

## Reduction in cryptosporidiosis associated with introduction of enhanced filtration of drinking water at Loch Katrine, Scotland

K. G. J. POLLOCK<sup>1</sup>\*, D. YOUNG<sup>2</sup>, C. ROBERTSON<sup>2</sup>, S. AHMED<sup>3</sup>  
AND C. N. RAMSAY<sup>1</sup>

<sup>1</sup>Health Protection Scotland, Clifton House, Clifton Place, Glasgow, UK

<sup>2</sup>Statistics Department, University of Strathclyde, Glasgow, UK

<sup>3</sup>NHS Greater Glasgow & Clyde Health Board, Glasgow, UK

Received 8 November 2012; Final revision 25 February 2013; Accepted 27 February 2013;  
first published online 16 April 2013

### SUMMARY

Previous evidence has suggested an association between cryptosporidiosis and consumption of unfiltered drinking water from Loch Katrine in Scotland. Before September 2007, the water was only micro-strained and chlorinated; however, since that time, coagulation and rapid gravity filtration have been installed. In order to determine risk factors associated with cryptosporidiosis, including drinking water, we analysed data on microbiologically confirmed cases of cryptosporidiosis from 2004 to 2010. We identified an association between the incidence of cryptosporidiosis and unfiltered Loch Katrine drinking water supplied to the home (odds ratio 1·86, 95% confidence interval 1·11–3·11,  $P=0\cdot019$ ). However, while filtration appears to be associated with initially reduced rates of cryptosporidiosis, evidence suggests it may paradoxically make those consumers more susceptible to other transmission routes in the long-term. These findings support implementation of similar treatment for other unfiltered drinking-water supplies, as a means of reducing cryptosporidiosis associated with drinking water.

**Key words:** *Cryptosporidium*, epidemiology, parasitic disease epidemiology and control, water (quality).

### INTRODUCTION

Drinking water contaminated with *Cryptosporidium* oocysts is an established risk factor for human illness [1–3]. Contamination of drinking water can arise from a variety of sources [4] including raw water and post-treatment contamination with oocysts from infected humans, livestock and feral animals. Oocysts can remain infectious in the environment and water for prolonged periods and are resistant to most

disinfectants used to treat drinking water. Inadequate treatment of drinking water can permit transmission of infectious oocysts to susceptible consumers [5–7]. Although infection in immunocompetent people generally results in a self-limiting diarrhoeal illness, cryptosporidiosis in the immunodeficient can result in extra-intestinal disease and life-threatening wasting and malabsorption [8].

The response by the water industry to *Cryptosporidium* contamination of drinking water has been to establish effective multiple barrier water treatment systems, using a variety of methods, in an effort to reduce this pathogen from drinking-water supplies. These include coagulation, rapid gravity filtration (RGF), dissolved air flotation (DAF) and membrane

\* Author for correspondence: Dr K. G. J. Pollock, Health Protection Scotland, Meridian Court, Glasgow G2 6QE, UK (Email: Kevin.Pollock@nhs.net)

filtration [3]. We previously performed a retrospective analysis of *Cryptosporidium* incidence in a population supplied with drinking water, where introduction of both coagulation and RGF had a demonstrable association with a reduction in local incidence rates of cryptosporidiosis [6].

Low levels of oocysts have been detected in 65–97% of surface-water supplies, suggesting that many populations may be at risk of waterborne infection [7, 9]. In Scotland, the majority of drinking-water supplies to urban areas have had effective forms of water treatment, capable of significantly reducing the *Cryptosporidium* load in final drinking water. Until September 2007, the Loch Katrine system, which supplies much of the Glasgow and Clyde population had only micro-straining and chlorination but not filtration (Fig. 1). In 2000, an outbreak of cryptosporidiosis in Glasgow residents was associated with drinking water from Loch Katrine [10]. The catchment area around Loch Katrine had a large sheep population, as well as cattle. Livestock were removed from the catchment area by the water authorities, following the outbreak although there was no microbiological evidence that these animals were the source of the outbreak [11]. Furthermore, in order to eliminate *Cryptosporidium* from Loch Katrine-sourced water, coagulation and RGF was introduced as a treatment to the Loch Katrine supply in September 2007.

In order to determine whether Loch Katrine-sourced water was associated with the local incidence of cryptosporidiosis, we performed a retrospective cohort study of all laboratory-confirmed cases in the Glasgow & Clyde area between 2004 and 2010, 3 years either side of the introduction of water filtration. Our hypothesis was that if a proportion of clinical illness was attributable to drinking unfiltered Loch Katrine water, then the incidence should have decreased after the introduction of enhanced water treatment. However, rates of reported cryptosporidiosis in the Glasgow & Clyde NHS Board area were known to be much lower than the mean rate for Scotland. Detecting small changes in incidence might therefore be difficult.

## METHODS

The methodology has been previously described elsewhere [6]. Briefly, a retrospective cohort study of microbiologically confirmed cases of cryptosporidiosis was conducted involving the population of the Glasgow & Clyde NHS Board area. The period of

study (2004–2010) was chosen to enable the detection of any epidemiological trends in cases and in risk factor exposures associated with cryptosporidiosis, straddling the introduction of enhanced water treatment. In order to improve the potential power of the analysis and to improve the ability to detect a true difference, if one existed, we pooled all cases.

Both demographic and potential risk factor data from original NHS Health Board case investigation records were reviewed, and were entered into an Excel database. Using home location postal codes, we linked cases to their drinking-water supply zone and water source, details of which were supplied by Scottish Water. For analysis, cases were divided into two groups according to the source of drinking water at the home address (Loch Katrine or other source, i.e. not Loch Katrine). Water treatment for the ‘not Loch Katrine’ drinking water supply did not change from 2004 to 2010. We used the date of laboratory confirmation to code the variables before/after filtration, based on 1 October 2007 as the cut-off.

The potential waterborne oocyst exposure during the study period was addressed using weekly data collected on the levels of oocyst contamination detected in the Loch Katrine drinking-water supply. Large sample volumes (about 1000 litres) were used for oocyst detection to reduce scope for sampling bias, taken at points before and after filtration, i.e. raw and final, treated water. ‘Genera’ filters were used for final water sampling; ‘Cuno’ filters (3M, UK & Ireland) were used for raw water samples due to the higher turbidity of these samples.

Descriptive statistics were used to describe the characteristics of cases and the distribution of cases by water source, before and after the introduction of enhanced physical treatment at Loch Katrine. Cases associated with different water sources were compared using Mann–Whitney tests for quantitative variables and  $\chi^2$  tests for qualitative variables. A  $\chi^2$  test was then performed to investigate any difference in the distribution of cases in people who received water from Loch Katrine or from other sources, before and after the introduction of enhanced treatment of Loch Katrine water. The factors with  $\chi^2$  *P* values < 0.10 were included in a multiple logistic regression model and we used backward selection by removing the least significant factor at each stage. Unless otherwise stated, Minitab statistical software, version 14 (www.minitab.com) was employed at a significance level of 5% for all analyses.

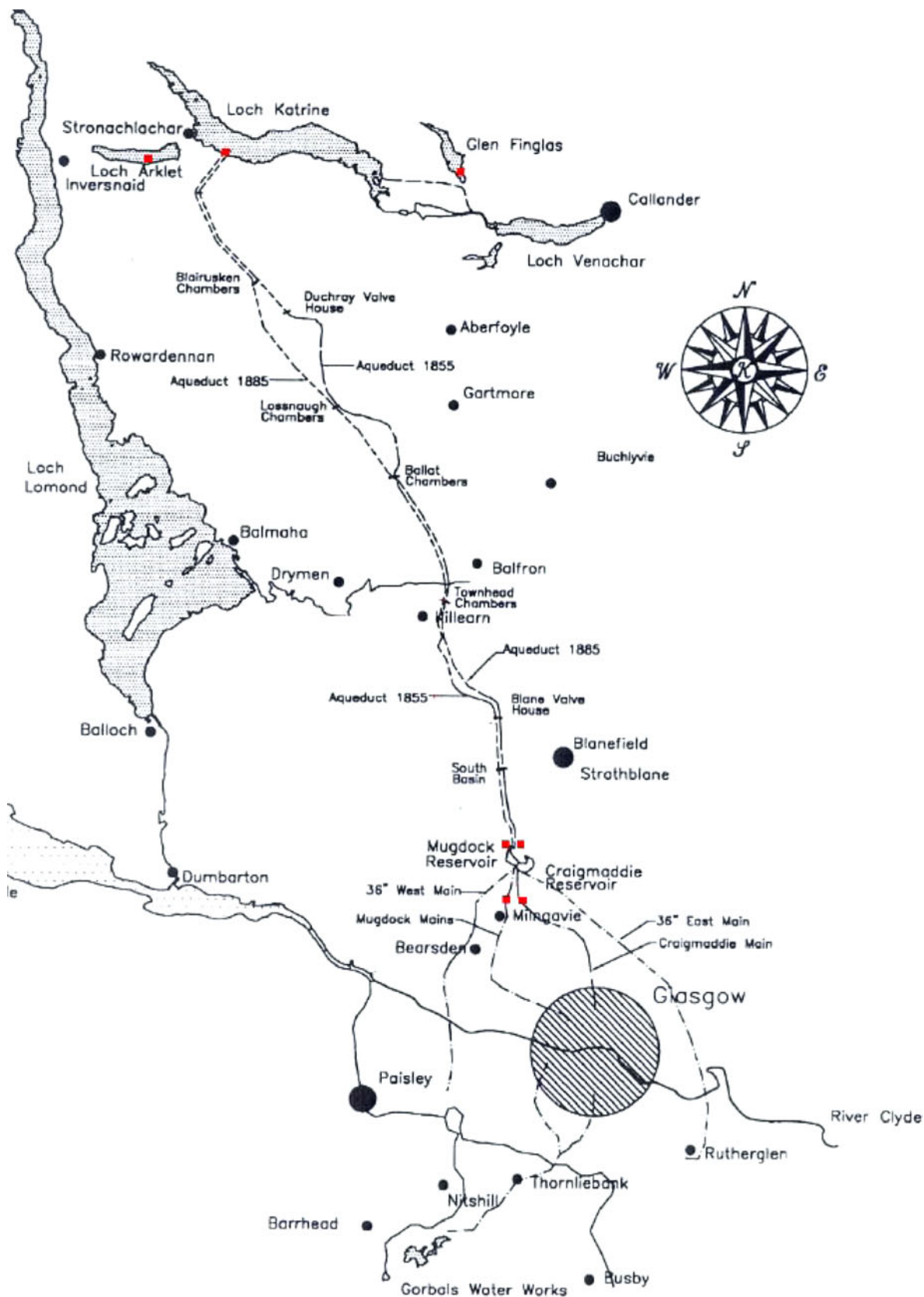


Fig. 1 [colour online]. Layout of Loch Katrine supply to Glasgow.

Table 1. *Distribution of cases of cryptosporidiosis in Greater Glasgow & Clyde NHS Board area by drinking-water source, before and after the introduction of filtration on Loch Katrine water, Scotland*

Relation to enhanced water filtration*	Water source, no.†		
	Loch Katrine	Other	All
Before	89 (78)	118 (129)	207
After	60 (71)	128 (117)	188
All	149	246	395

\* Coagulation and rapid gravity filtration, introduced in September 2007.

† Numbers in parentheses are cases expected under the null hypothesis (i.e. no association between water supply and filtration).

Table 2. *Period incidence of cryptosporidiosis cases/100 000 population in Greater Glasgow & Clyde (GG&C) NHS Board area and Scotland, 2004–2010*

Cases by drinking-water source	Jan. 2004–Sept. 2007	Oct. 2007–Dec. 2010
GG&C, Loch Katrine	7.5	5.0
GG&C, non-Loch Katrine	9.9	10.7
Rest of Scotland	35.7	33.8

## RESULTS

Of the 452 microbiologically confirmed cases, 15 had no date of laboratory confirmation and were excluded from further analysis. Although there were no reported outbreaks in Glasgow and Clyde between 2004 and 2010, there was a large outbreak in 2005, associated with a wildlife centre in Perthshire, 50 km distant from Glasgow [12]. Nineteen cases from Glasgow and Clyde were known to have visited this wildlife park during the exposure period; both microbiological and epidemiological investigations defined these as outbreak-associated cases and so these were also excluded. A small number of cases ( $n=23$ ) had no defined water source or were supplied via a private water source, therefore the actual number in the final analysis was 395 (Table 1).

There was no association between water source, gender or age, i.e. those living in the area supplied by Loch Katrine had a similar demographic profile to non-Loch Katrine distributed water. This did not change after filtration was introduced in September 2007. The period incidence of cases supplied with water from Loch Katrine was 7.5 cases/100 000 population before filtration (September 2007) and

Table 3. *Period incidence of Cryptosporidium spp., per 100 000 population in Loch Katrine supply area of Greater Glasgow & Clyde NHS Board, 2005–2010*

Species	Apr. 2005*–Sept. 2007	Oct. 2007–Dec. 2010
<i>Cryptosporidium parvum</i>	1.4	1.3
<i>Cryptosporidium hominis</i>	2.0	3.4

\* Health Protection Scotland only received speciated data from April 2005 via the Cryptosporidium Reference Unit, Swansea.

Table 4. *Rate of oocysts in raw and final water before and after the installation of a filtration system in Glasgow*

Period	Concentration of oocysts/10 litres		
	Raw	Final	Reduction (%)
Pre-filtration	0.004 219	0.001 320	69
Post-filtration	0.000 855	0.000 000	100

5 cases/100 000 after filtration (Table 2). The period incidence of cases who received water from other water sources in the Glasgow & Clyde region was 9.9/100 000 before October 2007 and 10.7/100 000 after. By comparison, the period incidence of cases in the rest of Scotland was 35.7/100 000 before October 2007 and 33.8/100 000 after.

The period incidence of cases of *Cryptosporidium* spp. for the Loch Katrine supply area in the Glasgow & Clyde region was analysed for cases before and after filtration (Table 3). Comparison of the observed and expected data illustrates that fewer cases of *C. parvum* occurred in the area supplied by Loch Katrine after enhanced treatment than would have been expected ( $P=0.025$ ). Consequently, there were more cases of *C. hominis* in the Loch Katrine supply area, after filtration.

Before September 2007 (the introduction of filtration to Loch Katrine) the oocyst detection rate in Glasgow (final water) averaged  $13.2 \times 10^{-4}/10$  litres (Table 4). Following the introduction of filtration at Loch Katrine, the oocyst count decreased to zero in final water, representing complete removal of waterborne oocysts.

Comparison of the observed and expected values (Table 1) illustrates that fewer cases occurred in the area supplied by Loch Katrine after enhanced treatment than would have been expected if there had been no

Table 5. Reported exposure variables for cryptosporidiosis, Greater Glasgow &amp; Clyde NHS Board area – univariate analysis and logistic regression analysis

Variable	% of cases within Loch Katrine water source	% of cases not within Loch Katrine water source	<i>P</i> value	OR (95% CI)	Adjusted <i>P</i> value
Pre-filtration	59.7	48.0	<b>0.023</b>	1.86 (1.11–3.11)	<b>0.019</b>
Travel in the UK	14.2	24.4	<b>0.022</b>	0.45 (0.23–0.88)	<b>0.019</b>
Travel outside UK	40.8	42.0	0.822		
Contact with farm animals	13.1	16.5	0.404		
Contact with farm land	13.2	20.2	0.108		
Ownership of pets	28.7	41.5	<b>0.019</b>	0.51 (0.30–0.88)	<b>0.016</b>
Recreational water contact	35.5	69.7	<b>&lt;0.001</b>	0.23 (0.14–0.38)	<b>&lt;0.001</b>
Consumption of bottled water	53.8	69.2	<b>0.005</b>	0.56 (0.33–0.97)	<b>0.037</b>
Contact with confirmed case	7.1	11.3	0.289		

OR, Odds ratio; CI, confidence interval.

association between the incidence of cases and introduction of drinking-water filtration. The analysis shows a significant association between being a case and living in the area supplied by Loch Katrine water before filtration was introduced ( $P=0.023$ ) (Table 5).

There were a number of other putative risk factors associated with being a confirmed case of cryptosporidiosis (Table 5). Fewer cases than expected from the Loch Katrine-supplied area were associated with travel within the UK ( $P=0.022$ ), while fewer cases than expected supplied by Loch Katrine, owned pets ( $P=0.019$ ). Fewer cases than expected from the Loch Katrine area had recreational water contact compared to cases from non-Loch Katrine areas ( $P<0.001$ ). Finally, cases from the Loch Katrine supply area were significantly less likely to consume bottled water compared to their non-Loch Katrine counterparts ( $P<0.005$ ).

In the logistic regression analysis, the most significant factor associated with being a Loch Katrine-supplied case was consumption of unfiltered drinking water ( $P=0.019$ ) (Table 5), people who consumed Loch Katrine water prior to the introduction of filtration (*vs.* other studies) were significantly more likely to be a confirmed case of cryptosporidiosis [odds ratio (OR) 1.86, 95% confidence interval (CI) 1.11–3.11]; significantly less likely to have consumed bottled water (OR 0.56, 95% CI 0.33–0.97), owned pets (OR 0.51, 95% CI 0.30–0.88) or had recreational water contact (OR 0.23, 95% CI 0.14–0.38).

## DISCUSSION

In Scotland, there has been an intense public interest in the microbial quality of drinking water, largely

due to a number of high-profile outbreaks of cryptosporidiosis and incidents resulting in boil-water notices [10, 13, 14]. The legacy of the Central Scotland *E. coli* O157 outbreak in 1996 [15] still prompts significant media and political interest in cases of infectious intestinal disease. Multiple barrier methods have been developed for source and treatment especially in urban areas [16]. The Loch Katrine distribution system supplying much of the Glasgow and Clyde population was an exception until September 2007.

In order to reduce the risk of waterborne cryptosporidiosis from Loch Katrine, coagulation and RGF was introduced to the Loch Katrine distribution system in September 2007. A sero-epidemiological study was conducted to determine whether there was evidence of an association between the seroprevalence to *Cryptosporidium* and the type of treatment (Ramsay et al., unpublished data). As a follow-up to this work, we collated data on all laboratory-confirmed cases of cryptosporidiosis in the Greater Glasgow & Clyde NHS Board area from 2004 to 2010 and performed a retrospective cohort study to ascertain whether there was an ecological association between exposure to unfiltered Loch Katrine-sourced drinking water and the incidence of cryptosporidiosis.

The study provided evidence that consumers were being exposed to infectious oocysts in minimally treated tap water. Upgrading the water treatment, using coagulation and RGF was associated with significantly reduced numbers of laboratory-confirmed cases. This is consistent with findings in similar natural experiments involving other drinking water supplies in the UK [3, 6, 17]. The concurrent findings that bottled water consumption, recreational water contact and pet ownership were inversely associated

with being a Loch Katrine supply area case are of interest. While consumption of bottled water might suggest reduced consumption of tap water and therefore less potential for waterborne oocyst exposure in the Loch Katrine case cohort, the 'protective' element of recreational water contact and pet ownership is unclear although there is some evidence to suggest that pet ownership may be protective [18].

Chappell and others [19, 20] have postulated that protective immunity may be acquired by repeated low-level exposure to oocysts. However, the same group suggests that clinically apparent infection may develop with ingestion of as little as 10 oocysts [21]. Since recreational water exposure is considered one of the strongest aetiological risk factors for infection with *Cryptosporidium* [22, 23], our finding of previous recreational water exposure being apparently 'protective' against developing cryptosporidiosis in the Loch Katrine case cohort, is paradoxical but substantiated elsewhere [24]. Host immunity is multi-factorial and depends on both infectious dose and viability of the consumed oocysts, as well as the immunological status of the host at the time of ingestion and during the incubation period. It is probable that susceptibility/resistance to infection is due to key interactions in the immunological milieu (both cellular and physical) being significantly influenced by subtle extrinsic and intrinsic factors, e.g. steroidal medications for underlying disease or endogenous stress cortisol levels [25, 26]. This argument could be applied to pet ownership but the situation is likely to be even more complex [27].

We previously performed a study assessing filtration in the Loch Lomond supply to Glasgow and other parts of Central Scotland and observed that the period incidence decreased from 12.6/100 000 to 6.5/100 000 [6]. It is interesting to note that the period incidence per 100 000 for the Loch Katrine case cohort (before and after filtration) was lower than that for the non-Loch Katrine case cohort. Given that the non-Loch Katrine case cohort received filtered water from Loch Lomond and other sources in the Glasgow area and has done for at least 7 years, this would suggest that rates of cryptosporidiosis are actually increasing in these areas (from 6.5/100 000 for 2000–2003, to 10.7 cases/100 000 for 2007–2010).

While filtration appears to be associated with initially reduced rates of cryptosporidiosis, it may paradoxically make those consumers more susceptible to other transmission routes as demonstrated by an increasing period incidence of *C. hominis* in the area

supplied by Loch Katrine. This finding corroborates previous studies [20] and suggests that a reduction in exposure to one species (*C. parvum*) through improved physical treatment of drinking water might lead to increased susceptibility to other species (e.g. *C. hominis*) through other transmission routes such as foreign travel and swimming [28, 29].

Given the ecological study design, our conclusions are valid only at the population level; the study was based on the water supply solely to the home location of cases. Data on the water consumption patterns of individuals were insufficient for us to be able to comment on other sources of drinking-water exposure. The ecological study method is, however, widely accepted in environmental epidemiology, as opportunities to collect precise environmental exposure data on individuals are often limited. The inferences gleaned from this study highlight the complexities associated with exposure, infection and immunity to *Cryptosporidium*.

While all known outbreak cases were excluded from this study, it is plausible that we may have included cases that were associated with undetected mini-outbreaks. Since 2005, most positive isolates for *Cryptosporidium* spp. have been forwarded to the *Cryptosporidium* Reference Unit for speciation and/or genotyping. In summary, our data suggest that drinking unfiltered tap water supplied from Loch Katrine was associated with infection with *Cryptosporidium* spp. at the population level. Upgrading the treatment using a RGF system was associated with a marked reduction in the numbers of cryptosporidiosis cases. These findings support implementation of similar treatment for other unfiltered drinking-water supplies, as a means of reducing clinical cryptosporidiosis.

## ACKNOWLEDGEMENTS

The authors acknowledge the Scottish Government (Drinking Water Quality Directorate) for their generous funding of this project.

## DECLARATION OF INTEREST

None.

## REFERENCES

1. McAnulty JM, *et al.* Contaminated drinking water in one town manifesting as an outbreak of cryptosporidiosis

- in another. *Epidemiology & Infection* 2000; **125**: 79–86.
2. **Goh S, et al.** Sporadic cryptosporidiosis, North Cumbria, England, 1996–2000. *Emerging Infectious Diseases* 2004; **10**: 1007–1015.
  3. **Goh S, et al.** Sporadic cryptosporidiosis decline after membrane filtration of public water supplies, England, 1996–2002. *Emerging Infectious Diseases* 2005; **11**: 251–259.
  4. **Chalmers RM, et al.** *Cryptosporidium* sp. rabbit genotype, a newly identified human pathogen. *Emerging Infectious Diseases* 2009; **15**: 829–830.
  5. **MacKenzie WR, et al.** A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. *New England Journal of Medicine* 1994; **331**: 161–167.
  6. **Pollock KGJ, et al.** Cryptosporidiosis and filtration of water from Loch Lomond, Scotland. *Emerging Infectious Diseases* 2008; **14**: 115–120.
  7. **Mason BW, et al.** A *Cryptosporidium hominis* outbreak in north-west Wales associated with low oocyst counts in treated drinking water. *Journal of Water and Health* 2010; **8**: 299–310.
  8. **Hunter PR, Nichols G.** Epidemiology and clinical features of *Cryptosporidium* infection in immunocompromised patients. *Clinical Microbiology Reviews* 2002; **15**: 145–154.
  9. **Juranek DD.** Cryptosporidiosis: Sources of infection and guidelines for prevention (<http://www.cdc.gov/crypto/prevention.html>). Accessed 26 May 2012.
  10. **National Health Service for Scotland.** Greater Glasgow Outbreak Control Team. Report of an outbreak of cryptosporidiosis in the area supplied by Milngavie Treatment Works–Loch Katrine water. Glasgow: Department of Public Health, Greater Glasgow Health Board, 2001.
  11. **Elwin K, Chalmers RM.** Contemporary identification of previously reported novel *Cryptosporidium* isolates reveals *Cryptosporidium bovis* and the cervine genotype in sheep (*Ovis aries*). *Parasitology Research* 2008; **102**: 1102–1105.
  12. **McGuigan CC, Steven K, Pollock KGJ.** Cryptosporidiosis associated with wildlife center, Scotland. *Emerging Infectious Diseases* 2010; **16**: 895–896.
  13. **Smith HV, et al.** An outbreak of waterborne cryptosporidiosis caused by post-treatment contamination. *Epidemiology & Infection* 1989; **103**: 703–715.
  14. **Mukherjee A.** Outbreak of cryptosporidiosis in Grampian NHS Board area (January–March 2002). National Health Services Grampian (<http://www.nhsgrampian.org/grampianfoi/files/CryptoAberdFRRep2002.pdf>). Accessed 2 July 2011.
  15. **Cowden JM, et al.** Epidemiological investigation of the central Scotland outbreak of *Escherichia coli* O157 infection, November to December 1996. *Epidemiology & Infection* 2001; **126**: 335–341.
  16. **Cassady JD, et al.** Beyond compliance: environmental health problem solving, interagency collaboration, and risk assessment to prevent waterborne disease outbreaks. *Journal of Epidemiology & Community Health* 2006; **60**: 672–674.
  17. **Department of the Environment and Department of Health.** *Cryptosporidium* in water supplies: second report of the group of experts. London: HMSO, 1995.
  18. **Robertson B, et al.** Case-control studies of sporadic cryptosporidiosis in Melbourne and Adelaide, Australia. *Epidemiology & Infection* 2001; **128**: 419–431.
  19. **Chappell CL, et al.** Infectivity of *Cryptosporidium parvum* in healthy adults with pre-existing anti-*C. parvum* serum immunoglobulin G. *American Journal of Tropical Medicine and Hygiene* 1999; **60**: 157–164.
  20. **Frost FJ, et al.** How clean must our drinking water be: the importance of protective immunity. *Journal of Infectious Disease* 2005; **191**: 809–814.
  21. **Chappell CL, et al.** *Cryptosporidium hominis*: experimental challenge of healthy adults. *American Journal of Tropical Medicine and Hygiene* 2006; **75**: 851–857.
  22. **Yoder JS, Harral C, Beach MJ.** Cryptosporidiosis surveillance – United States, 2006–2008. *Morbidity and Mortality Weekly Report (Surveillance Summary)* 2010; **59**: 1–14.
  23. **Valderrama AL, et al.** Multiple risk factors associated with a large statewide increase in cryptosporidiosis. *Epidemiology and Infection* 2009; **137**: 1781–1788.
  24. **Schijven J, de Roda Husman AM.** A survey of diving behaviour and accidental water ingestion among Dutch occupational and sport divers to assess the risk of infection with waterborne pathogenic microorganisms. *Environmental Health Perspectives* 2006; **114**: 712–717.
  25. **Tatar G, Hazirolu R, Hascelik G.** *Helicobacter felis* as a co-factor alone or together with stress in cryptosporidial infection in mice. *Journal of International Medical Research* 1995; **23**: 473–479.
  26. **Gopal R, Birdsell D, Monroy FP.** Regulation of toll-like receptors in intestinal epithelial cells by stress and *Toxoplasma gondii* infection. *Parasite Immunology* 2008; **30**: 563–576.
  27. **Overgaauw PA, et al.** Zoonotic parasites in fecal samples and fur from dogs and cats in The Netherlands. *Veterinary Parasitology* 2009; **163**: 115–122.
  28. **Mayne DJ, et al.** A community outbreak of cryptosporidiosis in Sydney associated with a public swimming facility: a case-control study. *Interdisciplinary Perspectives on Infectious Diseases* 2011; 341065.
  29. **Chalmers RM, et al.** Long-term *Cryptosporidium* typing reveals the aetiology and species-specific epidemiology of human cryptosporidiosis in England and Wales, 2000 to 2003. *Eurosurveillance* 2009; **14**: 19086.