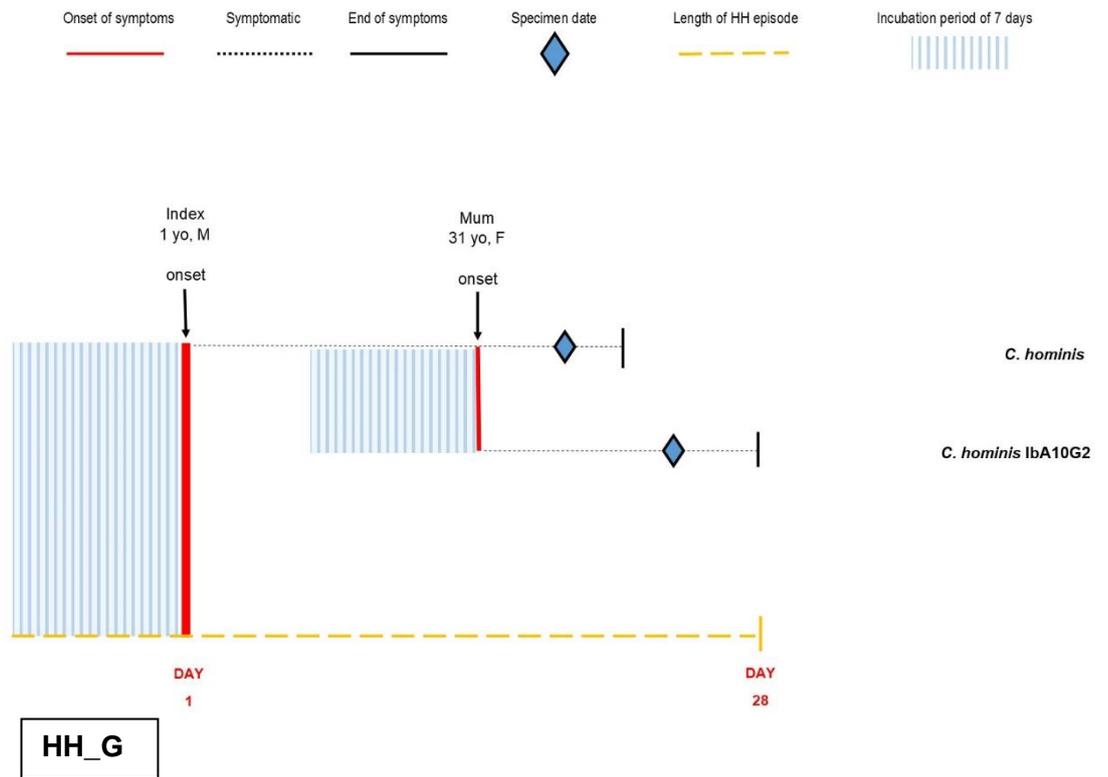


Figure 45: Depiction of course of illness in the home, with laboratory results_Household G

3025

3026 **Household H**

Index: <i>C. hominis</i>	gp60 lbA10G2
HH sample 1: <i>C. hominis</i>	gp60 lbA10G2

3027 The household H index case was five-year-old female with confirmed *C. hominis*
 3028 lbA10G2. One household sample of a sibling, an eight-year-old female, was returned
 3029 to the laboratory and confirmed as *C. hominis* lbA10G2. This household contact
 3030 specimen had previously been submitted as part of routine surveillance, so it is likely
 3031 that this child also reported symptoms.

3032 There were 12 days between the two specimen dates: this could represent
 3033 transmission or two co-primary cases with a common exposure. The household did
 3034 not return a questionnaire and no further examination of this home was obtainable.

3035

3036 **Household J**

Index: <i>C. hominis</i>	gp60 lbA10G2
HH sample 1: <i>C. hominis</i>	gp60 lbA10G2
HH sample 2: <i>C. hominis</i>	gp60 lbA10G2

3037 The household H index case was *C. hominis* gp60 lbA10G2. Two additional
 3038 household contact samples were linked to this household and were indistinguishable
 3039 by gp60The index case was a seven-year-old boy, and the two household contact
 3040 samples were taken from dad and a two-year-old sibling. The sibling reported illness
 3041 (length of time unknown) but dad did not report any compatible symptoms. The index
 3042 was ill for 90 days, and additionally reported poor appetite: with two young children
 3043 ill, this is likely to have been a serious burden on this home. Common exposures were
 3044 reported: swimming, farm animal contact, and exposure to pets. The time between
 3045 onset and specimen collection for the index was 34 days, and for the sibling, 16 days.
 3046 There were 26 days between index onset and sibling’s illness. No BSS data were
 3047 available, but the index case’s sample would have been taken during a symptomatic
 3048 period. Anecdotally, for this household, mum reported that the case often stays at
 3049 another parent’s home, and there had also been additional illness there. (Figure 46)

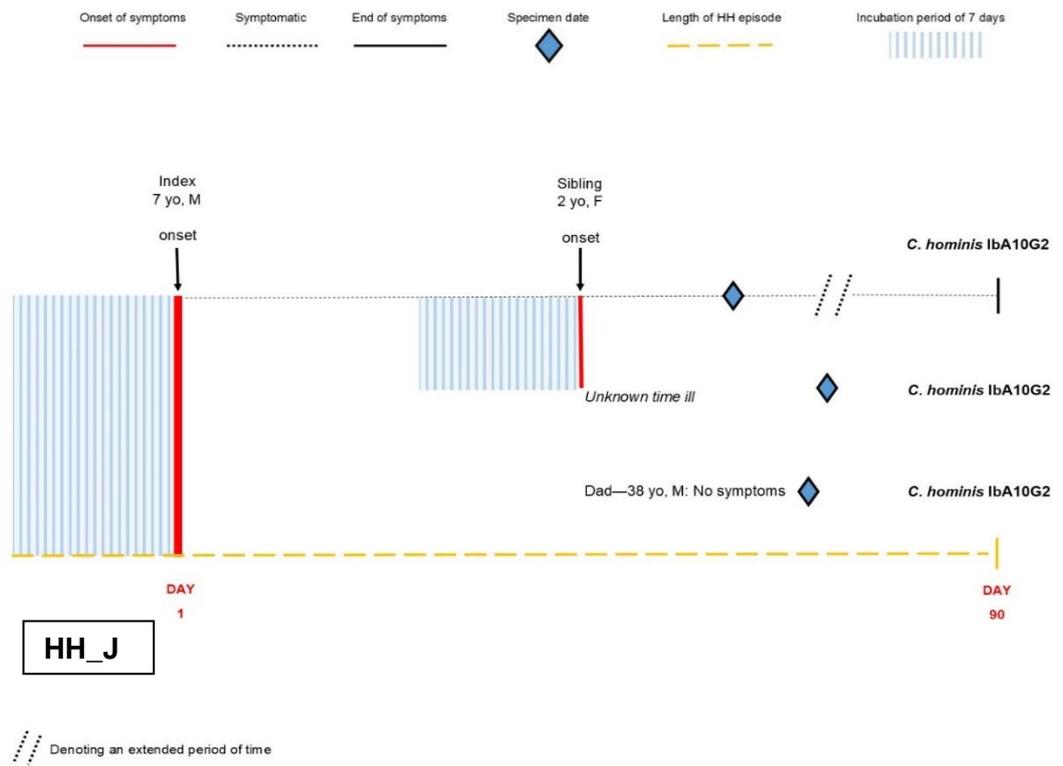


Figure 46: Depiction of course of illness in the home, with laboratory results_Household J

3050

3051 **Household K**

Index: <i>C. hominis</i>	gp60 lbA10G2
HH sample 1: <i>C. hominis</i>	No typing

3052 The household K index case was a four-year-old female with confirmed *C. hominis*
3053 lbA10G2. One household sample was returned to the laboratory, was speciated, and
3054 confirmed as *C. hominis* (no typing). The positive household contact was a 38-year-
3055 old male, possibly dad. The questionnaire was returned for this household but poorly
3056 filled in and most of the data were missing. The index was reportedly ill for one day
3057 and the household did not report any other illness in the home within two weeks of
3058 the index. The index specimen date was the same as the onset, and there were 39
3059 days between the two specimen dates.

3061 Discussion

3062 I undertook this study in order to begin to describe factors within the home
3063 environment that might be associated with spread of *Cryptosporidium*. My primary
3064 research objectives were to estimate the amount of secondary spread of infection that
3065 occurs in homes with a case and examine any associated factors. This exploratory
3066 study has highlighted several characteristics of cases, and of the environment in
3067 which they live, that might be correlated with spread of infection. Significant
3068 independent factors in multivariable analysis included attending nurseries and being
3069 infected with *C. hominis*.

3070 Representativeness

3071 We enrolled 128 index cases into the study, most of which were *C. parvum* or *C.*
3072 *hominis*. This represents about 10% of the cases reported in those regions over the
3073 study year. Our recruitment uptake was good for a study of this type, with around 18%
3074 of those contacted taking part and returning one or more elements of the study packs
3075 (Tam *et al.*, 2012). I think that one of the reasons our participation was so good was
3076 due to the resource of the CRN nurses, who were able to directly contact participants:
3077 a somewhat similar study to the epiCrypt study, conducted by Waldram and
3078 colleagues reported a massive participation rate over 60%, using environmental
3079 health officers to present study information face-to-face with families and to retrieve
3080 samples at the point of contact (Waldram *et al.*, 2017). More generally, a decrease in
3081 'volunteerism' and perception of participant burden has been reported among
3082 observational studies over recent years (Morton *et al.*, 2012). Additionally, recent
3083 changes in privacy laws and rules on recruitment, often dictated by ethics committees,
3084 can lead to reduced participation rates and possible selection bias (Galea and Tracy,
3085 2007; Morton *et al.*, 2012). The complexity of the epiCrypt study most definitely lay in
3086 the design, with much navigation required to accommodate legal and ethical
3087 requirements for participant approach and data handling, and cross-organisational
3088 contract obligations. I expand on some of my personal thoughts on this later in the
3089 discussions.

3090 Whilst an understand of the participation is informative, I do not think in isolation it
3091 can proxy the study validity, and despite a definite lean towards recruitment of families
3092 rather than other household compositions, the characteristics of our included
3093 participants were fairly typical of both North West England and Wales. Participants

3094 were mostly comprised of 25 to 44-year olds but we also had a decent proportion of
3095 young children represented (21% under 5 years), with male cases tending to be
3096 younger. This fits with what we know about the descriptive epidemiology of
3097 *Cryptosporidium* in England and Wales (Chalmers *et al.*, 2009).

3098 The age of the index cases ranged from 9 months to 78 years old with a
3099 preponderance of cases among the younger age groups. The index case was a child
3100 under five years old in almost 30% of the recruited households and two-thirds of those
3101 were male. This is supported by other examinations of cases in the UK and beyond
3102 that demonstrate an increased prevalence in infants and young children (Abubakar
3103 *et al.*, 2004; Hunter, Hughes, Woodhouse, Syed, *et al.*, 2004; Chalmers *et al.*, 2009;
3104 Cacciò and Chalmers, 2016).

3105 There were more *C. parvum* (n=69) cases than *C. hominis* (n=43) in the study. This
3106 is quite likely representative of the circulating species over the year, although there
3107 were two periods of reduced recruitment in January and June. This is unlikely to have
3108 affected the species results too much as both months are still in the *C. parvum* period,
3109 with the increase in *C. hominis* happening in late summer, from August (Nichols *et al.*,
3110 2006; Chalmers *et al.*, 2019; Douglas *et al.*, 2019). In line with what is expected,
3111 in the rural areas, most cases were *C. parvum*, while most *C. hominis* cases were
3112 reported from urban areas (McLauchlin *et al.*, 1999; Deshpande *et al.*, 2015).

3113 The households that took part originated from all socioeconomic areas, but there were
3114 slightly more households from the less deprived geographies. This is consistent with
3115 the profile of *Cryptosporidium* infection across England and Wales where the most
3116 deprived areas appear slightly underrepresented (I. I. R. Lake *et al.*, 2007). This might
3117 reflect difference in access to, or use of, services, or may be a reflection of differences
3118 in recruitment and participation (Snel, Baker and Venugopal, 2009; Ellis *et al.*, 2017).

3119 *Profile of illness*

3120 More than a quarter of index cases reported symptoms other than diarrhoea and
3121 vomiting, including nausea, abdominal pain, and headaches. Moreover, vomiting was
3122 not frequently reported at all, occurring in less than half of the index cases, which has
3123 been noted previously (Hunter, Hughes, Woodhouse, Raj, *et al.*, 2004; Johansen *et al.*,
3124 2014; Adler *et al.*, 2017) Symptoms differed somewhat by age with nausea,
3125 headache, and stomach pain occurring more among older cases and vomiting in the
3126 younger cases, particularly males. This could be due to differences in the symptom
3127 profile of species. Differences in symptom presentation have been identified before,

3128 in an outbreak of *C. hominis*, where headache and abdominal pain were more
3129 common in female cases (Adler *et al.*, 2017). However, as a high proportion of the
3130 cases were children, it is important to remember that we are relying on secondary
3131 reports of illness, usually via parents. Self-reported illness can be fraught with
3132 reliability issues, especially with non-clinically obvious symptoms like pain or nausea,
3133 which may be difficult for a young child to describe or articulate.

3134 Of additional interest, this study revealed there were some differences in length of
3135 illness by sex and infecting species: males were more likely to report a longer illness
3136 ($p=0.003$) as were cases of *C. hominis* ($p=0.004$). As discussed in Chapter 2 Part A
3137 (Clinical manifestation), a study of sporadic disease in the UK reported a mean
3138 duration of symptoms for patients with *C. hominis* that was two days longer than *C.*
3139 *parvum* cases (Hunter, Hughes, Woodhouse, Syed, *et al.*, 2004). One curious finding
3140 in this study was that additional, and possibly secondary, cases in the home were not
3141 as long-lived as those in the index cases and might point to a decreased virulence in
3142 person-to-person spread (R. Chalmers *et al.*, 2016). This has been evidenced before
3143 in homes with transmission of gastrointestinal pathogens, where secondary cases'
3144 average duration of illness was more than half that of primary cases (Perry *et al.*,
3145 2005). However, this study did not confirm aetiology of cases, and additionally
3146 excluded secondary cases with onsets >5 days after cessation of index symptoms,
3147 which might exclude a reasonable amount of *Cryptosporidium* infections, given its
3148 longer than average incubation time.

3149 A browse of the free text in the questionnaires revealed that case symptoms were
3150 extensive: those most frequently reported included foul-smelling stool, sleep
3151 disturbances, lethargy and exhaustion, loss of appetite, and joint pain. In addition,
3152 several cases reported persistent and recurring illness and two people reported a
3153 hospital admission. This demonstrates a substantial burden of illness on the individual
3154 as well as on the home overall and is well corroborated in other literature which has
3155 revealed duration of symptoms far beyond IID of other aetiologies (Robertson *et al.*,
3156 2002a; Kortbeek, 2009; Shirley, Moonah and Kotloff, 2012; Carter *et al.*, 2019).
3157 Crucially the longevity of illness might also amplify spread, by potentially increasing
3158 the length of time the oocysts are shed (Jokipii and Jokipii, 1986; Chappell *et al.*,
3159 1996; Chalmers; *et al.*, 2016), although here there was no association between length
3160 of illness and burden of additional cases in the home. Nonetheless, complications this
3161 long lasting and potentially burdensome warrant further examination.

3162 There is certainly a public health, and economic, argument for interventions to reduce
3163 not only primary infections with *Cryptosporidium*, but also subsequent spread. This
3164 might include work to provide more targeted advice for individual *Cryptosporidium*
3165 patients or during outbreaks, and these strategic and population-level approaches are
3166 critical given the lack of licensed treatment for this infection in the UK. This evidence
3167 does reinforce the importance of speciation and subtyping of isolates where at all
3168 possible, in order to better understand the clinical course of disease for the patient or
3169 population and administer appropriate interventions and advice.

3170 *Asymptomatic infections*

3171 This work did not reveal any considerable proportion of asymptomatic infection.
3172 *Cryptosporidium* was detected in 12/259 (4%) of household members' samples of
3173 whom two were asymptomatic, giving a prevalence of asymptomatic infection of 2%.
3174 Both households were diagnosed with cases of *C. hominis* IbA10G2. From the small
3175 number of relevant studies, carriage of *Cryptosporidium* appears to be low at between
3176 0.1-1.3% (Tompkins *et al.*, 1999; Davies *et al.*, 2009) although this has once been
3177 demonstrated as high as 9% following an outbreak of *C. parvum* in Norway (Johansen
3178 *et al.*, 2014). Identification of true carriage is difficult as we tend to capture diarrhoeal
3179 cases and it is likely that all of the index cases here will have sought clinical
3180 assessment following symptoms. In addition, recrudescence of symptoms
3181 complicates the identification of differences between true asymptomatic infection and
3182 shedding of oocysts in an asymptomatic period. In this study, because I used a
3183 question covering compatible illness at any time, it is plausible that those who do not
3184 report symptoms truly did not ever have symptoms. An asymptomatic prevalence of
3185 2% would be in line with carriage expected for the UK (Davies *et al.*, 2009) but the
3186 design of this work did not allow any examination of this contribution to spread. We
3187 do not know the immune status of household members, but none of the index cases
3188 reported any immune compromise or deficiency.

3189 The time between initial onset of illness in the home and sample retrieval from others
3190 was variable, and often long. This does raise some uncertainty about the capability
3191 of the tests used to confirm infection. For this reason, we included both IFM and PCR.
3192 Given that we have already demonstrated differences in length of illness by species,
3193 it would not be implausible that asymptomatic, or indeed less protracted secondary
3194 infections, might lead to shorter shedding times (Perry *et al.*, 2005). If this were the
3195 case, perhaps by the time that the laboratory had received samples the detection
3196 power of the tests, and especially IFM was reduced. Also, with such a small sample

3197 size the power to truly detect asymptomatic infections would be fairly weak. In this
3198 sense, the true asymptomatic infection burden may well be under ascertained here
3199 and we know from previous work that lack of detection by routine diagnostic methods
3200 does not necessarily equate to lack of infection (Chalmers *et al.*, 2011; Chalmers *et*
3201 *al.*, 2016). Due to the study design, I was not able to determine if being asymptomatic
3202 meant a reduced compliance with submitting stool samples, as I only had the
3203 information that the household filled in on the questionnaire: if this excluded members
3204 of the home I was unaware.

3205 Whilst fairly confident about this result representing at least the minimum
3206 asymptomatic carriage, it is important nonetheless to recognise issues that arise from
3207 using self-reported data, and the differences in possible perception and experiences
3208 of illness by individuals, affecting validity and possibly over estimating this effect
3209 measure. Whilst we are selecting our at-risk individuals from the same homes as
3210 cases, and thus will have certain similarities to cases, the generalisability of this is
3211 questionable. There are complex biological and social factors that affect surveillance
3212 data capture, of which illness severity has been shown to be important (Tam,
3213 Rodriguez and O'Brien, 2003) and one person's idea of 'being ill' might differ from
3214 another's. Nonetheless, this result is not insignificant. If asymptomatic infections are
3215 indeed few, rather than this being something to be dismissed, actually this indicates
3216 that if infected you are more than likely to be ill, and we know that with this illness
3217 comes considerable symptomatic burden, and the risk of longer term sequelae
3218 (Hunter, Hughes, Woodhouse, Raj, *et al.*, 2004; Stiff *et al.*, 2017). As such, this makes
3219 tackling preventable secondary transmission of infection a crucial issue of public
3220 health importance.

3221 *Additional illness in the home*

3222 We were able to confirm infection microbiologically in a small proportion of household
3223 contact samples (4%; n=12). However, at least one additional case of self-reported
3224 compatible illness within two weeks of the index case was identified in 27 households
3225 (27%). Almost half of the homes for which data were available (39/82; 47.6%)
3226 reported some additional illness at any other time in the home (not necessarily within
3227 two weeks). Given the lengthy and often variable incubation period for
3228 *Cryptosporidium* (Bouzid *et al.*, 2013; Chalmers and Caccio, 2016) these cases
3229 outside the two week window could still be due to onward spread from an index case.

3230 An analysis by species revealed that less than 20% of the *C. parvum* index cases
3231 reported additional illness in their household (19.6%), compared to 48% of the *C.*

3232 *hominis* indexes ($p=0.01$). This result is in line with similar studies evidencing *C.*
3233 *hominis* as a species particularly associated with people, and probably the person-to-
3234 person transmission pathway. A case-control study in the Netherlands (Nic Lochlainn
3235 *et al.*, 2019) found that *C. hominis* cases in particular were three times more likely
3236 than controls to have been exposed to a case in the home and were less likely to live
3237 in homes with lots of adults. Also, in those years where *C. hominis* was the
3238 predominant circulating species, other risk factors such as food items were reported
3239 as associated with decreased odds of illness. This work adds to the body of evidence
3240 that sources for *C. hominis* may be exclusively human and that person-person
3241 transmission is the most likely pathway (Hunter, Hughes, Woodhouse, Syed, *et al.*,
3242 2004; Hunter and Thompson, 2005).

3243 *Burden on the home*

3244 Almost two additional cases occurred, on average, in households with an index case.
3245 Additionally, the analyses suggested that almost a third (31%) of people in the home
3246 could be expected to get ill from transmission of infection. This burden was even
3247 greater in households where the index case was infected with *C. hominis* or the index
3248 case was under five years old. Risk of infection in settings with young children has
3249 previously been demonstrated and is known to facilitate spread (Hannah and Riordan,
3250 1988; Hunter, Hughes, Woodhouse, Syed, *et al.*, 2004; Johansen *et al.*, 2014). This
3251 work further highlights that person-to-person is a specific transmission pathway but
3252 is first study to quantify the burden that this exacts on the home.

3253 I examined case contact as a risk factor for illness in the systematic review chapter
3254 revealing that this pathway does seem to contribute quite heavily to cases, albeit not
3255 well understood. If we consider this in light of the results here, it makes sense that
3256 underlying case contact is direct person-to-person transmission. If most index cases
3257 are young children, and mums make up the burden of secondary cases, then it is
3258 plausible that the driver here is direct contact, in a caring capacity; undertaking
3259 activities which likely put the main carers in the home at high risk. Additionally, siblings
3260 were also affected considerably by secondary infection, but where the adult was an
3261 index case, their children were less frequently the secondary case. It has been
3262 documented before that mums and siblings are most at risk of *Cryptosporidium*: in a
3263 follow-on study in Norway, with 12 and 13-year-old index cases, a 17% secondary
3264 transmission rate was mainly comprised of female caregivers and siblings (Johansen
3265 *et al.*, 2014). This is mirrored among other gastrointestinal aetiologies (Perry *et al.*,

3266 2005), and indeed for *E. coli* O157 it has been suggested that separation of siblings
3267 might be a key intervention in reducing secondary cases (Werber *et al.*, 2008).

3268 Gender roles influence both patterns of exposure to infectious agents and the
3269 treatment of infectious disease (World Health Organization, 2007). Caring for the sick
3270 carries an increased risk of exposure, especially for diseases that are spread directly
3271 from person-to-person and in most societies females are more likely to care for the
3272 sick than males (Anker, 1998). The heterogeneity of contact within the home has been
3273 examined in respiratory diseases such as Influenza and Pertussis, and studies found
3274 that contacts between mother and children and between siblings are most prevalent
3275 (Goeyvaerts *et al.*, 2018).

3276 Some of the individual home analyses among the laboratory confirmed contact
3277 isolates exposed a considerable burden on the home, with one home reporting illness
3278 for 90 days. As these illnesses were often in young children, and mums represented
3279 about 30% of additional illness (followed by siblings), this could signify a real societal
3280 burden. Several cost and burden of illness studies have been undertaken in the
3281 Netherlands, which have considered the economic and societal impact of
3282 gastrointestinal infections. Overall, there is a considerable burden on productivity due
3283 to absence from work for the ill or the caregiver(s) (Pijnacker *et al.*, 2019), and one
3284 study estimated that in 15% of cases where a child was ill, a parent had to remain off
3285 work (Mughini-Gras *et al.*, 2016). An additional analysis considering the longer-term
3286 manifestations of *Cryptosporidium* in particular reported similar burdens on
3287 productivity, with additional impact on disability adjusted life years (DALYs) due to
3288 recurring diarrhoea and long-term joint pain (Monge *et al.*, 2019). Further work
3289 confirming and examining this disparity for *Cryptosporidium* would be a welcome
3290 addition to work to describe the economic and societal burden of this disease.

3291 The demonstration of a *C. hominis*-specific burden provides another argument for
3292 swift and complete characterisation of isolates, with results fed into local and national
3293 surveillance data. If we are able to identify a high-risk individual or population this
3294 might help highlight those at specific risk of secondary infections and allow particular
3295 clinical and public health advice to be followed.

3296 *Clinical assessment/Health-seeking*

3297 Fewer than 20% of secondary cases saw a clinician for their illness (17.5%). In
3298 addition, secondary cases tended to report shorter symptom times than index cases,
3299 and there were ten incidences where the index was not the first in the home. If we

3300 consider that almost a third of people in a home could get ill, but only a fifth of these
3301 seek medical assessment this definitely represents an under-ascertainment of true
3302 cases. However, these data are based on self-reported illness which can be
3303 unreliable (Hunter and Syed, 2002; Hunter *et al.*, 2005), and we cannot confirm that
3304 similar symptoms were indeed *Cryptosporidium*.

3305 *Detecting Cryptosporidium in household contact stool samples*

3306 *Cryptosporidium* was detected in 12/259 (4%) of household members' samples of
3307 whom two were asymptomatic, giving a prevalence of asymptomatic infection of 2%.
3308 This is much smaller than expected if the self-reported clinical illness does truly
3309 represent secondary infections. An explanation for this may be the lag time from
3310 illness to receiving household samples and the likelihood of detecting
3311 *Cryptosporidium*. Despite a range of laboratory testing methods, including PCR, the
3312 results demonstrated that confirmation was more likely in specimens taken during, or
3313 soon after, a case's symptomatic period. The average time between the index cases'
3314 specimen date and the first household member specimen was 43 days. Oocysts
3315 might also be shed intermittently but the study design did not allow for repeat
3316 sampling.

3317 Additionally, using a clinical indicator of BSS made no difference to microbiological
3318 confirmation: all of the diarrhoeic specimens were subsequently unable to be
3319 confirmed as infected with *Cryptosporidium*. Conversely, all those household member
3320 samples that were confirmed had formed stools. This supports our prior
3321 recommendation (Chapter 2: Part B) to eliminate stool consistency as a testing
3322 inclusion criterion in local laboratories.

3323 The laboratory was able to further characterise a large proportion of the
3324 *Cryptosporidium* gp60 or MLVA profiles, which were representative of those
3325 circulating in the study areas (Hunter *et al.*, 2007; Chalmers *et al.*, 2008, 2019; R M
3326 Chalmers, RP Smith, *et al.*, 2011). The *C. parvum* subtypes of IIaA15G2R1 and
3327 IIaA17G1R1 are reported as most prevalent in England and Wales (using outbreak
3328 data) and are mostly associated with animal contact. The *C. hominis* most prevalent
3329 subtypes are IbA12G3 and IbA10G2, mostly associated with water. These profiles
3330 were indistinguishable from the index case in all homes with confirmation, suggesting
3331 direct transmission or at least common exposures.

3332 This study detected possible household transmission of *C. ubiquitum*, with an index
3333 case and a confirmed household infection. Unfortunately, the questionnaire element

3334 of the study was not returned for this home and so further examination of exposures
3335 was not possible. But this was an interesting find: it is an unusual subtype, sources
3336 of infections in humans are not entirely clear and transmission between people has
3337 never been demonstrated (Li *et al.*, 2014).

3338 *Factors associated with transmission in households*

3339 The variable most strongly associated with additional cases in the home was the
3340 infecting species of the index case. Among homes that reported transmission, there
3341 was a significant preponderance of *C. hominis* cases versus *C. parvum* cases and
3342 this was four times more likely to be reported in homes with additional cases (OR =
3343 3.78). The Netherlands recently reported similar results (Nic Lochlainn *et al.*, 2019),
3344 specifying that *C. hominis* cases were more likely than controls to have been exposed
3345 to a case in the home. Additionally, the authors reported corroborating indicators
3346 supportive of a person-to-person pathway, including living in smaller homes, and
3347 living with children. Although not independently associated with transmission in the
3348 logistic regression model, this study did highlight similar associated exposures, with
3349 homes with additional cases twice as likely to report the index case being a child less
3350 than five years old or attending a nursery (OR = 2.5). Although not statistically
3351 significant, being in a home with three or fewer people was reported in a greater
3352 proportion of those homes without additional cases and this has been demonstrated
3353 previously for gastrointestinal infection transmission in the home (Perry *et al.*, 2005).
3354 This is additionally supported by the other crowding indicators; fewer than one toilet
3355 per person (OR = 3.29) and fewer than one bedroom per person (OR = 1.96) were
3356 both more prevalent in homes where there was transmission, although they did not
3357 remain in the final model.

3358 This work continues to buttress the existing literature but highlights quite clearly that
3359 differences in species and transmission are quite likely. At risk homes can be
3360 identified as those where the index is less than five years old and/or is infected with
3361 *C. hominis*. Of particular risk are mums and caregivers, and siblings, and targeted
3362 hygiene advice should be specifically directed here.

3363 **Limitations**

3364 *Accurately identifying transmission*

3365 A study of this kind is not without its limitations. The main objective of this work was
3366 to identify transmission in the home environment, and this is arguably the most

3367 evasive and difficult part of the project overall. Generally, contact with other cases is
3368 used as a proxy measure for spread or transmission of infectious diseases between
3369 people, but this does not always happen, or cannot be proven to happen, in one
3370 specific environment (Bloomfield *et al.*, 2012; Iyengar *et al.*, 2015; Nic Lochlainn *et*
3371 *al.*, 2019). Studies oftentimes are unable to examine further underneath this to
3372 ascertain specific differences in cases or identify modifiable risk factors, and very
3373 specific study designs would be needed to examine this. Proving transmission is a
3374 difficult task, and the ubiquitous nature of *Cryptosporidium* and of its exposures make
3375 untangling these exposures and demonstrating causality difficult in a study of this set-
3376 up. Nevertheless, this study was intended as an exploratory piece, and the evidence
3377 presented suggests that *Cryptosporidium* does transmit readily in the home
3378 environment, and that person-to-person is the transmission pathway. This further
3379 supports my assertion in Chapter 3 (Conclusions) that this pathway needs to be
3380 considered for sporadic cases and warrants further investigation and work.
3381 Demonstrating causation is fraught with problems, and more in-depth research
3382 should take place prompted by this low-resolution piece of work.

3383 Additionally, our limited understanding of the background prevalence of
3384 asymptomatic infection of *Cryptosporidium*, and its effectors, make it difficult to
3385 identify its importance in spread of disease in contained settings, and this study did
3386 not reveal a large amount of asymptomatic infection. In addition, the study design was
3387 not appropriate to demonstrate if an asymptomatic carrier was shedding oocysts or
3388 was infectious to others in the home. However, previous work on secondary
3389 transmission data has mainly stemmed initially from outbreaks, and data rarely
3390 include laboratory confirmation of secondary cases (Johansen *et al.*, 2014). The
3391 epiCrypt study is unique in that it has allowed for an examination of secondary cases
3392 at both species level and with further typing. A larger scale study of sporadic infections
3393 would continue to build on our understanding of species-specific risks of spread and
3394 also could examine heterogeneity in subtype populations (Morris *et al.*, 2019).

3395 *Truly capturing secondary infections*

3396 Additionally, difficulties arise distinguishing between primary and secondary
3397 infections as close contacts often have similar exposures (Johansen *et al.*, 2014) and
3398 the clinical course of *Cryptosporidium* infection can result in variable incubation,
3399 symptoms, and onset between individuals making verifying person-to-person
3400 transmission challenging. I had initially intended to use the clinical and exposure data
3401 from the questionnaire to examine likely co-primary cases, but on reflection these

3402 data were just not adequate for this purpose. The design of the questions meant that
3403 where participants left these blank, I was unable to assume this meant that they were
3404 not reporting that exposure. It was curious that stool samples were returned more
3405 readily than questionnaires, and in hindsight I think I would have perhaps had a better
3406 return if the questionnaires had been shorter and more focused. By not being able to
3407 accurately combine exposures and onsets to define possible co-primary cases, we
3408 may be overestimating the amount of transmission, and actually, these additional
3409 cases might be due to variable incubation periods from common exposures.

3410 The considerable lag time from index symptom onset to household contact sample
3411 collection meant that some infections may have been tertiary or beyond, as well as
3412 secondary infections. We did initially consider a way to identify this in the first round
3413 of study design. However, with limitations on the data access, time, and budget, and
3414 the anonymity and self-reporting nature of the design (i.e. not having, for example,
3415 Environmental Health Officers or research nurses able to go into the home and ask
3416 questions) I felt that large matrix data collection like this would be tough to gather,
3417 and may actually decrease participation. However, the primary research objective of
3418 this study was simply to identify other infections in the home. Following this study, the
3419 proposal will be for a more compact, but more in-depth, study, but initially this high-
3420 level, large-footprint study was undertaken as the first step to contribute to the
3421 evidence base. Additionally, we had secured external funding for further genotyping
3422 of some samples, which may support household level investigations of directionality
3423 and population mixing, but due to the impact of COVID-19 this work has been delayed
3424 indefinitely.

3425 *Study design biases*

3426 Some elements of the study design were retrospective in nature, as the index case
3427 must have already been ill and been tested in order to be selected. As a result, some
3428 ascertainment bias may lead to a skewed sample from which to choose the index
3429 cases (The burden of illness pyramid). We may have captured more severe disease
3430 as these cases are more likely to seek health care and be tested, and perhaps more
3431 likely to test positive, given the variability of local testing methods (**Error! Reference**
3432 **source not found.**) (Adams *et al.*, 2018).

3433 The study was designed using a model specifically for household transmission
3434 studies, but these are most often applied in practice to respiratory disease. Of course,
3435 respiratory illnesses have the advantage that you know the source of the infection in
3436 a closed setting, but in this that is less clear. The sources of infection in this study, as

3437 well as being the index case, could additionally be ubiquitous or common sources. Of
3438 course, other methodological biases do exist in this approach, mainly the inability to
3439 estimate population-level rates or extrapolate results to a wider community, but in the
3440 main this design suited the objectives of this work. I was interested in exploring
3441 household transmission and examining if it was indeed likely to be driven on the
3442 person-to-person transmission pathway, using associations in context to assess this.
3443 It was not an objective of this study to present causation and thus, although not
3444 perfect, this study design presented a feasible option in terms of resource, while
3445 allowing me to meet the objectives of the study. The epiCrypt study has presented an
3446 investigation of possible secondary infections in the home with some confidence and
3447 with this I hope to be able to profile homes where this occurs, in order to springboard
3448 further work.

3449 A large proportion of our participants represented families with young children which
3450 may have led to over-representation of these households. In addition, we might
3451 expect that having young children who were ill, or being severely ill themselves, may
3452 incentivise cases to participate in the study, more than adult, less severe cases.
3453 Without the baseline data on all cases contacted and opting out or not returning study
3454 materials there is no way to accurately measure this.

3455 *Case definitions*

3456 For ease the case definitions used were quite broad – anyone with compatible
3457 symptoms in the prior two weeks to the index case, or two weeks after, was classed
3458 as symptomatic and probably a case. In the restraints of this design it would be
3459 impossible to know for sure (they would have to attend a healthcare facility AND get
3460 tested AND have a positive sample) whether this additional case was truly secondary,
3461 co-primary, recrudescence of a previous infection not picked up, or a previous
3462 symptomatic case, now in a asymptomatic period of the same infection. These are all
3463 limitations, but mostly logistical due to the size and scope of this study. For the
3464 purposes of this work, identifying any additional reported or confirmed illness in the
3465 home was enough, but these considerations would certainly be applied in a more
3466 focused piece of work.

3467 This study design did not allow us to look for other enteric pathogens that cause
3468 diarrhoea, which could lead to both misattribution of index case illness to
3469 *Cryptosporidium*, and to overestimation of *Cryptosporidium* household transmission
3470 rates. We are not excluding from the study cases of *Cryptosporidium* where another

3471 organism has also been identified, and we would not be able to access that data. The
3472 ethical approval only allowed testing of household samples for *Cryptosporidium*.

3473 *Estimating the prevalence of asymptomatic infection in households*

3474 As secondary transmission was the primary objective, the sample size calculation
3475 was based on this. It is unlikely that this study was sufficiently statistically powered to
3476 uncover the prevalence of asymptomatic infection, but there is little previous work in
3477 the UK to estimate what the likely prevalence of this might be.

3478 *Time from onset of illness to identifying and recruiting the participants*

3479 One of the most noticeable issues I came across in this work was the time that
3480 elapsed from identifying participants until their recruitment, which could create recall
3481 bias, or affect the likelihood that people will take part. The most impactful of these
3482 issues is arguably the time from illness to specimen collection, which might have
3483 affected the detection of *Cryptosporidium* in the stool samples.

3484 The study design only allowed for one sample from each household member, and not
3485 re-sampling the index case, for time and resource reasons. This could lead to missing
3486 intermittent shedding of oocysts and/or misclassifying recurring illness. The lab
3487 confirmation rate was low, and perhaps with more samples detection would have
3488 been better.

3489 In this design, index cases were download from the system weekly, so that might be
3490 10 days from specimen to our capture of the case (I know from a preliminary analysis
3491 of the Public Health England surveillance data that the mean time from specimen date
3492 (not onset) to showing on the surveillance system is six days). Following the invite
3493 letter, it was a further 14 days at least before cases were contacted by the research
3494 nurses. Participants were given two weeks to return their materials (questionnaire,
3495 consent, stools), before a reminder was posted. The average time from index case
3496 specimen date to retrieval of household samples might be six weeks or more. This
3497 might have led to oocyst levels below a detectable level (Jokipii and Jokipii, 1986;
3498 Chalmers *et al.*, 2016). We tried to supplement this as far as possible by asking about
3499 clinical symptoms of household members in the questionnaire and recording stool
3500 consistency so some descriptive analyses could be carried out, even if
3501 *Cryptosporidium* was not detected in stools. This consideration also led to the
3502 implementation of the opt-out strategy for consent, which was a good and acceptable
3503 way of reducing burden on both participants and study staff, perhaps increasing
3504 uptake, and cutting out some time delay. Our recruitment uptake was good for a study

3505 of this nature, with around 18% of those contacted taking part and returning one or
3506 more elements of the study packs. Interestingly, return of stool samples was better
3507 than return of questionnaires. I think that some of the factors leading to this might
3508 include the opt-out approach, which allowed participants to be contacted directly and
3509 have the study explained to them personally.

3510 We know that time constraints are a major contributor to issues in epidemiological
3511 observational studies, and in research are due in some part to ethical considerations
3512 (Wilson, Draper and Ives, 2008; Bennett, Dolin and Blaser, 2015). Although it is
3513 imperative that as researchers, we retain the integrity and responsibility of our
3514 research, oftentimes undertaking observation epidemiological studies is burdensome,
3515 and the blanket application of rules can adversely affect the outcome of the work. A
3516 paper discussing a way forward to improve collaborations is being prepared
3517 separately.

3518 **Strengths of the work**

3519 A major strength of this study is that it identifies possible secondary cases of
3520 *Cryptosporidium* to a species or subspecies level, informing differences in
3521 transmission pathways and risk factors between genotypes. The results generated
3522 here have demonstrated that *Cryptosporidium* is a considerable burden on the home,
3523 identifying particular risk characteristics for targeted public health recommendations.

3524 The complexity of the design of this study illustrates a novel approach to widespread
3525 cross-organisational research and recruitment. Many lessons learned from approvals
3526 processes and ethical delays are being digested and a paper is being prepared to
3527 help change some of this for future work. The protocol for this work has already been
3528 published (McKerr *et al.*, 2019) and might provide a boiler plate template for some of
3529 the further research.

3530 **Conclusions**

3531 In conclusion, despite limitations that restricted analyses, this work met the primary
3532 objectives and demonstrated that additional cases of *Cryptosporidium* occur in over
3533 a quarter of homes with a case. I was also able to quantify this. Spread is likely to
3534 affect up to a third of the home and cause considerable burden of illness. This is
3535 especially common where the index is a young child, with mums and other siblings
3536 are most at risk of secondary infection, and where homes have cases of *C. hominis*.
3537 Eighty percent of those additional cases do not seek any help for their illness, and for

3538 those that do, we may not even detect them as their illnesses are shorter, and testing
3539 varies locally. Thus, we are likely under ascertaining cases of sporadic illness, under
3540 examining person-to-person spread, and under-advising where we could be giving
3541 specific clinical advice to high-risk households. Systematic changes that would
3542 support better examination of this should include 100% speciation of *Cryptosporidium*
3543 samples, fed routinely back into surveillance systems and local health protection
3544 teams across England and Wales, and the consideration of specific clinical advice on
3545 prevention for high-risk homes. This might include managing the patient's
3546 expectations on the length of illness, and the possibility of relapse, and giving specific
3547 advice on preventing person-to-person spread (Chalmers and Davies, 2010).

3548 **An opinion.....**

3549 Anecdotally, the research nurses in this study consistently reported that patients they
3550 spoke to the telephone complained of terrible burden of illness on the home. They
3551 often reported that others had been ill, that they (parents in particular) were astounded
3552 and surprised at how ill their children had been and how much time and effort it took
3553 to look after them and taking time off work. The considerable burden and nastiness
3554 of this illness was often the impetus for these families to take part – in part evidenced
3555 by our decent uptake and good response to returning stool specimens. Often parents
3556 would claim that they “didn’t want this to happen to another child or family”. A review
3557 of *Cryptosporidium* infection by Chalmers & Davies in 2009 included some
3558 experiences from a patient and a parent of a case: the review reported long-term and
3559 debilitating illness of several weeks alongside a period of worry and anxiety, not being
3560 able to look after children, and recrudescence of symptoms two weeks after apparent
3561 resolution (Chalmers and Davies, 2010). Whilst often dismissed as a mild and self-
3562 limiting infection, the burden can be considerable. I think that there a couple of things
3563 that we, as public health professionals, could do here. Another piece of work, deeper
3564 than this one, and perhaps using mixed methods, exploring physical, mental, and
3565 economic burden of this disease would be a welcome examination. Also, short-term,
3566 this work could help support clinicians to prepare patients, and their families if
3567 appropriate, for the likelihood of a long and possibly unpleasant illness. This might
3568 help with the management of these infections, in the family environment in particular
3569 and give parents a chance to prepare. Longer-term, this would require close working
3570 with laboratories to ensure all submitted stools are tested for *Cryptosporidium* and
3571 any positive samples are expedited to the CRU for species identification at least.
3572 Speciation might now not just be a requirement for research, but actually for public

3573 health action, intervention, and advice. Recently, changes to surveillance systems
3574 means that results of speciation are fed back into surveillance data, but a reliable
3575 pathway to health protection teams for action is still unavailable.

3576 Any participants who did get in contact with me to ask questions or confirm their
3577 participation were interested, involved, and keen to take part. In the questionnaires,
3578 participants reported considerable burden of this illness on the home overall and were
3579 really eager to be active in research that may make a difference. This does somewhat
3580 jar with some of the detailed ethical considerations and access obstacles that arose
3581 during the design of the study which led to a very difficult navigation of systems,
3582 complex design pathway for getting data from patients and recruits, and excess time
3583 delays which certainly had an impact on the results. This was largely down to the time
3584 taken to navigate certain ethical requirements such as invitation letters directly from
3585 health organisations prior to study invites with a minimum two week opt out period.
3586 Whilst it is important as responsible researchers that we implement responsible
3587 research designs, I found some aspects of the process challenging due to an inability
3588 to appreciate the rationale for some of the requirements, which I felt, were barriers to
3589 progress. I had undertaken a small piece of work looking at public involvement with
3590 research studies as part of my NIHR funding requirements (Appendix 7). Accessing
3591 data for research by well-trained staff in trusted organisations is generally well
3592 received and acceptable to the public. Personally, I think I would like to have seen
3593 some active researchers on the panel for the ethics committee: they gave excellent
3594 advice and made useful suggestions based on their plethora of extensive clinical
3595 experience, but demonstrated a lack of understanding about the 'doing'. I think the
3596 addition of researchers on ethics committees might be a route to attaining a better
3597 balance for this. Additionally, I felt that early input from the ethical committee, or a
3598 representative, in the infancy of the protocol would have minimised a lot of ineffective
3599 and often fruitless rounds of review. I was lucky – I had a great supervisory team with
3600 lots of experience on this, and yet still, many, many problems arose that took over a
3601 year to negotiate and navigate. When thinking about PhD programmes it is important
3602 for teams to consider this real burden and ensure that it does not get in the way of
3603 good, sound epidemiological studies.

3604 **Further work**

3605 Further work should expand on this research, which was only intended to be
3606 exploratory and low resolution. A better and closer examination of homes alongside
3607 a methodology to identify true secondary transmission more accurately should be the

3608 next step. This work should be designed in a way that allows correlations to be
3609 extrapolated more widely, and it is important that these are facilitated by all public
3610 health bodies across England and Wales.

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3628 Health and Care Research Wales, and all other public/lay contributors to the Public
3629 and Patient Involvement survey.

Chapter 7

Conclusions and personal reflections

3630 **Conclusions**

3631 The objectives of this overall piece of work and my attempts to address these are
3632 outlined below.

3633 **Objective 1**

3634 **To describe the distribution and burden of this infection in the UK, as well as**
3635 **considering detection and approaches to testing that underpin the surveillance**
3636 **data.** I presented the most recent surveillance data and included exposures examined
3637 in outbreaks, in particular. The main pathways were water, with both drinking and
3638 recreational exposures, animal contact, food, and person-to-person, in various
3639 settings. Outbreaks related to all of these have been reported, and the frequency of
3640 some are changing. However, we are not entirely sure if the contribution of these
3641 appears the same for sporadic disease. *Cryptosporidium* infection, and subsequent
3642 disease, can present in all groups of people, although the greatest burden occurs in
3643 the most vulnerable. The incidence of this infection is significant in England and
3644 Wales, but inconsistencies in testing do exist, leading to possible presentation and
3645 testing biases in specific groups (immunocompromised, younger children). The
3646 results from the laboratory audit were able to confirm this, but also suggest that
3647 changes to practices might see these inconsistencies reduce in the near future.
3648 Additionally, I reported that submission of samples for genotyping and
3649 characterisation is variable, leading to biases in our understanding of the different
3650 contribution of transmission pathways to species-specific disease burden.

3651 **Objective 2**

3652 **To examine and present exposures most associated with sporadic disease, and**
3653 **calculate how much these contribute to infection.** I conducted a literature review
3654 to describe the reported exposures examined and associated with sporadic disease
3655 in industrialised countries, and highlight any pathways to infection that should be
3656 further considered. Results from the systematic review reflected the bimodal age-
3657 related pattern that *Cryptosporidium* follows in UK; a peak in young children, and a
3658 further peak in adulthood, possibly driven by close contact with cases, with exposures
3659 such as parenting and caring activities responsible. I was able to conclude that the
3660 person-to-person transmission pathway is often, but not thoroughly, investigated.
3661 This pathway represents a consistent exposure for infection. This seems to apply
3662 particularly to the home environment, which is increasingly understood to be a
3663 significant setting for spread of *Cryptosporidium* infection.

3664 **Objective 3**

3665 **To explore transmission in the home environment, and calculate the burden**
3666 **this might have on people in the home, considering longevity and severity of**
3667 **illness.** Attempts at quantifying this in The epiCrypt study revealed that additional,
3668 and mostly uncaptured, cases of cryptosporidiosis might occur in around a quarter of
3669 homes with an index case. Spread of this infection in the home environment
3670 represents a considerable burden, individually and societally. Almost two additional
3671 cases are likely to occur, on average, in households with an index case and almost a
3672 third of people in the home could be expected to become ill. Impacts on the family
3673 can be great, with mums and siblings (often children) making up the burden of
3674 secondary cases. It is plausible that the driver here is direct contact, in a caring
3675 capacity; undertaking activities which likely put the main carers (often females) in the
3676 home at particular risk. Homes reporting spread of infection are most often those with
3677 *C. hominis* cases, and those with children under five years old. This knowledge can
3678 help support targeted public health measures.

3679 This work did not reveal any considerable proportion of asymptomatic infection, with
3680 a detected prevalence of 2% in the study population. It seems unlikely that
3681 asymptomatic carriage contributes to spread of infection in the home environment,
3682 although there are specific limitations with identifying true asymptomatic carriage and
3683 specifically designed studies are necessary to accurately measure this. Using
3684 additional clinical indicators, such as stool consistency, has no correlation with
3685 microbiological confirmation.

3686 **What does this work add overall?**

3687 • This work demonstrated that additional cases of *Cryptosporidium* occur in
3688 over a quarter of homes with a case. This is likely to affect up to a third of the
3689 home and cause considerable burden of illness. This is especially common
3690 where the index is a young child, with mums and other siblings most at risk of
3691 secondary infection, and where homes have cases of *C. hominis*.

3692

3693 • We are likely under ascertaining cases of sporadic illness and under
3694 examining person-to-person spread. The work supports recognition of this
3695 pathogen as burdensome and of considerable significance in
3696 immunocompetent individuals. Person to person spread should be considered
3697 in public health response to outbreaks.

3698

- 3699 • Using additional clinical indicators such as stool consistency has no
3700 correlation to microbiological confirmation. I have recommended that we
3701 continue to support the removal of criteria for testing, and test all stools for
3702 *Cryptosporidium*.
3703
- 3704 • The protocol for this work can provide a boilerplate template for complex
3705 cross-organisational research.

3706 **What are my recommendations?**

- 3707 • To eliminate stool consistency as a testing inclusion criterion in local
3708 laboratories and to support testing of all stools submitted for the investigation
3709 of gastrointestinal pathogens.
3710
- 3711 • Systematic changes to surveillance to include 100% speciation of
3712 *Cryptosporidium* samples, fed routinely back into local health protection teams
3713 across England and Wales.
3714
- 3715 • The consideration of specific clinical advice on prevention for high-risk homes
3716 to be added to public health response documentation. High-risk homes should
3717 be considered those with cases of *C. hominis* and/or children under five years
3718 old. This might include managing the patient's expectations on the length of
3719 illness, and the possibility of relapse.
3720
- 3721 • A further study with a specific methodology to identify true secondary
3722 transmission with increased accuracy. Work should be designed to allow
3723 correlations to be extrapolated more widely, exploring exclusions of co-
3724 primary cases. This might include working with partners on the production of
3725 protocols which can be triggered during outbreaks, to follow up homes where
3726 we know contacts have not been exposed to the source of infection.
3727

3728 **How might some of the limitations be addressed in further work?**

3729 It is quite possible that asymptomatic infections contribute somewhat to spread of
3730 infection on this pathway, although these were tricky to determine in the design of this
3731 work. I would like to see a specifically designed study, looking at this in the general

3732 population and in homes with cases. This would need to specifically to consider the
3733 timing of specimen collection, and perhaps utilise follow-up and repeat sampling.

3734 The systematic review was a large and demanding piece of work, that ultimately grew
3735 beyond the scope of this work and restrictions were enforced. It would be interesting
3736 to see this work followed up: a piece of research explicitly considering differences
3737 between sporadic and outbreak cases in terms of transmission pathways would be
3738 welcome. I am intending to take this forward myself in the future.

3739 Variations in testing practices can have an impact on our understanding of the
3740 incidence of this infection, and our confidence in the surveillance data. The audit I
3741 undertook had a low response rate, and I would be keen to follow up on this work
3742 later. Ongoing flexibility and evolution of the guidance for standard practice is key for
3743 oversight of this pathogen in England and Wales, and I would hope that the upcoming
3744 publication of the laboratory audit, and any further work, would be an impetus for this.

3745 Despite an attempt at detailed collection of data in The epiCrypt study, some of it was
3746 unusable for detailed examination of the home. Nonetheless, there was some
3747 anecdotal evidence for differences in risk of secondary infection according to role in
3748 the home. I would be keen to work with qualitative researchers to design a mixed-
3749 methods study to examine the impact of this infection in families; perhaps measuring
3750 patient experiences, assess disproportionate gender burden, and economic impact in
3751 England and Wales.

3752 Some additional characterisation of samples was negatively impacted by the
3753 occurrence of the global COVID-19 pandemic, which affected staff time, access to
3754 equipment, and organisational priorities. The samples needed for WGS are prepped
3755 and complete, and the CRU and I are hoping to continue with this work later. This
3756 might allow for further understating of the populations of *Cryptosporidium* in this
3757 study, and in homes with spread.

3758 **Personal reflections on the PhD experience**

3759 I began my PhD experience late in my career, and perhaps in my life compared to
3760 most. It began with my desire to build a research piece alone, with my own hands,
3761 and a massive leap of faith from four potential supervisors that I could actually do it!
3762 I started out nervous, and tried everything to remain in my comfort zone. I ignored
3763 advice that tried to impress on me the difficulties in implementing an observational
3764 study. I thought I knew better. Well, as the process went on, I realised I really didn't!

3765 And far from keeping me in my comfort zone, with every step I was further and
3766 further at sea. I had to quickly navigate ethics procedures for two countries, get staff
3767 from other organisations to engage with this on nothing but goodwill, and manage
3768 many, many disagreements in between. I had to learn to stand on my own two feet
3769 pretty quickly. Add to this a systematic review, which in hindsight was way too big
3770 for this work (learning point!), changing reviewers once, and losing two supervisors
3771 (but who remained ever on hand, much to my gratitude).

3772 But, I loved it. I was always stretched. Always pushed. I was given every opportunity
3773 to make more of my PhD than just a thesis. I went to conferences, published
3774 papers, wrote funding bids for public engagement ideas and delivered them all over.
3775 I was given time to pursue, and achieve, my FLTHE (Foundations for Learning and
3776 Teaching in Higher Education) qualification to teach. This PhD is bigger than the
3777 sum of its parts for me. Whilst I feel proud of this thesis, and especially the epiCrypt
3778 study, I believe that I have embarked on a journey that goes beyond this; of self-
3779 discovery, of confidence and independence, and one that I hope is just beginning.

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Appendices

Appendix 1: Laboratory audit questionnaire

An Audit of Laboratory Practices for Cryptosporidium Diagnosis in England and Wales 2018/19

1. Name

2. Position

3. e-mail address

4. Laboratory name

5. Laboratory address

6. Laboratory region (highlight or delete)

North of England - North East

North of England - Cumbria & Lancs

North of England - Yorks & the Humber

North of England - Greater Manchester

North of England - Cheshire & Merseyside

Midlands & East of England - Lincolnshire, Leicestershire, Nottinghamshire & Derbyshire

Midlands & East of England - W Midlands

Midlands & East of England - Norfolk, Suffolk, Cambridgeshire & Essex

Midlands & East of England -S Midlands & Hertfordshire

London (all)

South of England - Sussex, Surrey & Kent

South of England - Thames Valley

South of England – Wessex

South of England - Devon, Cornwall & Somerset

South of England - Avon, Gloucestershire & Wiltshire

Wales

7. Does your laboratory ever test stool samples for Cryptosporidium?

Yes

No

8. Stool samples tested for Cryptosporidium are:

All stool samples

All non-formed stool samples

All except inpatient for >3 days (3-day rule)

Other criteria applied

9. If you have answered 'other' and apply criteria, then please answer the rest of the questions on this page, indicating under which criteria you would test:

Choice based on history and given clinical details

Yes

No

If Yes, please specify the history and clinical details that you use in your testing criteria, e.g. overseas travel, immunocompromised etc.

10. In specific age groups

Yes

No

If Yes, please give age groups in which you test/upper and lower range

11. Other criteria not already recorded (check all that apply)

Overseas travel

Immunocompromised

Eosinophilia

Employment

Exposure history

If requested

Other, please specify

Testing methodology

Please indicate which of the following methods you currently use as the primary test for Cryptosporidium within your laboratory (there is a question later about any upcoming changes you have planned)

12. Enzyme immunoassay (EIA)

Yes

No

If Yes, Please specify which EIA kit you use

Do you confirm positive reactions?

Yes

No

If Yes, specify how

13. Polymerase chain reaction (PCR)

Yes

No

Please specify which DNA extraction kit or platform you use

Please specify which PCR assay you use

14. Bright field microscopy stain e.g. modified Ziehl-Neelsen or Kinyoun

Yes

No

15. Fluorescent microscopy stain e.g. auramine phenol

Yes

No

16. Please give details of any other diagnostic method you currently employ for Cryptosporidium

17. Have you recently made any changes to the test method?

Yes

No

If Yes, please indicate the relevant method and add date if known, below

18. Are you planning any future changes to the test method?

Yes

No

If Yes, We are changing our test method to.....

EIA

PCR

Other

If other, please specify

Will the change in method mean that your criteria for testing will change?

Yes

No

If Yes, which of the following will be tested?

All stool samples

All non-formed stool samples

All except inpatient for >3 days (3-day rule)

Other

If other, please specify

When are these changes due to take place?

19. Do you refer Cryptosporidium-positive stools to the reference lab for genotyping?

Yes

No

If Yes, In what circumstances do you send positive Cryptosporidium samples to the reference lab for genotyping?

Highlight all that apply

All positive samples are sent routinely

Only in an outbreak situation

At the request of a Health Protection Team

Never

Other

If other, please specify

Do you update your LIMS with the genotyping result?

Yes

No

If No, If you don't presently send samples for genotyping, would you be willing to do so, either when they are diagnosed or in batches?

Yes – when diagnosed

Yes – in batches

No

Any comments

20. Any other comments

Appendix 2: Systematic review protocol manuscript

Please see attached additional file

Appendix 3: Final search terms for systematic review

Question components and search terms	Term type		Boolean operator
	Free	Mesh	
Population: cases of cryptosporidiosis 1 cryptospor* 2 humans 3 1&2	X	X	AND
Exposure: risk factor(s) 4 epidemiolog* 5 risk factors 6 exposure 7 transmission 8 association 9 or/4-8	X X X X X		OR
Outcome: study endpoints 10 cohort 11 case-control 12 “case control” 13 case-crossover 14 “disease outbreaks” 15 meta-analysis 16 longitudinal 17 ecological 18 or/10-17	X X X X X X X	X	OR

Appendix 4: Minimum data set for extraction for included papers in systematic review

Bibliographic detail	Study detail
Name of reviewer	Study design
Date of extraction	Number of cases reported
Publication type	Age/sex cases/participants
Country of origin/language	Case definition (& any known co-infections)
Study title	Definition of exposure(s)
Names of authors	Definition of activities
Journal/source reference	Species identified
Year published	Incubation period
	Exposure window(s)
Study outcomes	General methodological
Number (%) exposed among groups	Confounders
Types of exposures	Likely biases
Comparator(s) (well controls, other infection)	
Selection and recruitment methods	
Availability of appropriate controls (from the same source population as the cases)	
Interview methods	
Effect measures (type and result)	

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Appendix 5: The epiCrypt study protocol manuscript

Please see attached additional file

Appendix 6: The epiCrypt study invite letter to index cases

PUBLIC HEALTH ENGLAND OR WALES HEADED PAPER

Public Health England/Wales

ADDRESS

POSTCODE

Date ____/____/____

Name/Parent/Guardian of Name

Street

Address

Postcode

Study ID

Dear Name/Parent/Guardian of Name

The epiCrypt Study – investigating how *Cryptosporidium* infection spreads at home

We are inviting your household to take part in a study because you/your child recently had the *Cryptosporidium* bug. You may have already been contacted by someone from Public Health England/Wales or your local environmental health team, and asked to fill in a questionnaire. It is no problem if you have already done this, you can still take part in this study.

If you have not been made aware of this diagnosis yet you can contact your GP or clinician who took your sample, to confirm this with them.

The study

We are a team of researchers at the University of Liverpool, Public Health England, and Public Health Wales who are interested in finding out more about the spread of a common tummy bug called *Cryptosporidium*.

Some people pick up the bug and feel fine. Other people can feel poorly for some time. We want to find out if *Cryptosporidium* spreads between people in the same house, and look for clues as to how this might happen. This will help us to stop others getting sick.

Why have we chosen your household?

You or your child have recently had *Cryptosporidium*. We are asking everyone who lives in the North West of England or in Wales, who has recently had the *Cryptosporidium* bug, to take part along with other people in their household.

What we would like you to do

We would like to test the poo of everyone else in your household to see if anyone has had *Cryptosporidium*, even if they haven't been sick. You will not need to give another poo sample as you have already been tested.

There is also a short questionnaire to fill out asking some general questions about your/your child's illness and your household.

Further information

A member of our team from the National Institute for Health Research (NIHR) Clinical Research Network will be in touch in the next few days to explain the study and chat things over. This gives you a chance to take your time to decide if you want to take part and talk to us about the study itself.

You may be contacted by post if we cannot reach you.

If you would rather not be contacted about this study, you can let us know by:

- email: FES.NorthWest@phe.gov.uk OR Surveillance.data@wales.nhs.uk
- 'phone: 0344 225 0562 - Option 6 [Field Epidemiology Service] OR +44 2920 10 4488
- post: Just return the slip below in the envelope provided

Quote your study ID when you contact us.

If you have any further questions please feel free to contact a member of the team on 0344 225 0562 - Option 6 [Field Epidemiology Service] OR +44 2920 10 4488

Yours faithfully

Dr. Roberto Vivancos

Consultant Medical Epidemiologist

Dr Christopher Williams

Consultant Medical Epidemiologist

✂.....

ID | | | | | | | |

I do not wish to be contacted about this study

.....

Appendix 7: The epiCrypt study - justification of recruitment approach and short PPI survey

Justification of approach to recruitment

Our approach to the recruitment process has been driven by necessity and feasibility, and previous research supports the acceptability of this method. In addition, following valuable comments from the CAG on the application, we undertook a short survey among the general public and specific PPI groups to gauge general attitudes toward accessing data prior to consent, to support recruitment to research.

This document outlines the main considerations, in brief, in our decision to recruit using the methods described in the protocol. We feel that our approach to recruitment is well established and understood, and is supported not only by previous research in other settings, but also by the small PPI activity undertaken for this piece of work.

Ethics committee feedback

Whilst the researchers recognise the importance of patient and public involvement (PPI) in driving and shaping research, the scope for a large PPI study within this project, as part of a 3-year doctorate, is limited. The researchers were questioned on their reasons for the absence of engagement (The NHS REC Committee - Liverpool NE REC – committee meeting 19 April 2018) and outlined that scope, time and resources, including expertise in the methods, were significant limiting factors. After discussion on the resource requirements for this, and patient response and engagement with similar studies²⁰, the committee were satisfied that this was not feasible or required. In addition the CAG recognised that as detection of *Cryptosporidium* was retrospective and undertaken by laboratory staff, there was no opportunity to consent individuals at the time of diagnosis and the recruitment process could not be achieved without access to confidential patient information. However, the CAG report contained useful comments and suggestions on this, and recommended that we gauge the feeling on his approach by testing the acceptability of using confidential patient information without consent in order to facilitate the study recruitment procedures.

²⁰ Studies recruiting based on disease surveillance are common for GI infections, and many projects have taken this approach – the methodology for the epiCrypt Study has been influenced by design aspects of large-scale studies such as Enigma, IID2, and Integrate.

Considerations when drafting the protocol

We considered the acceptability of many recruitment process in our decisions at the protocol drafting stage of the project and explored several options. We used previous literature and experience of other studies to help us to achieve the best model for recruitment, which balanced the data needs with patient choice. Several publications exist which support our approach to recruitment and confirm that although not always the preferred approach to consent, such an approach is nonetheless acceptable.

Because our inclusion criteria, and indeed means of identifying a case, can only be met using laboratory records it is essential that we access these records in order to get contact details to approach potentially eligible participants. This step is necessary as cryptosporidiosis is only confirmed via laboratory testing and as such there is no other method of identification of cases available to the study team. We designed our approach based on several key factors known to be important considerations for the public when engaging in research:

1. General acceptance of accessing data, but with opt-out routes

While generally the public support health research, some research does suggest that most people would find data use without any possibility of objection, to be unacceptable (Willison *et al.*, 2008). However, people also tend to recognise that an approach of 'consent for each use' is burdensome for both researcher and participant, and can hinder research. In addition, a recent study in England and Wales (Taylor and Taylor, 2014) reported that people who would prefer to be asked explicitly before data were would however accept an 'opt out' model of consent *if* the reasons for not seeking explicit consent are clear. In this work, a model similar to ours emerged as the preferred and most acceptable model, where explicit consent is not necessarily disclosed but participants are given a route to opt-out, for sufficient/any reason. In our model, participants are given opt-out options at each contact and it is emphasised that they can withdraw at any time, even if they have provided information, this can be deleted at any point.

2. Differences in acceptability of accessing data, with and without explicit consent, according to who is accessing it

Research suggests that in general the public do have some concerns relating to the appropriateness of access, irrespective of the model of consent adopted. But with a less favorable but acceptable model, if an individual's data are to be used without

explicit consent, then it appears to be considered important that research was carried out by somebody owing a duty of confidentiality:

“We are talking about data that is only going to researchers. These researchers inherit a duty of confidentiality by dealing with this. If it was being passed on to a third party who did not have a duty of confidentiality under the NHS, then that would be very different”. [TH7, Group 3] (Taylor and Taylor, 2014).

Other work suggests that alternatives to explicit consent may be acceptable if participants are confident that data are used by persons, and for purposes, that they trust and accept as reasonable (Wilson, Draper and Ives, 2008)

In our methods, we do not use any existing data prior to consent, and are only accessing for purposes of recruitment contact details. In addition we have specifically set this up so that the contact details are shared with NHS staff who are researchers but with a clinical background, in order to improve acceptability and instill trust in participants. Evidence from a pan-European survey looking particularly at the transfer of contact details to a research team reported that the acceptance of this scenario appeared to be based upon the choice patients had to return a questionnaire once contacted by researchers, and that only names and addresses were being released (Patil *et al.*, 2016). Some concerns were expressed around ensuring and believing in the credibility of the “researcher” amid concerns about their duty of confidentiality. It seems that NHS and/or clinical staff are more acceptable to the public when in a model of accessing data for contact, prior to consent, and using a “research nurse” to contact patients was favourably greeted. In our model, only NHS/Public Health staff access laboratory records initially – then if the case does not opt out via the mechanisms provided, their details are shared only with NHS research nurses in order that they can be contacted. The University research staff only access data following consent, and only the data that the participant provides themselves.

3. Participants’ study information

A 2008 paper recommended that good practice for recruiting primary care patients into research studies should include detailed patient information that describes the proposed research and emphasizes that withdrawal of consent, or refusal to participate, will not affect their clinical care (Wilson, Draper and Ives, 2008). Our study pack contains a tri-fold pamphlet outlining the study, as well as links to the study webpage hosted by NHS Wales. There is also a cryptosporidiosis health advice

leaflet and participants are expressly informed that their rights to health/clinical care are not affected by any decision about participating in the study. They are also given several points of contact and means of contacting the study team and chatting anything over. The research nurses' call also gives participants another opportunity to discuss and understand the study and again opt out of participation or further contact if they choose.

4. The trust in the confidentiality of patient data and security of systems.

The pan-European survey showed that there is general support for using data for research, and storing more detailed electronic health data was generally preferred, but respondents were averse to wider access to and sharing (Patil *et al.*, 2016). Another study looking at attitudes of lay persons in South Wales, UK., reported that unauthorised access to data by external agencies was a specific, common fear (Robling *et al.*, 2004).

Our model of recruitment is set up using only established, secure internal systems. Any data transfers (contact details) are supported by contracts and data sharing agreements which have been reviewed and authorised appropriately. The methods for accessing patient details, transferring, and recording opt-out preferences are laid out in the protocol and staff members will all be trained in the process. Data are not used for any other purposes, nor are they retained beyond required terms, and no data are shared with any other person/study/team. Recruitment data are only accessed by existing NHS/PHE trained staff bound by confidentiality and who would access and see the records anyway. There are agreed data transfer protocols in place between PHE/PHW and the Clinical Research Network.

Comments from the CAG and subsequent PPI

The CAG report contained useful comments and suggestions on this, and recommended that we gauge the feeling on his approach by testing the acceptability of using confidential patient information without consent in order to facilitate the study recruitment procedures.

We drafted a short survey which outlined the approach to recruitment and the framework of the study. We accessed a lay PPI group from the Infection and Global Health panel at the University of Liverpool, and one from Health and Care Research Wales. The link was circulated via email and participants were also encouraged to email or call the research team directly if they had specific comments. Participants

were asked to think generally about the method of recruitment and how they felt about this approach, about who was accessing the data, and about what information materials they would like to be provided with if they were taking part.

This was a quick approach to gathering some feedback in support of our work. We were unable to gather personal data on participants and so cannot make any conclusions about representativeness or sampling. In addition the survey questions and responses have been simply posed and analysed. A large scale PPI study, although suitable, is beyond the scope of this study and far outside the resource and expertise available to this team. Nonetheless, it is hoped that the responses will help support the CAG and ethical approvals, and also may help buttress our recruitment process decisions, and study materials.

These data have been analysed as of 12/06/2018.

More responses may be due in and in addition, the study lead has two further appointments face-to-face with PPI groups at the University of Liverpool, and at Royal Liverpool and Broadgreen Hospital. These group discussions may well reveal further thoughts and comments, and the team will put these together in a broader document and submit these with the study write-up. We were unable to access these meetings prior to the CAG deadline of 30/06/2018.

Survey users were asked to read and consider the scenario and then answer questions, with the option of additional comments.

There were also some broader, free-text questions reported at the end. Individual results are available in the appendix.

In general the feeling echoes some of the previous research we have touched upon. It is generally considered acceptable to access data for recruitment, especially in order to support much needed research. However, considerations and worries include the person accessing data, with NHS staff, or at least those with a clear science/medical link, generally viewed as more acceptable. Contact from researchers (non-NHS) is generally not viewed as favourably. All users reported that they would be happy to take part in this piece of work if contacted. Participants would like to receive as much information as possible and would like health and disease advice and information. One user stated in particular that they would like to see the benefits/objectives of the research and the proposed dissemination. We are happy that we fulfil these needs/requirements in our study packs as they currently stand.

Conclusions

Extracting cases from surveillance data is often normal practice for gastrointestinal disease research and techniques are well studied and agreed.

In this study, participants can only be identified using surveillance data as *Cryptosporidium* is diagnosed via laboratory confirmation. No identifiable data will be shared with the University study team members until consent is given. Only those staff in NHS or in public health will be able to access contact data and data are managed and held securely and in line with security and protection requirements of each organisation. We feel that this attention to detail and integrity of our approach to accessing personal information from surveillance data helps to support acceptability.

The study is low risk but has many benefits – results may highlight the possibility of secondary illness pathways in this disease: Participants may be better placed to stop future spread of illness in the home.

By identifying and understanding secondary transmission, public health measures can be reiterated or improved to stop onward transmission so participants are involved in supporting longer-term research and knowledge

We feel that our approach to recruitment is well established and understood, and is supported not only by previous research in other settings, but also by the small PPI activity undertaken for this piece of work.

We will continue to work on this PPI activity; we aim to present this further and gather more information in support of our ongoing work, and other research in the department.

Appendix A: The survey questions and current results

Participants were given this scenario

SCENARIO FOR RECRUITMENT TO A RESEARCH PROJECT

This research project is about *Cryptosporidium*.
This parasite causes over 4,000 cases of diagnosed illness in England and Wales every year. It particularly affects children and can spread between family members.

This project will be a year-long study to identify secondary transmission in households and to examine risk factors and likely mechanisms for spread.

We will recruit 400 cases across England and Wales, ask general questions about their household composition and behaviours, activities, and pets. We will test stool samples from consenting household members.

Cryptosporidium is only diagnosed by testing a stool sample. This positive lab report then goes into a national disease surveillance database.

So, in order for this research team to identify potentially eligible study participants, we need to *get all* the lab reports of Cryptosporidium cases in England and Wales, and get their name and contact details from the surveillance records. This way, we can contact these cases to ask them if they are interested in taking part in our study. The data are only downloaded and viewed by existing, trained NHS or Public Health staff – not researchers at the University.

Cases will be then contacted initially by research nurses (part of the NHS/National Institute for Health Research).

These data are only accessed for contact purposes and no data are retained or used for research. Staff only see the necessary contact details and all access will have been approved by the relevant ethics committees.

After the case is contacted, if they want to participate they then fill out a consent form to take part and we collect fresh data from a questionnaire.

In order to support good, transparent research, and alongside our CAG/ethics applications, the research team would like to understand the views of the public on this approach.

Think of the scenario you have been given and then say how much you agree with the statements

	Strongly Agree	Agree	Neutral	Disagree	Strongly Disagree	Total
It is acceptable for a member of NHS staff to access your records so that they can contact you about a study you would be eligible to take part in	5	2	0	0	0	7
It is acceptable for a non-NHS researcher to access your records so that they can contact you about a study you would be eligible to take part in	3	1	2	1	0	7
It is acceptable for a researcher to access your existing records and use that data for research purposes, without seeking consent	3	0	1	2	1	7
I would be happy about participating in this research if I was contacted by Public Health England or Public Health Wales	6	1	0	0	0	7

I would feel more comfortable with NHS staff accessing my data and contacting me than non-NHS researchers	3	0	2	1	1	7
I have heard of disease surveillance systems	3	3	0	1	0	7
I understand that when I have a lab test the results go into national surveillance databases	2	5	0	0	0	7
This method is acceptable in an outbreak or emergency situation	4	3	0	0	0	7
This method is acceptable if there is no other way of identifying potential participants	4	2	1	0	0	7
I trust those in our health and research organisations to handle my confidential data with respect	4	2	1	0	0	7
This approach is acceptable (Accessing lab records, contacting and asking if you would like to take part. If so, then taking consent through a consent form)	4	2	1	0	0	7

Free-text responses

How do you feel (in general) about researchers accessing data without explicit consent in order to identify potential study participants?

- This should not happen, these records are confidential and this should be respected no matter the importance of the research.
- As long as my details are not shared further
- If this is the only way to identify them then I think this is acceptable and appropriate, especially if you are seeking consent later
- If the data is there and used correctly And anonymously I think it's ok
- I feel that such research is done in the public interest and so am happy with the method

Is this more or less acceptable in this case where there is no other way of identifying this cohort of cases?

- For this to be done by NHS staff I feel is more acceptable, but this should not be from anyone else.
- Yes
- More acceptable
- More (acceptable)
- More acceptable

How do you feel about who accesses your data? Does it matter? Would it matter to you if non-NHS research staff could see your records? Is it more or less acceptable if it is clinical and/or NHS staff?

- Yes because it is the principal of privacy.
- As long as it's for research purposes and identifiable details are not shared further
- NHS don't always has the resources to conduct these surveys so it is only natural that outside agencies need to complete them. Would prefer ones who have a medical/scientific background
- It is more acceptable if it is clinical or NHS staff ordinarily. However, I have an understanding that such staff have not got the time to do this important work and so a clear letter of explanation in lay terms to explain why I am being contacted I would feel re assured.

If you were taking part in this study, is there anything specific you would like to see included in your study information pack? Any certain documentation or explanations? Would you rather have posted materials or speak to a research nurse on the 'phone or in person?

- I would like both an information pack and a contact with whom I could discuss any concerns or questions relating from the information pack
- How to control the spread within a family. How does one become infected in the first place? How can I protect myself and my family?
- Probably on the phone or post.
- clear letter of explanation in lay terms to explain why I am being contacted

Additional thoughts from others who did not fill in the survey but contacted us directly

- "A person's consent and confidentiality should always be paramount. However, I don't see that contacting people to ask their consent to be involved is a problem. They have the right then to say 'yes' or 'no'. I also feel that people would be more inclined to be participants in research if they were firstly approached by NHS staff, rather than researchers".
- "If I was taking part in a study myself, I would like information as to what the study hoped to achieve (i.e. the outcomes), what my participation would involve, and how the results would be disseminated".
- "Even if there was no other way to conduct this study, I would still have an issue with patient confidentiality and data protection - I would like to be asked first whether I agreed to disclose my personal details or not".

Appendix 8: The epiCrypt study participant study packs

Please see attached additional file for full study pack

Appendix 9: The epiCrypt study participant reminder letter

The epiCrypt study

Investigating how *Cryptosporidium* infection spreads at home

Clinical Research Network
NWC
2nd Floor
131 Mount Pleasant
Liverpool
L3 5TF

Date ____/____/____

Dear -----/parent/guardian of -----,

Re: **The epiCrypt Study** – investigating how *Cryptosporidium* infection spreads in the home

We recently contacted you about taking part in a study about the spread of tummy bugs. You should have had your information pack by now, and we are writing to remind you to send in your questionnaire and stool pots if you want to take part in the research.

It is completely up to you and your household to decide whether to take part. If you decide to take part you are still free to change your mind at any time and without giving a reason. You can contact us about anything you want to chat over. If you need another pack, or more sample pots, just contact the team on the below number or email, and let us know.

Further information

If you would like to discuss the study or have any questions or concerns, feel free to contact **The epiCrypt Study** team via email (c.mckerr@liv.ac.uk) or 'phone (0151 795 8334)

Thank you for helping us with our research!

CRN North West Coast

Appendix 10: Laboratory protocol for the processing, testing and recording of stool samples in the epiCrypt *Cryptosporidium* household transmission study

This study is being undertaken as part of a PhD by Caoimhe McKerr, University of Liverpool, in collaboration with PHE and PHW. It is being funded by the National Institute for Health Research (NIHR).

The aim is to estimate the frequency of transmission of *Cryptosporidium* in households and understand more about it.

This study is scheduled to run for 12 months. The first month will be a pilot phase and processes will be reviewed for any changes that need to be made. Samples from the pilot will be included in the main study in the event that there are no, or minimal, methodological changes. See below for the sample processing workflow.

Stool samples from consenting household contacts of index cases will be self-collected using Fe-Col kits and the pots sent to the CRU in the post. Index cases will have been identified through routine surveillance, recruited and consented with their household for the study.

Pots will have a sticker attached with a household-level study ID beginning NW (for North West England) or WS (for Wales). This is the study ID, used later to cross-reference CRU results with questionnaire data at Liverpool University. The label should also have the subject's age and sex and sample date, filled in by the subject themselves.

When an epiCrypt sample pot arrives in the laboratory:

1. Pre-print a series of stickers HH0001 - HH1000 to provide HH sample numbers
2. Date stamp the required number of pre-printed HH stickers with today's date dd/mm/yyyy
3. Allocate a dated, HH sample number sticker to each pot, but do not cover the handwritten information on the pot.
4. Book samples in using the project lab database (maybe one person reading from the pot, the other booking in). During the pilot phase an Excel spreadsheet will also be used, and reviewed at the end of the pilot phase. To record at booking in (compulsory fields are shown in bold):
 - **Date received as per sticker just applied dd/mm/yyyy**
 - **Unique HH sample number (HH0001-HH1000) as per sticker just applied.**
 - **The study ID (beginning NW or WS) which is on the stool pot**
 - Age in whole years (from the stool pot). Anyone <1 year is 0.
 - Sex (from the stool pot) M, F or U
 - Date of stool sample (from the stool pot) dd/mm/yyyy
 - **Consistency using the Bristol Stool Scale**
5. Store in Fridge 2 in the project tray until testing.

Screening the samples

Screen the samples in routine IFM batches as this avoids the need for new algorithms and paperwork and is an efficient use of QC. Samples don't have to be screened daily but don't leave them longer than a couple of weeks.

1. Screen samples using IFM (2 wells) (CRU SOP17). Use the routine microscopy form (CRU SOP17F1) to record the results but "report" on the project database. Enter the reason on the microscopy sheet as "HH study". Use the microscopy scores in CRU SOP17; if "scanty" record the actual number of oocysts seen:

No. of (oo)cysts seen per field of view (x40 objective)	Record as:
None in the entire well	Neg
1 or fewer (but at least 1 in the entire well)	Scanty, and record the number of oocysts seen
2-5	+
6-10	++
11-50	+++
50 or more	++++

2. For positive samples, add the HH sample number to the batch record sheet for salt float, CRU SOP 004 F1
3. For negative samples, dispose of immediately and record this on the project database.

Further processing and testing IFM positive samples

1. For positive samples, do a salt float²¹ (batches of 8 or more, no need for pos or neg controls, using CRU SOP 65 i.e. re-suspend in 200 µl RO water).
2. On the same day, proceed to bleach treat all of the 200 µl oocyst suspension and do the freeze-thaw process as per CRU SOP 65. If there is no time to extract the DNA, store frozen.
3. Do Mini kit DNA extraction as per CRU SOP 65 using record sheet F3.
4. Store DNA in freezer 5 until use for species ID (see point 5 below), future subtyping and possible WGS.
5. For species ID, perform RT2 PCR (CRU SOP 61), then if RT2 is negative 18s (CRU SOP 28). Record results in the project database.
6. Once the species result is finalised, stool samples should be discarded in the usual way; the stool samples must not be retained for any further use. Record that the *Cryptosporidium* positive stools have been discarded when the species results have been entered on the sample database.

Other information

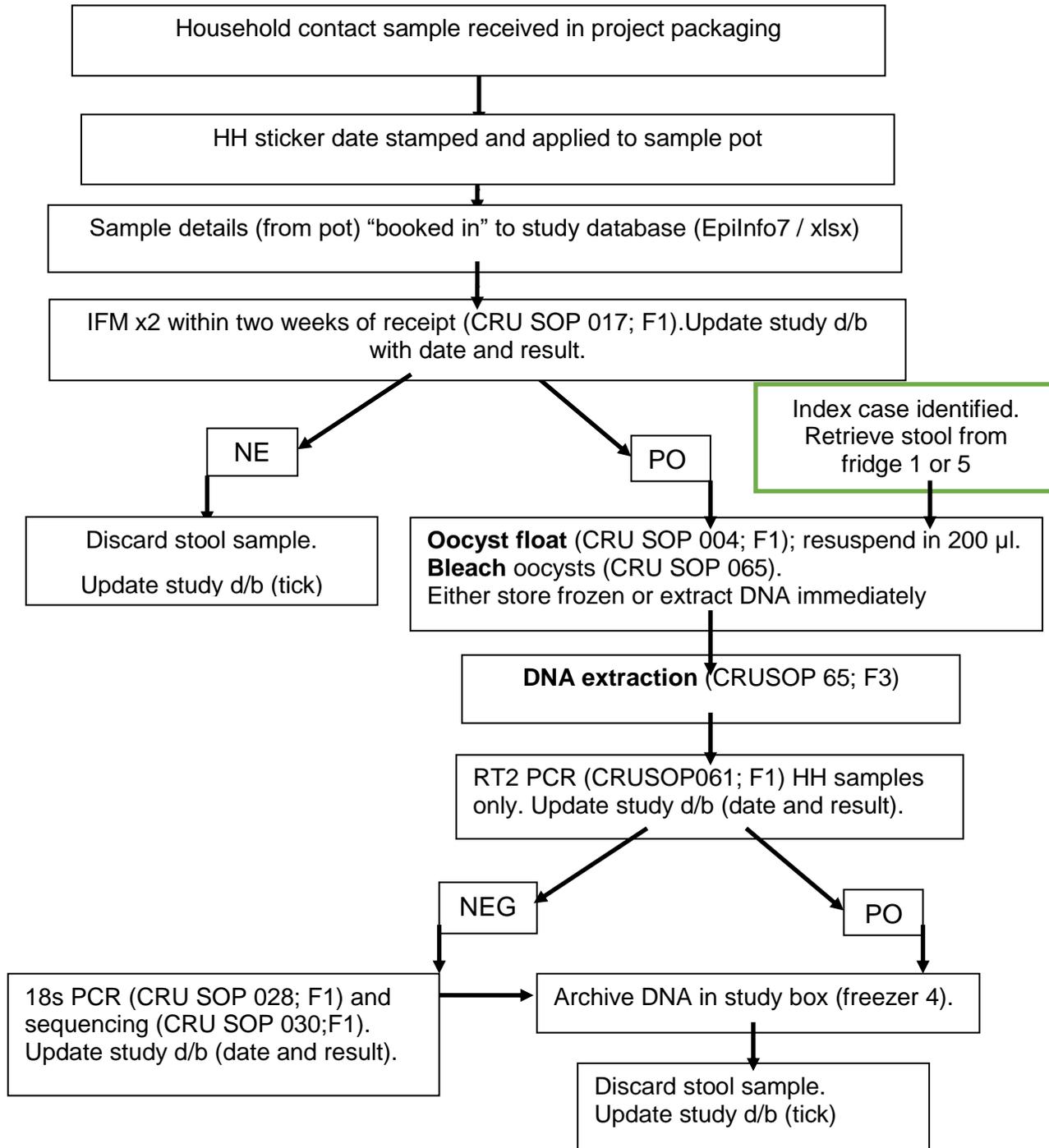
²¹ Benefits of salt float and bleaching before DNA extraction: separates parasites from human material; sample is ready for gp60/MLG and for WGS in a single preparation.

- **To identify the routine samples from index cases that have been enrolled in the study²²**
- Enter the sample in database, with accession number, the original sample receipt date, and the study ID. Record the Bristol Stool Scale as 99 i.e. not relevant²³. Enter the sample in into the workflow for salt float, bleach, DNA extraction, testing and storage of DNA.
- **Notification.** As *Cryptosporidium* is a causative agent listed in Schedule 2 of The Health Protection (Notification) Regulations 2010 and The Health Protection (Notification) (Wales) Regulations 2010, the operator of a diagnostic laboratory has a duty to notify detection in human samples. In Wales, notification is to the proper officer of the relevant local authority (i.e. that of the person soliciting the test). Once a week, send an email notifying of the number of *Cryptosporidium* positive HH stools identified in the previous 7 days.

²² Health protection/CDSC staff from PHE/PHW will keep a record of those Index cases who have consented (according to protocol agreed between CMCK and the HP teams). Once a known Index case has consented to their sample being used in the study, the HP staff will look up the corresponding specimen number on the original case record. They will share this, age, sex, date of sample, and the study ID via monthly email with the CRU. The laboratory ID will allow staff at the CRU to identify and locate the index case sample. Once retrieved, include the sample in the salt float batch for subtyping and possible WGS.

²³ The patient would have had diarrhoea to visit GP in the first place, and the sample will have been mixed, stirred and possibly water added by this stage

epiCrypt study sample workflow v1



Bristol Stool Scale

Bristol stool chart	
	Type 1 Separate hard lumps, like nuts (hard to pass)
	Type 2 Sausage-shaped, but lumpy
	Type 3 Sausage-shaped, but with cracks on surface
	Type 4 Sausage or snake like, smooth and soft
	Type 5 Soft blobs with clear-cut edges (easy to pass)
	Type 6 Fluffy pieces with ragged edges, mushy
	Type 7 Watery, no solid pieces (entirely liquid)



PROTOCOL

Open Access



Exposures associated with infection with *Cryptosporidium* in industrialised countries: a systematic review protocol

Caoimhe McKerr^{1,2,5*} , Sarah J. O'Brien¹, Rachel M. Chalmers³, Roberto Vivancos^{1,2,4} and Robert M. Christley^{2,5}

Abstract

Background: *Cryptosporidium* is a protozoan parasite of humans and other animals worldwide and is one of the greatest contributors to human diarrhoeal illness. Transmission can occur indirectly via contaminated food or water, or directly via contact with animals or other infected people. Risk exposures are often identified from outbreak investigations, but a subset of cases remains unexplained, and sources for sporadic disease and pathways to infection are still unclear.

Given the few systematic syntheses of reported evidence in industrialised populations, the aim of this review is to consolidate the literature to describe exposures associated with human cryptosporidiosis in industrialised countries, specifically including the UK, and describe any differences between outbreak-associated and sporadic disease.

Methods/design: Where relevant, methods will follow the recommendations made in the Cochrane Handbook for Systematic Reviews of Interventions. Three steps will be used to identify the literature including electronic database searching using PubMed, Scopus, Embase and Web of Science; reference list trawling; and an exploration of the grey literature. Screening of results will be undertaken by two reviewers using pre-defined criteria. Studies conducted in industrialised countries and reporting on human subjects will be included. All observational studies will be included where they report exposures and relevant quantitative results.

Data will be extracted using a standardised form. Study quality will be assessed using the ROBINS-I tool. Data will be summarised presenting the papers' main findings including population under study, outcomes, and exposures, and whether these were considered outbreak or sporadic cases. A narrative summary will also be included. Where populations are appropriate, available data will be pooled in a meta-analysis combining the significant exposures across studies.

Discussion: This review aims to consolidate the evidence for transmission routes and exposures for *Cryptosporidium* in industrialised countries, with particular reference to how these may apply to the UK. In addition, the review will seek to describe differences between outbreak and sporadic cases. This will help to identify those most vulnerable, highlighting pathways where interventions and public health response may be appropriate.

Systematic review registration: PROSPERO number [CRD42017056589](https://doi.org/10.1186/1745-7256-42017056589).

Keywords: Cryptosporidium, Protozoa, Outbreaks, Sporadic disease, Zoonoses, Gastrointestinal infection, Risk factors, Epidemiology, Parasite, Foodborne diseases, Waterborne diseases

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Background

Cryptosporidium is a protozoan parasite which can infect humans and other animals, and the most prevalent species identified in humans are *Cryptosporidium parvum* and *Cryptosporidium hominis* [1, 2]. *Cryptosporidium* is distributed worldwide and is suspected to be one of the greatest contributors to human diarrhoeal illness [3]. *Cryptosporidium* is reported in 1–3% of immunocompetent patients with diarrhoea in industrialised countries and 7–20% in developing countries [4–8]. The dissimilarities are probably driven by variation in exposure due to sanitation, infrastructure, and housing and health factors such as acquired immunity and nutrition. The highest prevalence is observed among children under 5 years old, in particular the under twos [3, 9]. The parasite has a complex life cycle and characteristics which favour the faecal-oral transmission route, which may facilitate outbreaks via person-to-person (*C. hominis* and *C. parvum*) or animal-to-person (*C. parvum*), as well as indirect transmission through ingestion of water and food contaminated with infectious oocysts [10].

Reported risk exposures for both *C. parvum* and *C. hominis* often overlap and include consumption of contaminated drinking water [11–15] and exposure to recreational waters [16–18] and food-related outbreaks (likely contaminated via water or by food handlers) [19–22].

C. parvum is frequently associated with exposure to farm animals [23, 24] due to its zoonotic nature and *C. hominis*, more anthro-zoonotic, with person-to-person spread [25–28] and foreign travel [29]. Risk factors and associated exposures are often hypothesised/identified from outbreak investigations; however, outbreaks may only represent a small proportion of cases. Estimates in the United Kingdom (UK) suggest, of all cases reported to national surveillance in England & Wales, <10% are likely to be linked to an identified outbreak [30]. However, the accuracy of the case numbers captured by surveillance may be imprecise [31, 32]. As a consequence, pathways may be under-reported and we cannot be certain that transmission routes for sporadic disease are the same as those which drive outbreaks [33]. Despite case-control studies which have investigated differences in risk for endemic and outbreak disease [34, 35], sources for sporadic disease and pathways to infection are still unclear and a substantial subset of reported cases remain unexplained.

Previous reviews

A search of PubMed and the Cochrane Library revealed five previous systematic reviews which have synthesised evidence on risk factors for infection, all published between 2006 and 2016.

Two reports dealt with only immunocompromised populations: a review of *Cryptosporidium* prevalence in HIV/AIDS patients [36] and another assessing the treatment in immunocompromised patients [37]. A 2006

global review by Gualberto and Heller of drinking water sources found that unboiled water was associated with an increased risk of endemic cryptosporidiosis [38]. Another paper looked at seasonal patterns of five gastrointestinal pathogens together, including *Cryptosporidium*, in the Organisation for Economic Co-operation and Development (OECD) countries [39]. The paper hypothesised that environmental factors, e.g. land use, rainfall, temperature, and host characteristics, e.g. social contact, travel, and animal proximity, were drivers for seasonal patterns of cryptosporidiosis, and this was further buttressed by the existence of comparable evidence from New Zealand for other enteric pathogens [40]. However, these reviews were unable to report results by *Cryptosporidium* species, which may impact on risk factors, or investigate separately sporadic and outbreak-related cases for any variation in associations.

Given the absence of any systematic synthesis of reported evidence in the UK, and the few reviews in the rest of the industrialised countries, the aim of this review is to search the literature, including unpublished work, and describe the purported exposures associated with infection with *Cryptosporidium* in industrialised countries, specifically including the UK. In addition, there may be scope for a meta-analysis to support assessment of the available evidence and to explore differences that may exist in exposures or associations between sporadic and outbreak-related cases.

Research question

In industrialised populations, what exposures are associated with human infection with *Cryptosporidium* and are these different for outbreak-associated and sporadic disease?

Methods

To improve the transparency and completeness of the protocol, a copy of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses for Protocols 2015 (PRISMA-P 2015) [41] checklist can be found in Additional file 1. This protocol is written following this checklist and guidance.

Population

The review will include human populations only.

To avoid missing papers that may be useful to this review, a decision was made not to exclude key at-risk groups, where known, such as HIV/AIDS patients. The wealth of literature available indicates that these are well-studied groups and may act as good sentinels for the identification of transmission risks or pathways for immunocompetent populations. At the data collection and analysis stages, high-risk or highly susceptible populations, where known, can be separated for a more nuanced interpretation.

Searches will be restricted to reports from industrialised countries given that the literature suggests that transmission pathways and exposures, as well as susceptibility of populations, are different between these and countries with less infrastructure [42]. An industrialised country will be defined using OECD category of countries based on membership (Table 1) [43]. Where studies report results from a mix of industrialised and non-industrialised countries, and it is not possible to disentangle outcomes and exposures, the study will be excluded.

Exposure

All exposures, including food, water, animal, environmental, and human, will be considered for inclusion.

Outcome

Primary outcomes will include identifying exposures associated with *Cryptosporidium* infection and/or disease among both sporadic disease and outbreak-related cases. Outcomes among exposed populations will be compared to those in unexposed populations, where the study design allows. We are also interested in capturing molecular detail such as species where possible, as risk factors and exposures may vary.

Inclusion/exclusion criteria (Table 2)

Only studies conducted in industrialised countries (as previously described) and reporting on human subjects will be included. All observational studies will be included where they report risk factors and relevant quantitative results. To allow us to capture the most relevant

Table 1 Current membership—OECD

Australia	Japan
Austria	Korea
Belgium	Latvia
Canada	Luxembourg
Chile	Mexico
Czech Republic	Netherlands
Denmark	New Zealand
Estonia	Norway
Finland	Poland
France	Portugal
Germany	Slovak Republic
Greece	Slovenia
Hungary	Spain
Iceland	Sweden
Ireland	Switzerland
Israel	Turkey
Italy	United Kingdom
	United States

Table 2 Criteria for inclusion in the search

Inclusion criteria	Exclusion criteria
Any language—abstract (if available) in English	Cases known/defined as travel-related/acquired in non-industrialised country
Publication period—any	Individual case reports
Human cases	
All <i>Cryptosporidium</i> sp. including mixed	
Industrialised countries	
Known immunocompromised groups where risk factors are reported	
Known outbreaks	

and robust information on risk factors at a population level, individual case reports will be excluded.

Where the information is clearly communicated, we will exclude information describing cases who acquired their infection in a non-industrialised country and there is no further follow-up, for example, reporting on secondary spread. Where we cannot accurately determine country of infection, these will be excluded.

To capture any changes in incidence and factors associated with *Cryptosporidium* over time, there will be no limitation on publication period. We are also interested in capturing molecular detail, such as species, where possible, as risk factors and exposures for these may vary and this may be pertinent for comparisons of pathways and of value to the knowledge of zoonotic transmission routes.

There are no restrictions on language, provided the abstract can be made available in English for the first round of screening.

Search strategy and terms

Where relevant, methods will follow the recommendations made in the “Cochrane Handbook for Systematic Reviews of Interventions” [44].

The search strategy proposed comprises three approaches, designed to collect as much relevant literature as possible from both peer-reviewed and grey sources.

The choice of databases was following advice from a University of Liverpool Medicine and Dentistry Liaison Librarian, as those deemed to be most relevant to the research question and likely to yield the highest number of relevant papers.

Step one—peer-reviewed literature

One reviewer (CMCK) will conduct electronic searches in the following databases of published literature considered most likely to yield the relevant papers:

- PubMed
- Web of Science
- Scopus
- Embase

The search terms were developed initially for PubMed and piloted in an iterative process ahead of commencing the review to ensure they successfully captured relevant papers. Where possible, terms were exploded to broaden the search. In the review, terms will be adapted as per the functionality of each database.

A more complete documented approach to developing the choices and finalising search terms is available on request.

Terms include the following categories:

- Organism terms: e.g. crypto*, *Cryptosporidium*, cryptosporidiosis
- Population term: e.g. “human”, patients, population,
- Transmission terms: e.g. transmission, risk factor, exposure, sporadic, infection, outbreak(s)
- Outcome terms: e.g. multivariate analysis, odds ratio, risk*, relative risk

Additional file 2 is an example of final search terms used for PubMed.

Search terms will be sought within the title, abstract, and keywords of the documents contained in each database. Filters within the three databases will be applied if required to restrict the results as appropriate according to inclusion criteria.

The publications captured using the final agreed search terms will be exported into reference managing software (Mendeley) and duplicates removed. The remaining publication titles will then be screened for relevance by two reviewers (CMCK and AW), using the inclusion and exclusion criteria.

Step two—hand-searching in papers

Reviewers (CMCK and AW) will search reference lists to identify any further literature or relevant publications not previously captured in the other strategies. The abstracts of any references considered potentially relevant will be sought and screened for inclusion using the inclusion and exclusion criteria.

Step three—accessing grey literature

One reviewer (CMCK) will access grey literature relevant to the review question using published online resources which may include bulletins and reports from relevant agencies, conference proceedings, and other relevant published outputs.

A search of Google Scholar (and any other relevant agencies' sites, e.g. WHO) will be undertaken by entering

the term ‘cryptosporidium’ with ‘risk factors,’ ‘outbreak(s)’; ‘sporadic,’ ‘endemic,’ and/or ‘transmission’ into the application and reviewing the first 100 results for relevance. Using the same search terms and inclusion criteria, the same reviewer will carry out an additional search for unpublished theses work in the ProQuest database.

Abstracts (or relevant variations thereof) will be shared with the second reviewer (AW). Following agreement on inclusion, the work will be reviewed as per protocol.

To refine and clarify the inclusion criteria and search terms and ensure that the criteria can be applied consistently by all reviewers, the selection process will be piloted by applying criteria to a sample of papers.

Abstract and paper selection

Following title selection, abstracts of the final included publications will be screened independently by two members of the review team (CMCK and AW) to ensure consistency in the application of the inclusion and exclusion criteria. Any discrepancies will be discussed and re-examined until an agreement is reached. A third reviewer is available for irreconcilable opinions on inclusion.

The full texts for all included works will be retrieved via the online library where possible and, if required, with the help of the University Liaison Librarian or by contacting authors. All full-text studies will be screened independently by the same reviewers (CMCK and AW) to ensure that they conform to the inclusion and exclusion criteria and discrepancies tackled as before.

Full-text papers which appear in a language other than English will be shared with colleagues in the Health Protection Research Unit (HPRU) and wider university teams for assistance with translation. An online translation tool (Google translate) will be used for initial screening where needed and where electronic papers are available for input.

Searching will cease when no further relevant and/or not previously identified work is being discovered.

Data collection

A standardised data collection form will be developed in Covidence software. Each reviewer will be able to input data and update this as they each extract data from the papers. A minimum dataset of information from each paper will be extracted and recorded in duplicate, by each reviewer and, where information is available, will include variables outlined in Table 3.

Studies will be allocated a unique identifier (automatically generated) and will be categorised according to the following groups:

- Included studies—studies that meet the eligibility criteria and are included in the review
- Excluded studies—studies that do not meet the eligibility criteria and are excluded from the review

Table 3 Minimum data set of information extracted from included papers

Bibliographic detail	Study detail
Name of reviewer	Study design
Date of extraction	Number of cases reported
Publication type	Age/sex cases/participants
Country of origin/language	Case definition (and any known co-infections)
Study title	Definition of exposure(s)
Names of authors	Definition of activities
Journal/source reference	Species identified
Year published	Incubation period
	Exposure window(s)
Study outcomes	General methodological
Number (%) exposed among groups	Confounders
Types of exposures	Likely biases
Comparator(s) (well controls, other infection)	
Selection and recruitment methods	
Availability of appropriate controls (from the same source population as the cases)	
Interview methods	
Effect measures (type and result)	

- Studies awaiting classification—relevant studies that have been identified but cannot be assessed for inclusion until additional data or information are obtained
- Ongoing studies—studies that are ongoing and meet (or appear to meet thus far) the eligibility criteria

Disagreements will be discussed and, if required, rely on the input of a third reviewer as previously described.

Assessing risk of bias

The ROBINS-I tool (Risk Of Bias In Non-randomized Studies - of Interventions) will be used as the framework for assessing quality of the studies. This instrument is well piloted and is specific to non-randomised study types [45]. The instrument provides an overall judgement on risk of bias using signalling questions across seven domains including bias, confounding, and missing data. Following assessment, each reviewer will label a study as 'low', 'moderate', 'serious', or 'at critical' risk of bias.

Strategy for data synthesis

Search results and numbers of titles selected will be presented in the PRISMA 2009 flowchart [46].

In order to accurately report on the content of papers and to explore relationships between disease outcomes and risk factors, data will be summarised in a table presenting the main findings of each paper individually, including population under study, outcomes (infection with *Cryptosporidium sp.*), exposures, and general results (rates, prevalence, number of cases, odds, relative risks). A narrative summary of the characteristics and quality of the papers will also be included, alongside, and in the context of the strength of evidence results from ROBINS-I.

Meta-analysis

A certain level of heterogeneity is expected between studies which may include outcomes measured, population groups, type of study, and measures of association. Following these results, and a discussion about comparability of studies reported, a decision will be made regarding moving forward with a meta-analysis.

Where the populations are appropriate, and study quality allows, data will be pooled in a meta-analysis combining the significant exposures, and categories, across studies and presented as a summary of effects in their individual groupings, for example, ORs or RRs. Forest plots will be created for each exposure category (where paper numbers are high enough to retain validity) and examined to identify heterogeneity. Odds or risk of exposure among cases of *Cryptosporidium* will be presented according to the study design and outcome measured.

The summary measure and I^2 statistic will be used to assess heterogeneity in the studies and will inform the use of meta-analysis techniques and the choice of a fixed or random effects model. Values of 30 to 60%, 50 to 90% and 75 to 100% will be used to denote moderate, substantial, and considerable levels of heterogeneity according to the Cochrane Handbook for Systematic Reviews of Interventions [44].

Data analyses will be carried out using RevMan, MS Access and Stata v12.0.

Data analysis plan

The data analysis will include a description of the cases and putative risk factors/exposures in each study, including the overall proportion of studies which report each exposure and the number of times a transmission pathway or risk factor is associated with illness.

Where possible, analyses of subgroup data may include:

- Outbreak vs non-outbreak disease
- Urban vs rural residence/populations
- Region of world
- *Cryptosporidium* species/genotype (e.g. *C. parvum* and *C. hominis*)
- Age groups of cases/non-cases

- Study design (such as cross-sectional, prevalence studies with risk factors, case-control, cohort, and other observational study designs, outbreak investigations, or surveillance analyses with risk factor information)

Aggregated study data by subgroup will be reported according to data type (e.g. mean and SD and percentages, ratios) and outcome measures (e.g. incidence, odds ratios, and relative risks). Studies will be further grouped by outcome measurement for consistency; studies reporting odds ratios will be aggregated separately to those reporting relative risk, for example. Exposures will be defined as per the paper under review, but where possible, they will be grouped into categories to allow for meaningful exposure group analyses. Categories are likely to include environmental exposures, water, animal exposures, exposure to a case, etc., and may also, where possible, include settings such as home, hospital, or nursery. Where data and number of papers allow these will be sub-grouped as much as possible.

Where data are missing or not reported in disaggregate form, the authors may be contacted in order to assist with further analyses. If the data allow, a more granular grouping of the studies may be undertaken to accurately address the research question.

Interpretation of findings

Given that we have not included any element of study design as part of the selection criteria for inclusion, interpretation of findings will begin with a description of the publication bias funnel plots where numbers of papers are sufficient. Discussions will include an exploration of all the strengths and weaknesses of the studies and a summary of the quality of evidence, using the Grading of Recommendations, Assessments, Development and Evaluation approach [47]. Most of the initial studies will likely be classed a priori as 'low' due to being observational in nature but may be upgraded after assessment of various domains of the tool, including bias, effect size, and precision. Papers will then be assigned a final grade for the quality of evidence as 'high', 'moderate', 'low', or 'very low' for all the critically important outcomes. Results will be reported using summary tables.

Interpretations of measures of effect may be stratified by study quality, and aggregated analyses of measures of effect will be assessed in the context of the populations under study.

Dissemination

The protocol and the report will be prepared for peer-review publication.

The review will form part of a larger project submitted in partial fulfilment of a Doctor of Philosophy degree at the University of Liverpool.

Where appropriate, data may be presented as conference proceedings.

Discussion

Many of the putative risk factors for cryptosporidiosis can have high exposure proportions and cases often report multiple risk factors, so well-designed studies are key in trying to elucidate clear pathways for transmission. More accurate understanding of the drivers behind continued apparent sporadic cryptosporidiosis has implications for public health intervention, control, and targeted treatment. This systematic review aims to describe the epidemiology and transmission of *Cryptosporidium* infection in industrialised countries, with particular reference to how this may apply to the UK. In addition, the review will seek to describe differences between outbreak and sporadic cases, investigating changes in prevalence and patterns among species and subtypes over time, and explore mechanisms for transmission of disease.

The results of this review will help support current knowledge and add to the evidence base on transmission pathways and risks for cryptosporidiosis, identifying those vulnerable and highlighting pathways where interventions may be of use.

The review will also help inform the development and direction of an analytical study as part of a PhD project.

Additional files

Additional file 1: PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol. (PDF 170 kb)

Additional file 2: Search terms. (PDF 108 kb)

Abbreviations

AIDS: Acquired immunodeficiency virus; GRADE: Grading of Recommendations, Assessments, Development and Evaluation; HIV: Human immunodeficiency virus; NIHR: National Institute for Health Research Health Protection Research Unit; OECD: Organisation for Economic Co-operation and Development; OR: Odds ratio; PHE: Public Health England; PHW: Public Health Wales; PRISMA-P: Preferred Reporting Items for Systematic Reviews and Meta-Analyses for Protocols; ROBINS-I: Risk Of Bias In Non-randomized Studies - of Interventions; RR: Relative risk/risk ratio; UK: United Kingdom

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Authors' contributions

All authors conceived the initial idea for the study. CMCK wrote the protocol. RChR, RChA, RV and SOB reviewed and revised the protocol and paper. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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BMJ Open Cross-sectional investigation of household transmission of *Cryptosporidium* in England and Wales: the epiCrypt study protocol

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ABSTRACT

Introduction Infection with the *Cryptosporidium* parasite causes over 4000 cases of diagnosed illness (cryptosporidiosis) in England and Wales each year. Risk factors are often estimated from outbreak investigations, and in the UK include ingestion of contaminated water and food, farm/animal contact and person-to-person spread in institutions. However, reported outbreaks only represent about 10% of cases and the transmission routes for sporadic disease may not be the same. Contact with other people has been highlighted as a factor in the transmission of *Cryptosporidium*, but the incidence of sporadic disease has not been sufficiently established, and how frequently this arises from contact with other infected people is not well documented. This project will estimate the amount of secondary spread that occurs in the home and potentially identify asymptomatic infections which might have a role in transmission. Risk factors and characteristics associated with secondary spread will be described including any differences in transmission between *Cryptosporidium* species.

Methods and analysis The study will prospectively identify cryptosporidiosis cases from North West England and Wales over 1 year and invite them and their household to take part. Each household will complete a questionnaire and each household member will be asked to provide a stool sample. Clinical, demographic and home variables will be described, and further analyses undertaken to investigate associations with secondary spread in the home. *Cryptosporidium*-positive stool samples, identified by immunofluorescence microscopy, will be characterised using molecular methods to describe patterns of transmission. Data collection is expected to take 1 year, beginning in September 2018.

Ethics and dissemination The study has been approved by the North West–Liverpool East NHS Research Ethics Committee (Reference: 18/NW/0300) and the Confidentiality and Advisory Group (Reference 18/CAG/0084). Outputs will include scientific conferences and peer-reviewed publications. In addition, a short, lay report of findings will be produced for participants, who can opt to receive this when they take part.

Trial registration number CPMS ID: 39458.

INTRODUCTION

Cryptosporidium is a protozoan parasite which can infect humans and other animals, and

Strengths and limitations of this study

- This prospective household study will provide detailed information on the incidence of, and risk factors for, secondary spread of cryptosporidiosis in the home.
- This study will characterise *Cryptosporidium* isolates to ascertain likely mechanisms of spread by species.
- This study will potentially identify the prevalence of asymptomatic infections with *Cryptosporidium*.
- Common exposures across households may present problems with accurately identifying true secondary spread.
- Biases may lead to a skewed sample of index cases because of under-ascertainment and bias in health-seeking behaviours.

the most prevalent species identified in humans are *Cryptosporidium parvum* and *Cryptosporidium hominis*.^{1 2} Cryptosporidiosis is the subsequent diarrhoeal disease following infection with *Cryptosporidium*. The disease affects all ages and although generally self-limiting, can be life threatening in some immune-compromised patients. Acute diarrhoea follows an incubation period of between 2 and 10 days (mean 7 days) and symptoms can include non-bloody diarrhoea, abdominal cramps, vomiting and/or nausea, low grade fever, lethargy and general malaise.

Public Health England (PHE) receive laboratory reports of over 4000 diagnosed cases per year (2000–2012 data) in England and Wales; however, research indicates that many infections may go undiagnosed, and the true incidence of disease may be much greater.^{3 4}

The parasite has a complex life cycle and characteristics which favour faecal-oral and environmental transmission routes, which may facilitate outbreaks via person-to-person (*C. hominis* and *C. parvum*) or animal-to-person

(*C. parvum*) contact, as well as indirect transmission through ingestion of water and food contaminated with infectious oocysts.⁵

Risk factors and associated exposures are often hypothesised/identified from outbreak investigations, however recognised outbreaks may only represent a small proportion of cases; estimates in the UK suggest, of all cases reported to national surveillance, <10% are likely to be linked to an identified outbreak⁶ and contact with other people is highlighted as a factor in the transmission of *Cryptosporidium*. In a 1988 paper, onward transmission of *Cryptosporidium* was reported in households in the UK following a nursery outbreak, probably propagated by person-to-person spread in the home.⁷ An analysis of outbreak reports from surveillance data in Ireland reported that ingestion of water and person-to-person spread were the most important mechanisms of transmission in outbreaks.⁸ In the USA, in a case-control study evaluating sporadic cryptosporidiosis among immunocompetent persons, risk factors associated with increased odds of being a case were international travel, contact with cattle and contact with a child with diarrhoea.⁹ In 2001–2002, a case-control study conducted in the North West of England examined species-specific risk factors for sporadic cryptosporidiosis.¹⁰ The authors compared risk factors for infection with genotypes 1 and 2 (currently recognised as *C. hominis* and *C. parvum*, respectively) and found that contact with another person with diarrhoea was a risk factor for infection with *Cryptosporidium*, and that changing children's nappies was a specific risk factor for infection with *C. hominis* whether the child was symptomatic or not symptomatic. Studies of *Giardia*, another gastrointestinal parasite, similar in terms of likely transmission routes, have recently been undertaken in the UK, and secondary spread and person-to-person transmission seems a likely and under-recognised route of transmission.^{11 12} In a 1988 paper, onward transmission of *Cryptosporidium* was reported in households in the UK following a nursery outbreak, probably propagated by person-to-person spread in the home.⁹ An analysis of outbreak reports from surveillance data in Ireland reported that ingestion of water and person-to-person spread were the most important mechanisms of transmission.¹⁰ In the USA, a study evaluated sporadic cryptosporidiosis among immunocompetent persons using a case-control design. Risk factors associated with increased odds of being a case were international travel, contact with cattle and contact with a child with diarrhoea. In 2001–2002, a case-control study conducted in the North West of England examined species-specific risk factors for sporadic cryptosporidiosis. The authors compared risk factors for infection with genotypes 1 and 2 (currently recognised as *C. hominis* and *C. parvum*, respectively) and found that contact with another person with diarrhoea was a risk factor for infection with *Cryptosporidium*, and that exposure through changing children's nappies was a specific risk factor for infection

with *C. hominis* whether the child was symptomatic or not symptomatic.

Asymptomatic spread

The burden of asymptomatic infection is less well documented in *Cryptosporidium* research than for other infections but may be an important factor in household spread. A study in the UK reported a point prevalence of 1.3% among asymptomatic pre-school children attending daycare¹³ suggesting that asymptomatic infection does occur. A Norwegian study looking at follow-on spread after two outbreaks found both asymptomatic and symptomatic infections in the households, which were likely to have been a result of secondary transmission.¹⁴ Overall though, most of the work examining household spread has been undertaken in *Cryptosporidium*-endemic countries, where a high prevalence and repeated exposure to the organism might facilitate transmission, although immunity following repeat exposure is still poorly understood.^{15 16} Newman *et al* undertook a prospective cohort study in Brazil to examine the transmission of *Cryptosporidium* infection in households where there was an identified case.¹⁷ Secondary cases of infection occurred in 58% of households, and around a quarter of the identified secondary cases had diarrhoea, indicating the presence of asymptomatic infection in almost three-quarters of the participants. Similar results were reported from a longitudinal study in Bangladesh, where asymptomatic infection was more prevalent than diarrhoeal disease.¹⁸ The same authors followed up with a case-control study in which the secondary attack rate was over 35%, and evidence of transmission in the home was further supported by genotyping results.¹⁹ If person-to-person spread is driven by both cases and those with asymptomatic infections of *Cryptosporidium*, then sporadic cases may subsequently arise following exposure to either, and outbreaks in close settings such as the home or institutions may happen more frequently than is currently recognised. Few studies exist which refute or confirm this, especially in industrialised countries.

AIMS AND OBJECTIVES

The aim of this study is to estimate the amount of onward spread of *Cryptosporidium* that happens in the home, and to describe associated factors and case characteristics. (We use the term 'secondary spread' to mean any apparent onward transmission of disease originating from a case, while recognising that this may be secondary or even tertiary levels of spread.) This study will support our understanding of continued apparent sporadic cryptosporidiosis in England and Wales and has implications for appropriate public health messages to help mitigate spread and infection. Further molecular characterisation of *Cryptosporidium* isolates may also help define the likelihood of secondary transmission by infecting species.

Objectives

- ▶ To estimate the number of secondary cases in households with an index case.

- ▶ To calculate the secondary transmission rate in households.
- ▶ To estimate the prevalence of asymptomatic carriage in households with an index case.
- ▶ To identify specific household-level and personal characteristics associated with secondary spread.
- ▶ To determine if factors and characteristics associated with secondary spread vary by species of *Cryptosporidium*.

METHODS

Study population

The study population will comprise residents of North West England and Wales.

The North West of England has a population of over seven million people and is the third-most populated region in the UK.²⁰ In 2016, over 600 laboratory-confirmed *Cryptosporidium* isolates were reported from the North West (8.4/100 000 population).²¹

Wales has a total population of over three million people.²² In 2016, over 400 laboratory-confirmed *Cryptosporidium* isolates were reported from Wales, the highest rate of *Cryptosporidium spp* laboratory reports per 100 000 population in England and Wales (15/100 000).²¹

Surveillance/sampling frame

The sampling frame will be taken from the two relevant surveillance systems which capture laboratory confirmed reports of *Cryptosporidium*: The Second-Generation Surveillance System in PHE, and Tarian in Public Health Wales (PHW). Systematic national surveillance of laboratory confirmed *Cryptosporidium* in England and Wales has been established for many years.²³ In the UK, *Cryptosporidium* is a notifiable causative agent, meaning laboratories have a statutory duty to notify the relevant public health authority of its identification in any human samples.^{24 25} Cryptosporidiosis may present similarly to other causes of gastroenteritis, and laboratory confirmation of infection with *Cryptosporidium* is necessary for a diagnosis. Clinical practice may differ, and clinicians would likely submit a sample to a primary diagnostic microbiology for a diagnosis of gastroenteritis. Local diagnostic laboratories across the UK use different methods to test for *Cryptosporidium*, and various criteria to decide whether to test for this parasite, including stool consistency, history or clinical details, duration of hospitalisation or clinician requests.²⁶ Positive samples identified in the diagnostic laboratories are routinely forwarded to the national *Cryptosporidium* reference unit (CRU) which provides expert management, prevention and control advice as well as *Cryptosporidium* typing and confirmation services for speciation and surveillance.²⁷

All cases of laboratory confirmed *Cryptosporidium sp.* reported from primary diagnostic microbiology laboratories in North West England and Wales, in the study year, will initially be eligible.

Study type

The identification of cases, and their subsequent recruitment, is cross-sectional, although the study also involves

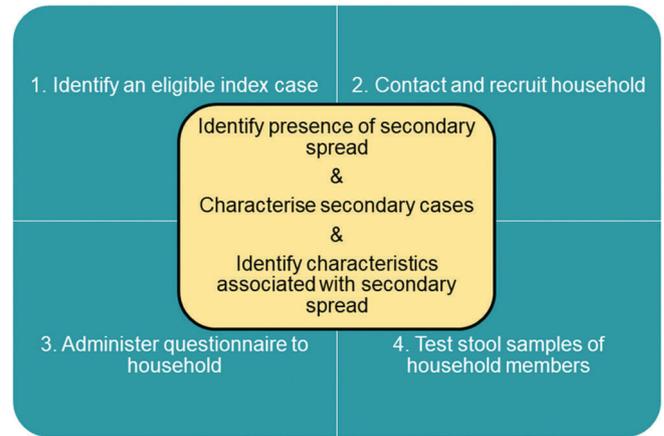


Figure 1 Outline of study.

retrospective data collection and some prospective sampling (figure 1).

Cases of cryptosporidiosis will be identified via the relevant surveillance system(s). Once participants are recruited, they will complete a questionnaire (one per household), collecting clinical (onset date, symptoms of any household member, other illnesses of index case), demographic (age, sex, relationship to the index case) and household composition (type, number of bathrooms and bedrooms, animals) information. In addition, consenting household members (excluding the index case) will be asked to supply a stool sample.

Study period

The study period will be 12 months, to account for seasonal variation and allow maximum enrolment, up to 400 households. The study is expected to begin with a pilot phase of 1–2 months in autumn 2018.

Sample size

Given that the North West & Wales report around 1000 cases per year (PHE data, 2015) and assuming a participation rate of 40%–60%, and some exclusions (based on similar studies/approaches^{11 28}) we anticipate a sample size of 400 households. Using 2011 Census indications of 2.4 persons on average per household,²⁰ we can expect to recruit around 960–1000 participants in total. Assuming that the rate of household transmission, defined as the proportion of households with more than one case, is between 0% and 20%,^{11 14 17 29–31} a range of sample sizes was estimated (118–402). Recruitment of 400 households is feasible and is sufficient to allow us to demonstrate a statistically significant minimum odds/risk ratio of 2.0, with type 1 error 0.05 and type 2 error at 0.20.

Case definition(s)

Boxes 1 and 2 outline case and household definitions used to categorise household members and define secondary transmission.

Box 1 Case definitions**Index case**

The first case from a household identified in the surveillance system (person reported to a Public Health England/Public Health Wales surveillance system(s) following detection of *Cryptosporidium* sp. in a faecal sample, with a specimen date in the study year). The first case from a household identified in the surveillance system (person reported to a Public Health England/Public Health Wales surveillance system(s) following detection of *Cryptosporidium* sp. in a faecal sample, with a specimen date in the study year).

Household case

Any household member of an index case who reports symptoms consistent with *Cryptosporidium* (diarrhoea and/or vomiting) and/or has a *Cryptosporidium* positive stool sample. Any household member of an index case who reports symptoms consistent with *Cryptosporidium* (diarrhoea and/or vomiting) and/or has a *Cryptosporidium* positive stool sample.

Secondary case(s)***Probable secondary case**

A person in a household of an index case, with symptoms: of diarrhoea and/or vomiting

AND

that started after another case's onset date in the household.

Confirmed secondary case

A person in a household of an index case, with symptoms: of diarrhoea and/or vomiting

AND

that started after another case's onset date in the household

AND

a *Cryptosporidium* positive stool sample.

Asymptomatic

A person in a household of an index case with: no reports of gastrointestinal illness

AND

a *Cryptosporidium* positive stool sample.

*We use the term 'secondary spread' to mean any apparent onward transmission of disease originating from an index case, while recognising that this may be secondary or even tertiary levels of spread.

Recruitment approach**Overview and rationale**

Cases of cryptosporidiosis are identified from routine surveillance (from diagnostic laboratories) and are contacted via post, by the relevant public health organisation, in the first instance. Following this, if they do not opt-out, they are contacted via telephone by a National Health Service (NHS) research nurse at the local Clinical Research Network (CRN) to chat about the study and determine if they would like to take part.

Our approach to the recruitment process was driven by necessity and feasibility and we explored several options at the protocol drafting stage of the project, balancing data needs with patient choice. As our capture of cases in the surveillance systems is retrospective and diagnosis of *Cryptosporidium* in the stool sample is undertaken by laboratory staff, there is no opportunity to consent individuals at the time of diagnosis and the recruitment process could not be achieved without access to patient

Box 2 Household definition**Household**

Two or more people (not necessarily related) living at the same address in North West England or Wales who share cooking facilities and share a living room or sitting room or dining area.¹⁸ Two or more people (not necessarily related) living at the same address in North West England or Wales who share cooking facilities and share a living room or sitting room or dining area.¹⁸

Household member

A person who normally resides in the household and regularly shares food or toilet facilities.³⁸ A person who normally resides in the household and regularly shares food or toilet facilities.³⁸

Household contact

A household member where an index case has been identified. A household member where an index case has been identified.

Household with transmission

A household that has more than one case. A household that has more than one case.

Household without transmission

A household that has one case (the index case). A household that has one case (the index case).

information. In our model, participants are given opt-out options at each contact and it is emphasised that they can withdraw at any time. Previous research supports the acceptability and understanding of this method, recognising that an approach of 'consent for each use' is burdensome for both researcher and participant,^{32 33} as does patient response and engagement with similar studies. (Studies recruiting based on disease surveillance are common for GI infections, and many projects have taken this approach – the methodology for the epiCrypt Study has been influenced by design aspects of large-scale studies such as Enigma, IID2 and Integrate.)

Public and patient involvement

Patients and public were not involved in the overall design of the study, but we did elicit some public opinion when finalising our approach to recruitment. Following valuable comments from the ethical review board we undertook a short survey among the public and specific Patient and public involvement (PPI) groups to gauge general attitudes toward accessing data prior to consent, to support recruitment to research. We drafted a survey which outlined the approach to recruitment and the framework of the study. We accessed a lay PPI group from the Infection and Global Health panel at the University of Liverpool, and one from Health and Care Research Wales. Participants were asked to think generally about the method of recruitment and how they felt about this approach. In general, the feeling was that it is acceptable to access data for recruitment, especially to support much needed research. However, considerations and worries included the person accessing data, with NHS/public health staff generally viewed as more favourable than non-NHS (eg, university) researchers.

Identification and first contact with the index case

Laboratory diagnosed reports of *Cryptosporidium*, and the corresponding patient contact details, will be extracted from the relevant surveillance system by health protection staff and saved in a line list (the Master copy of a confidential, separate table (MS Excel spreadsheet) holding patient details of all downloaded cases).

All potentially eligible participants will be issued a unique sequential study ID by PHE/PHW staff. This will be on all relevant study documentation and stool pots and follow each person and household through the study journey. This will allow data to be linked pseudonymously and helps with data management.

Staff from either PHE or PHW (depending on case location) will send an invite letter through the post to these potentially eligible index cases. The invite letter outlines the study, describes why the case has been contacted and explains that a research nurse may be in touch over the coming weeks to discuss the study. The letter allows the case to choose several ways of opting-out of this contact (email, freepost, telephone) and provides a named, clinical study lead for each public health organisation should they wish to discuss any aspect of this.

Approaching to recruit

If a contacted index case does not opt-out within 2 weeks, their details will be shared securely (using internally agreed practices) with the NHS research nurses at the CRN North West Coast. The research nurses will attempt to contact the index case (or parent/guardian of) via telephone (using internally agreed practices) to inform them about the study and offer them the opportunity to participate, if eligible. A maximum of three attempts will be made, and nurses will not leave voicemails. If a case is unable to be contacted, or does not wish to participate at this stage, their details will be deleted from the line list. If the approached index case is successfully contacted via telephone and interested in participating, or would like more information, the research nurses will prepare and post a study pack. Index cases may be excluded at this stage where discussions with the case reveal that any of the following exclusion criteria apply:

- ▶ Index case is in a single-person household.
- ▶ Index case is a visitor to a household in the study area, but is registered with a general practice (GP) outside the study area.
- ▶ Household is outside the study area.
- ▶ The index case is resident in an institution: retirement home, nursing home, prison, barracks, boarding school or college/university halls of residence.

Study packs

The study packs contain:

- ▶ A study information pamphlet.
- ▶ A questionnaire booklet for the index case or a suitable representative (eg, parent, head of household) to complete, with a freepost envelope.

- ▶ A consent form for each participating household member to read, initial and sign (forms part of the questionnaire)).
- ▶ A stool sampling pack (Fe-Col) for each participating household member, with the required return postal envelope.
- ▶ An information leaflet on cryptosporidiosis and the relevant health advice.
- ▶ An information sheet on General Data Protection Regulation for health and care research.

Consent

The index case and any household members who wish to take part will sign and return the consent form at the front of the questionnaire. The return of study materials such as a completed questionnaire and/or stool samples will be considered implied consent.

Disenrollment

If study materials are not received within 14 days of posting the pack, a reminder letter will be dispatched by the research nurses at the CRN. If study documentation is not returned within 14 days of posting the reminder letter, no further attempt at contact will be made, and the index case will be removed from the study line list.

Participation

If the household wants to take part, all interested members will sign and return the consent form. At least one household member, as well as the index case, must consent.

Consent forms and questionnaires are returned to the University of Liverpool in a stamped addressed envelope provided in the pack. The unique study IDs of those consenting will be shared weekly with the research nurses at the CRN to cross-match those contacted index cases that have been recruited and enrolled. Each consenting household member will also be asked to provide a stool sample for testing, using the provided Fe-Col kits, which include a pre-addressed and secure postal bag (compliant with UN3373 regulations for mailing Cat B biological samples³⁴). Instructions are provided, and samples will be returned directly to the CRU.

Data management and oversight

Documentation

Questionnaire data will be inputted from the paper format to a corresponding MS Access database and held securely on a University of Liverpool drive in accordance with their security protocols. **Figure 2** shows the data flow expected. Double data entry will be undertaken on a sample of questionnaires and discrepancies resolved using internal validation checks. When data entry is complete the data will be exported to the final study database (MS Access) where the data are pseudonymised for analyses: Name, date of birth and full postcode will be removed and replaced with unique study ID, age and Lower Super Output Area (a type of geographic area in England and Wales, comprised >1000 residents³⁵).

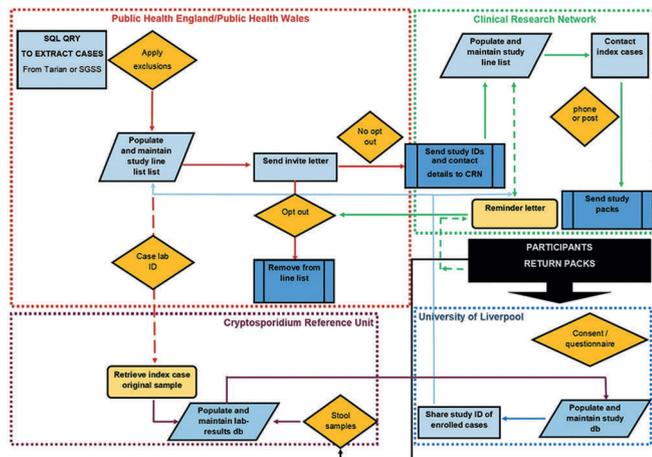


Figure 2 Study flow diagram.

All data storage, cleaning and analyses will be undertaken by the study team at the University of Liverpool. Data will be stored on institutional network drives with appropriate security measures in place. Hard copy records will be stored in a locked cabinet in a secure location and access to records and data are limited to study personnel. Paper documents will be stored separately from the corresponding electronic data. The sponsor and data controller for this study is the Clinical Governance Team at the University of Liverpool.

Data security protocol available on request

Stool sample management

For purposes of data confidentiality and governance, stool samples returned to the CRU are pseudonymised with the unique study ID, and participants will be asked to write their age, sex and date of sample on their sample pot before they collect the stool. Stool sample results will be added to a study-specific database held at the CRU. The stool sample results will be added to the study database at the end of data collection using unique study ID in a secure file transfer.

Identifying the index case samples

Original diagnostic laboratory numbers will be retained with the index case information in the original line lists at PHE/PHW so that the sample can be identified later at the CRU and grouped with the relevant household samples. This sample, when located at the CRU, will be processed in the same way as other study samples.

Table 2 An example table showing information collected in the questionnaire about activities

Activity	First case	Anyone else in the home
Travel outside the UK		
Swimming— outdoors in a lake, river, stream, etc (wild swimming)		
Swimming— in a treated swimming pool, either indoors or outdoors (such as a pool at a leisure centre or a lido)		
Other water activities/sport (such as surfing, rowing, water-skiing, etc)		
Other outdoor activities (such as camping, climbing, hiking, cycling, etc)		
Gardening (at home or elsewhere, such as an allotment)		
Contact with pets (at home or with pets at another house)		
Visiting or working on a farm or had contact with farm animals		
Visiting or working at a zoo or had contact with zoo or wild animals		

Full laboratory protocol available on request

Outcomes and measurements

Questionnaire data

The questionnaire is divided into sections and is mostly composed of dichotomous and multiple-choice questions. Section A asks questions to determine the composition of the household, the clinical details of the index case, and captures any other symptomatic household members. A table is used to collect information on any other symptomatic diarrhoeal illness in the house and will attempt to capture relationships to the index case (table 1).

Section B records activities of the index case, and others in the home, in the 2 weeks prior to the index case’s onset, based on known exposures for *Cryptosporidium*. Information on outdoor and leisure activities may help determine possible co-primary infections and distinguish them from those that are secondary (table 2).

Sections C and D collect household variables, including the number of bedrooms and bathrooms, and capturing those who share beds/baths, and asking about outside space and animals. We also ask about nappy changing and toilet training in the home, and about general hand-washing behaviour.

Table 1 An example table showing information collected in the questionnaire about other illness in the house

Age	Sex	Relationship to first case	Been ill with diarrhoea and/or vomiting (Yes/No/Don’t know)	When they became ill (Date if known, otherwise before/after the first case)	How many days were they ill with these symptoms?	Did they see a doctor about this illness?
39	F	Mother	Yes	18/12/2017	10	No
42	M	Father	Yes	Before	About 3 days	No
6months	M	Brother	No	–		
24	F	Lodger/housemate	Don’t know	–		



Figure 3 Fe-Col kit.

Full questionnaire available on request

Stool collection and genotyping

All consenting household members of the index case (but not the index case) will be asked to provide a stool sample using the Fe-Col kit (figure 3) provided in the study pack, and post to the CRU.

The stool pots will be labelled with the unique household ID which identifies them as part of the study but allows the samples to remain anonymous to the reference laboratory team. Samples will be scored against the Bristol stool scale and tested and quantified, only for *Cryptosporidium*, using immunofluorescence microscopy and real-time PCR. Positive samples will be speciated using validated PCR techniques which are part of normal practice.³⁶ *Cryptosporidium* DNA will be retained for subtyping and possibly whole genome sequencing at a later date.

Full laboratory protocol available on request

Analyses

The primary objective of this study is to determine the amount of spread that happens in the home where there is a case of *Cryptosporidium*. This will be established by testing stool samples of household members of a case for *Cryptosporidium* and reporting the numbers of other cases (according to our predetermined definitions in box 1). A household with more than one case of any type will be a household with transmission. As we are only able to capture cases of *Cryptosporidium* using laboratory confirmed cases reported to surveillance, we recognise that what we may define as an ‘index’ case, may not be, in true epidemiological terms, the first case that has driven transmission. While it is important that cases are identified and recruited based on the same diagnostic criteria, we accept that the identification of index cases is a pre-enrolment definition. Following enrolment of the

household into the study, and the return of documentation, an index case may be categorised differently, and may actually fit the definition for a secondary case. This will be analysed at the household level and depends on the accurate population of fields in the questionnaire. In doing this, we are able to more accurately describe transmission in the home, and this may well allow us to describe the characteristics of these true index cases, and why we do not pick them up in surveillance, for example, if they exhibit different health-seeking behaviour.

We will calculate the following:

- ▶ The secondary transmission rate/prevalence within households (number of cases in the home/numbers at risk in the home, number of households with secondary spread/number of households)).
- ▶ The amount of asymptomatic carriage among those exposed to symptomatic cases (number of asymptomatic cases/number at risk)).
- ▶ OR/RR of secondary illness according to activities and case/household characteristics;
- ▶ OR/RR of secondary illness according to organism species.

Confounding (eg, host factors such as age, comorbidity) will be considered, where known, using multivariable techniques. Also, we will, where possible, examine environmental level exposures using stratification, for example, those households/cases which are exposed to other known sources or risk factors, such as those living on farms.

Data will be analysed using Stata V.12.

Limitations and biases

Some elements of the study design are retrospective in nature, as the index case must have already been ill and been tested in order to be selected. As a result, some ascertainment bias may lead to a skewed sample from which to choose the index cases. We do not expect to capture the full profile of cases and households in the population that might have *Cryptosporidium* due to differences in risk or vulnerability, severity or health-seeking behaviour.³⁷ We may get an over-representation of severe disease as these cases are more likely to seek healthcare and be tested, and perhaps more likely to test positive.

We are only collecting one sample from each household member, and not re-sampling the index case, for time and resource reasons. This may well lead to missing intermittent shedding of oocysts, tertiary household infections and/or misclassifying recurring illness.

As *Cryptosporidium* is common in younger age groups, we expect a large proportion of the participants to represent families with young children which may lead to over-representation of these households. In addition, we might expect that having young children who were ill, or being severely ill themselves, may incentivise cases to participate in the study, more than adult, less severe cases.

Any likely over- or under-representation in the data collected will be considered when assessing and describing results. Further unidentifiable limitations may include

recall biases around dates of onset or activities, and classification biases as we are asking about self-reported illness and information may be inaccurate.

There is a possibility that we could see ongoing outbreaks in the study year. If this happens, we might try to identify these where possible and may consider excluding households from this study where all or most members have been exposed, including a definition of a co-primary case.

End of study

The study will be declared as ended when the database is closed to recruitment—after 1 year or when the maximum number of households has been enrolled.

Pilot arrangements

A pilot phase of 1 month is anticipated before data collection begins to assess and evaluate processes. Pilot data will be included as study data if no major methodological changes are proposed.

Ethics and dissemination

The study has been approved by the North West – Liverpool East NHS Research Ethics Committee (Reference: 18/NW/0300) and the Confidentiality and Advisory Group (Reference 18/CAG/0084). The project is registered on the National Institute for Health Research portfolio (CPMS ID: 39458).

Outputs will include scientific conferences and peer-reviewed publications. In addition, a short, lay report of findings will be produced for participants, who can opt to receive this when they take part.

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Competing interests None declared.

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The epiCrypt study

Investigating how *Cryptosporidium* infection spreads at home

How to collect and send a stool sample for laboratory tests

Your stool sample pack contains:

A Fe-Col kit - A stool pot with a blue lid, inside a strong plastic container

Instructions

Postal bag

Gloves

The stool pot (blue top) has a small plastic spoon fitted to the underside of the lid and can be found inside the strong plastic container.

Your household study ID will be written on the stool pot.

Please write the age and sex of the person providing the sample, and the date the sample is collected, on the label on the pot. Do this before collecting the sample.

Use a clean toilet which has been well flushed. Do not allow toilet cleaner or disinfectant to come into contact with the stool sample.

Follow the instructions provided in the kit. Try to get at least three or four scoops – but do not worry if you are under or over that! Whatever you can manage is fine.

To collect from a nappy, use the plastic spoon fitted to the container lid to scoop some poop out of the nappy and into the stool pot.

Once the sample has been taken, make sure the cap is screwed tightly onto the stool pot. Just leave the spoon attached.

Wash your hands thoroughly, using soap and running water, then dry well.

Put the stool pot into the strong, plastic container, then into the prepaid postal bag.

Only one sample should be added to each envelope.

Please ensure that the postal bag is securely closed.

Post the sample as soon as possible on the same day. If you are unable to do this, keep the package in a cool place (but not your fridge) and post as soon as possible the next day.

Thank you for helping us with our research!

General Data Protection Regulation for health and care research

The epiCrypt Study is sponsored by the University of Liverpool.

As a University we use personally-identifiable information to conduct research to improve health, care and services. As a publicly-funded organisation, we have to ensure that it is in the public interest when we use personally-identifiable information from people who have agreed to take part in research. This means that when you agree to take part in a research study, we will use your data in the ways needed to conduct and analyse the research study. Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally-identifiable information possible.

Health and care research should serve the public interest, which means that we have to demonstrate that our research serves the interests of society as a whole. We do this by following the [UK Policy Framework for Health and Social Care Research](#).

The University of Liverpool takes great care to abide by our legal and moral obligations when handling your personal and healthcare data. Due to changes introduced in the EU General Data Protection Regulation (GDPR), we are writing to provide you with information on the lawful basis on which we are processing your data. The lawful basis for the processing of your personal data for the research study which you have participated in is a task in the public interest.

The data you have provided for the epiCrypt Study will be stored for six years. You are free to withdraw your consent for your data to be collected, processed, or stored at any time. However if the data has already been anonymised it will not be possible to withdraw your data.

We will not share your data unless you have provided explicit consent for us to do so.

The data controller for this study is the University of Liverpool, Research Support Office, 2nd Floor Block D Waterhouse Building, Liverpool L69 3GL

Tel: 0151 794 8373

Email: sponsor@liverpool.ac.uk

The University Data Protection Officer, Mrs Victoria Heath, can be contacted on 0151 794 2148.

The University strives to maintain the highest standards of rigour in the processing of your data. However, if you have any concerns about the way in which the University processes your personal data, it is important that you are aware of your right to lodge a complaint with the Information Commissioner's Office by calling 0303 123 1113.

The epiCrypt study

Investigating how *Cryptosporidium* infection spreads at home

INFORMATION SHEET

Before you decide whether you wish to take part, please take time to read the following information carefully. Ask us if there is anything that is not clear or if you would like more information, and feel free to discuss it with your friends and family.

Who are we?

We are a team of researchers from the University of Liverpool, Public Health England, and Public Health Wales who are interested in finding out more about the spread of tummy bugs.

This study is being done to find out more about a common tummy bug called *Cryptosporidium*.

What are we doing?

Some people pick up the bug and feel fine. Other people can feel poorly for some time. We want to find out if *Cryptosporidium* spreads between people in the same house, and look for clues as to how this might happen. This will help us to stop others getting sick.

Why are we doing this?

Cryptosporidium is important because it affects many people, especially children, and some people may get very sick. Sometimes many people are affected at once, which is what we call an 'outbreak'.

Why have we chosen your household?

You or your child have recently had *Cryptosporidium*. We are asking everyone who lives in the North West of England or in Wales, who has recently had the *Cryptosporidium* bug, to take part with their household. We would like to test all the people in the household to see if anyone else has had it, even if they haven't been sick.

What will we want you to do?

We will ask you to read all the information we have given you and then sign the consent form. There is also a simple questionnaire to fill out – only one person needs to do this – asking some general questions about your household.

We would then like each person in the house to collect a sample of their poo and send it back to the lab to be tested, even if they haven't been sick. We will provide instructions, and stamped addressed envelopes.

It will not cost you anything to take part.

Do I have to take part?

It is completely up to you and your household to decide whether to take part. If you do decide to take part you are still free to change your mind later without giving a reason. Whatever you choose will not affect your medical care or legal rights. If some household members do not want to take part but others do, that is also fine. You can contact us if you want to chat about any of this.

How much time do I have?

If we do not get the documents back after two weeks, we may call or write to you again to remind you. If you do not get back to us after that, we will not try to contact you again.

What about my information?

There are strict laws that make sure we treat your information in confidence, in the same way as any other medical information. Only members of the small study team will know your details. When the results are written up, all personal details will be removed so you cannot be identified.

Can I change my mind?

Of course! You can change your mind at any time and just let us know – your details will be removed. You do not have to give a reason and it will not affect your care, or how any medical professional treats you. We will not tell anyone if you change your mind.

Has this study been approved?

This study has been ethically reviewed and approved by the Health Research Authority (HRA) and NHS/HSC R&D for Wales. The HRA publishes summaries of research approved by Research Ethics Committees in the UK and you can find these online at www.hra.nhs.uk. Our approval number is 18/NW/0300.

What happens after the study?

We hope that the results we find will help us improve the information and advice we give to people who get *Cryptosporidium* and stop more people getting sick.

The results will be put into reports and published in scientific journals and at conferences. In this way, other doctors and scientists can share the information and make recommendations to health organisations and the public.

We are not able to return individual sample results back to participants, but please read the information leaflet provided – this advises what you should do if you feel ill or if you are not getting better. You can get a summary of the research when we are finished – just tick the box on the questionnaire.

Who is paying for the study?

The research is being funded by the National Institute for Health Research (NIHR).

What if I have questions?

If you have any questions or comments about the study you can call us on 0151 795 8334 or e-mail us at c.mckerr@liv.ac.uk and speak to The epiCrypt Study team.

What happens next?

1. Your study pack includes this document, called an INFORMATION SHEET

Please make sure you have read it and you are happy to take part. Discuss this with others, and share it with the others in your home.

Make a note of our contact details in case you want to chat over anything.

2. Your study pack includes a includes a document called a QUESTIONNAIRE

Please open the questionnaire booklet to the CONSENT FORM on the first page.

Each adult (over 16) who wants to be in the study should initial after each statement, and sign in the table. A parent/guardian can consent, and sign, for those under 16 years old if needed. There is a box for this too.

Instructions for filling in the questionnaire and consent form are on the front of the booklet. This tells you who should fill it in and how. Fill in the questionnaire as well as you can on behalf of yourself/the first case and the household, and send it back to us in the pre-paid envelope provided.

Don't worry if you can't answer some of the questions - it is OK to put "don't know".

3. Your study pack includes STOOL SAMPLE PACKS

There is a stool pack for each person who wants to take part. The first case (you or your child) does not have to be tested again - just the others in the home.

Follow the instructions that are with the stool packs, and don't forget to add your details to your pot. Try to get at least three or four scoops into the stool pot, but don't worry if you get more, this is fine.

The pots have their own pre-paid envelopes to send back to the lab. Follow the instructions for packaging them up.

We have made sure we use only specialist equipment, which is safe to send in the post.

Do I need to stay off work or school?

Yes. While you are ill and have symptoms, you are infectious to others!

You should not return to work or school until you have not had any diarrhoea and/or vomiting for 48 hours.

If you work with vulnerable groups such as the elderly, the young, or those in poor health, or if you handle food, you should tell your employer you have had diarrhoea.

For further advice or if you have concerns about your health, contact or call your GP or **NHS 111**

Useful websites:

NHS Direct <http://www.nhsdirect.nhs.uk>

Public Health England – cryptosporidiosis

Public Health Wales – Cryptosporidium

Health and Safety Executive (HSE) Factsheets

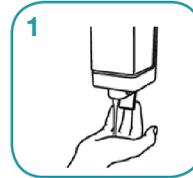
World Health Organization advice on hand washing

How do I wash my hands properly?

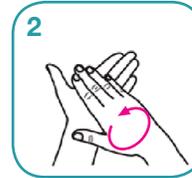
Washing your hands properly takes as long as singing 'Happy Birthday' twice, using the images below



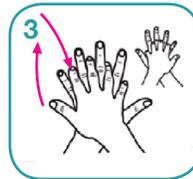
Wet hands with water



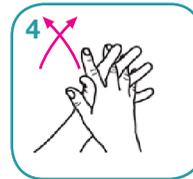
Apply enough soap to cover all hand surfaces



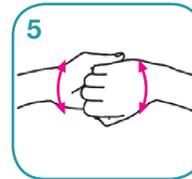
Rub hands palm to palm



Right palm over left dorsum with interlaced fingers and vice versa



Palm to palm with fingers interlaced



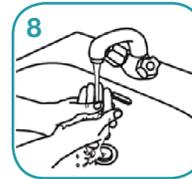
Backs of fingers to opposing palms with fingers interlocked



Rotational rubbing of left thumb clasped in right palm and vice versa



Rotational rubbing, backwards and forwards with clasped fingers of right hand in left palm and vice versa



Rinse hands with water



Dry thoroughly with a single use towel



Use towel to turn off tap



...and your hands are safe

The epiCrypt study

Investigating how *Cryptosporidium* infection spreads at home

Health Information & Advice

What is Cryptosporidium?

Cryptosporidium (sometimes called crypto) is a tiny parasite affecting people and farm animals. It causes an illness known as cryptosporidiosis. The disease is most common in spring and autumn.

What are the symptoms?

Not everyone will have symptoms but they usually include:

- diarrhoea
- stomach cramps
- fever

and sometimes:

- vomiting and loss of appetite
- headache

Who is at risk?

Anyone can get crypto, but it is most common in children under 5 years old. People who care for, or work with, young children or have contact with farm animals are more at risk. It can make children, the elderly, and those with immune problems, very ill.

How does it spread?

Transmission happens if you swallow the parasite, which is found in infected animal or human poo. You can get it directly from another person or animal by touching poo (for example when changing a nappy or stroking a dirty animal) and putting your hands near or in your mouth without washing them thoroughly.

You can also get infected from drinking contaminated water or eating contaminated food. Swimming pools and other recreational waters can get contaminated too.

What should I do if I am ill?

For most healthy people the illness is unpleasant but you will recover in a week or so. Drink plenty to replace lost fluids, and get lots of rest.

Antibiotics do not help.

You should contact your GP if symptoms become severe or if you have other medical conditions.

You can tell your doctor about this study and let them know that someone else in your home has had crypto.

People who have any problems with their immune system, or the very young or elderly, may have a more severe illness and should see their GP if they are ill.

How can I avoid spreading Crypto?

Do not go swimming until you have been free of symptoms for at least 14 days.

Clean toilet seats, toilet bowls, flush handles, sinks, and taps thoroughly, especially after someone at home has had diarrhoea or vomiting. If possible, poorly people should use a separate toilet.

Pay special attention to hygiene – especially hand washing. Make sure all members of your household wash their hands thoroughly with soap and hot water after going to the toilet, before preparing, serving, or eating food, and after handling soiled clothing or bedding.

Wash all soiled clothes and linen on as hot a machine wash as possible.

Do not share towels with other ill people.

STUDY ID NUMBER: _____

The epiCrypt study

Investigating how *Cryptosporidium*
infection spreads at home

Questionnaire & Consent Form

The epiCrypt Study_Ques & consent form V5.0 (25/06/2018)



Public Health
England



UNIVERSITY OF
LIVERPOOL

INSTITUTE OF INFECTION
AND GLOBAL HEALTH



GIG
CYMRU
NHS
WALES

Iechyd Cyhoeddus
Cymru
Public Health
Wales

Who should complete this document?

It is best that the questionnaire is completed by the FIRST CASE, or their parent/guardian if the first case is under 16 years old.

The FIRST CASE is the person who had Crypto and was contacted by the research team, or their parent/guardian if they were a child.

This may not have been the first person in the home to be ill - don't worry. Still answer the questions about the FIRST CASE that was contacted by the research team.

- **BEFORE COMPLETING THIS QUESTIONNAIRE PLEASE READ ALL THE INFORMATION IN YOUR STUDY PACK.**
- **PLEASE INITIAL THE STATEMENTS ON THE CONSENT FORM ON THE FIRST PAGE OF THIS BOOKLET AND SIGN.**
- **WHEN YOU HAVE COMPLETED THE QUESTIONNAIRE, PLEASE RETURN IT IN THE FREEPOST ENVELOPE PROVIDED.**

CONSENT FORM

HOW TO COMPLETE THIS FORM

Each person who wants to take part should read the statements on the following page and initial in the box to show they understand them and agree.

Then add your names AND sign in the table.

Children aged 16 & 17 must consent for themselves.

Children under 16 years old may consent for themselves if a parent or guardian feels it is appropriate, or a parent or guardian may consent on their behalf. Just initial and sign this portion of the form to confirm.

You do not need to detach this form. Just return the whole questionnaire booklet. Returning these documents will be taken as consent to take part.

Each person in the household who wants to take part or is consenting for others must initial a box beside each statement

I have read, and understand, the information pack explaining the study and have been given the contact details of the study team if I have any questions

I understand that taking part in this study is voluntary and I (or anyone I consent for) can leave at any time

I understand that any stool sample I provide (or any I consent for) will be tested for *Cryptosporidium* species and genotypes

I understand that all sample results are confidential, and my data may be shared securely with Public Health England/Public Health Wales to locate laboratory records relevant to this study

We agree to take part in this study

NAME	SIGNATURE

IF YOU ARE ALSO CONSENTING FOR ANYONE UNDER 16 YEARS

Please mark with parent/guardian's initial

I am the parent/legal guardian of the child/children in the household and I consent to my child/children taking part in this study

NAME OF PARENT/GUARDIAN	SIGNATURE	NAME OF CHILD

QUESTIONNAIRE

How to complete this questionnaire

Start at Section A on the following page

Write the date in the space at the top of the page

Some questions are about the first case and some are about the household. Follow the instructions to help.

You should:

- use pen to answer
- tick or cross your answers within the box like this:
- follow any 'if YES' or 'if NO' instructions and leave blank any questions you do not need to answer

Don't worry if you don't want to answer anything – you can leave it blank. But it helps our research to get as much of the information as possible.

Some of the questions are about others in the household - these will be clearly worded. You may want to get some information from others in the home or some help answering some of the questions. This is fine.

Try to think about when you/the FIRST CASE had their Crypto illness.

You may have already answered some questions like this on a questionnaire, or for your local environmental health officer, but we need to get these answers separately on this questionnaire.

Just answer as best you can – any information helps.

Remember: The FIRST CASE is the person who had Crypto and was contacted by the research team, or their parent/guardian if they were a child.

This may not have been the first person in the home to be ill - don't worry. Still answer the questions about the FIRST CASE that was contacted by the research team.

SECTION A

A1. What is the postcode of the home? _____

A2. How many people live in the household in total (including the first case)?

Please fill in the table below telling us how many people live in the household in each of the age groups

Aged less than 1 year	Aged 1-3 years	Aged 4-5 years	Aged 6-14 years	Aged 15-24 years	Aged 25-34 years	Aged 35-44 years	Aged 45-64 years	Aged 65 or over

A3. How old is the FIRST CASE?

Date of Birth / / OR Age _____

A4. What symptoms did the FIRST CASE have when they had Crypto?

(Tick all that apply)

- Diarrhoea (3 or more loose stools/runny poos in a day)
- Feeling sick/nausea Vomiting/being sick
- Stomach pain/Cramps High temperature/feverish Headache
- Other _____

A5. When did the FIRST CASE start to feel ill with these symptoms?

■ ■ / ■ ■ / ■ ■ ■ ■

A6. Is the FIRST CASE still ill with these symptoms?

Yes

No

If YES, and they are still unwell, for how many days have they had it now?

If NO, and they are better now, for how many days did the symptoms last?

A7. In the TWO WEEKS before symptoms started, did the FIRST CASE have close contact with anyone else who had diarrhoea and/or vomiting?

Yes

No

If YES, was that someone who lives:

In the home?

Elsewhere?

A8. In the TWO WEEKS before or after the FIRST CASE was ill, has anyone ELSE in the home been ill with the same kind of symptoms?

Yes

No

Who's who in the household and who else has been ill?

Please fill in **table A** on the next page, including all members of the household that are taking part in the study. This will be all the people on the consent form on the front of this booklet.

Please tell us whether others in the household have also been ill with diarrhoea or vomiting recently or around the same time as the **FIRST CASE**.

Try to think roughly about the month before and after the **FIRST CASE** was ill.

Don't worry if the dates are estimates – as accurate as you can get is fine. If you cannot remember, just let us know if you think it was before or after the first case started to get ill. This is still useful information.

Adding the age and sex helps us to identify the household members and match up their samples.

Look at the example to help:

Age	Sex	Relationship to first case	Been ill with diarrhoea and/or vomiting (Yes/No/Don't know)	When they became ill (Date if known, otherwise before/after the first case)	How many days were they ill with these symptoms?	Did they see a doctor about this illness?
39	F	Mother	Yes	18/12/2017	10	No
42	M	Father	Yes	Before	About 3 days	No
6	M	Brother	No	-	-	-
24	F	Lodger/ Housemate	Don't know	-	-	-

SECTION B

B1. Sex of FIRST CASE

- Female
- Male
- Other
- Prefer not to say

B2. Is the FIRST CASE:

(Tick all that apply)

- At nursery or pre-school
- In education (school/college/uni etc.)
- Working (paid work, either outside the home or from home)
- A stay at home parent or carer
- Retired
- Other

If OTHER, please describe

If the FIRST CASE is working, what is their main occupation?

B3. Does the FIRST CASE have any long-term medical problems or long-term illnesses?

- Yes
- No

If YES, please describe

Activities of the first case and others in the home in the 2 weeks before illness

Please fill in table B on the next page.

We want to find out if the household has been involved in any activities in the table.

Please fill in the first column in the table with a cross or tick if the **FIRST CASE** did any of these things in the **TWO WEEKS** before their symptoms started.

Also tick the second column if anyone else who lives in the home was there and did those things at the same time.

Don't worry if you're not too sure – as much as you can remember is fine. This is still useful information.

Look at the short example to help:

ACTIVITY	FIRST CASE	ANYONE ELSE IN THE HOME
<i>Travel outside the UK</i>	✓	✓
<i>Swimming – outdoors (lake, river, stream etc.)</i>		
<i>Swimming – in a treated swimming pool, either indoors or outdoors (such as</i>	✓	

TABLE B.

ACTIVITY	FIRST CASE	ANYONE ELSE IN THE HOME
Travel outside the UK		
Swimming – outdoors (lake, river, stream etc.)		
Swimming – in a treated swimming pool, either indoors or outdoors (such as a pool at a leisure centre or a lido)		
Other water activities/sport (such as surfing, rowing, water-skiing etc.)		
Other outdoor activities (such as camping, climbing, hiking, cycling etc.)		
Gardening (at home or elsewhere, such as an allotment)		
Contact with pets (at home or with pets at another house)		
Visiting or working on a farm or had contact with farm animals		
Visiting or working at a zoo or had contact with zoo or wild animals		

SECTION C

C1. How many bedrooms are there in the house? _____

Do any adults share a bedroom to sleep in at night?

Yes

No

Do any children (under 18 years old) share a bedroom to sleep in at night?

Yes

No

Do any infants (under 1 year) sleep in a bedroom with an adult at night?

Yes

No

C2. Does the FIRST CASE regularly (more than once a week) share a bed with anyone else in the home?

Yes

No

C3. How many bathrooms are there? _____

How many other people use the same bathroom at home as the FIRST CASE?

1 2 3 4 5 6 7 8 9 10

C4. How many toilets/WCs are there in total? _____

How many other people use the same toilet at home as the FIRST CASE?

- 1 2 3 4 5 6 7 8 9 10
-

C5. Does the FIRST CASE regularly (more than once a week) share a bath with anyone else in the home?

Yes

No

C6. Is your home on, or part of, a farm or smallholding?

Yes

No

C7. Does your home have any outside space/area?

Yes

No

If YES, what kind? (*Tick all that apply*)

Yard (no grass)

Front garden

Back garden

Other _____

C8. Is your home supplied by mains water (i.e. a water company that you get a bill from)?

Yes

No

C9. Are there any pets in the home?

Yes

No

If YES, what kind? (*Tick all that apply*)

Cat(s) Bird(s) Reptile(s)

Dog(s) Horse(s) Fish Other _____

C10. Are there any other animals at or around the home - such as livestock, chickens, cows, sheep etc.?

Yes

No

If YES, please describe

SECTION D

D1. Is the **FIRST CASE** a child under 5 years old?

Yes

No

If YES, are they? (*Tick all that apply*)

In nappies

Potty/Toilet training

Neither

If NO, are they an older child (more than 5 years old) or an adult that needs help with any of the following? (*Tick all that apply*)

Nappy changing

Assisting with going to the toilet

Bathing

None of the above

D2. Does the **FIRST CASE** regularly (more than once a week) help a child (or children) under 5 with any of the following? (this could be at work or in the home)

(*Tick all that apply*)

Nappy changing

Potty training

Assisting with the toilet

Bathing

None of the above/not applicable

D3. Does the FIRST CASE regularly (more than once a week) help a child over 5, or an adult, with any of the following? (this could be at work or in the home)

(Tick all that apply)

- Nappy changing
- Potty training
- Assisting with the toilet
- Bathing
- None of the above/not applicable

D4. Are there ANY children (under 5) living in the household who are in nappies and/or currently toilet training?

- Yes
- No

If YES, when changing nappies do you/others in the home generally use any of the following: *(Tick all that apply)*

Don't worry if it applies to more than one child – just tick if anyone in the home generally uses these things when changing nappies or helping the children with the toilet

- | | |
|--|---|
| <input type="checkbox"/> Disposable nappies | <input type="checkbox"/> Nappy cream |
| <input type="checkbox"/> Cloth nappies | <input type="checkbox"/> Hand gel |
| <input type="checkbox"/> Disposable baby wipes/wet wipes | <input type="checkbox"/> Nappy bags |
| <input type="checkbox"/> Water and cotton wool | <input type="checkbox"/> A nappy bin |
| <input type="checkbox"/> Top/Tail bowl | <input type="checkbox"/> A changing mat |

D5. Does the FIRST CASE regularly/usually prepare meals or cook for others in the household?

- Yes
- No

If YES, did they still prepare meals or cook for the rest of the household while they were ill with Crypto?

- Yes
- No
- Don't know

D6. When hand-washing, do household members normally use:

(Tick all that apply)

- Hot water
- Soap (bar or hand wash)
- Antibacterial hand wash
- Alcohol gel
- Hand wipes
- Don't know
- None of the above/Not applicable

D7. Generally, do household members wash their hands before and/or after any of the following?

If you're not sure, just try to think about what most people in the home do

Going to the toilet

- Before
- After
- Both
- Neither

Helping someone with the toilet

- (if applicable)
- Before
 - After
 - Both
 - Neither

Nappy changing (if applicable)

- Before
- After
- Both
- Neither

Eating

- Before
- After
- Both
- Neither

Preparing food

- Before
- After
- Both
- Neither

Handling dirty/soiled laundry

- Before
- After
- Both
- Neither

THANK YOU FOR COMPLETING THE QUESTIONNAIRE!

Please let us know if you would like a summary of the results when we have finished our study.

- Yes
- No

Please let us know if you would be willing to be contacted again if we had any further questions about your illness or your household.

- Yes
- No

If YES to either of the above, please give the preferred contact details:

- Email _____
- Post _____
- Telephone _____

Please place in the FREEPOST ENVELOPE PROVIDED and return to the epiCrypt study team. Thank you!