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Long-term effects of the *Cryptosporidium hominis* outbreak in Östersund

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Don’t drink the water, and don’t breathe the air.
-Tom Lehrer, 1965

Makalöst rent… även vattnet är det renaste och klaraste…
- Östersund municipality, 2009

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Abstract

Background
Waterborne infection with the parasite *Cryptosporidium* is a common cause of diarrhoea, particularly in low-income countries, and contributes significantly to diarrhoea-related morbidity and mortality. Young children and immunocompromised individuals are particularly vulnerable to such infections. In 2010, an outbreak of *Cryptosporidium hominis* in Östersund, Sweden, resulted in ~27,000 cases. Long-term symptoms, including abdominal and joint symptoms, headache, and fatigue, can persist for up to 5 years, but whether they extend beyond that time frame is unclear. Data on the long-term health-economic impact of *Cryptosporidium* outbreaks are lacking. In addition, physicians appreciate that the incidence of inflammatory bowel disease (IBD) and microscopic colitis (MC) has increased in Region Jämtland Härjedalen (RJH) since 2010, but a link between cryptosporidiosis and IBD or MC has not been established.

Aim
We aimed to understand the long-term consequences of the *Cryptosporidium* outbreak in Östersund by assessing symptoms after 10 years, as well as the health-economic impacts and incidences of IBD and MC.

Methods
In 2011, a randomly selected cohort of Östersund residents (n = 1,524) and 500 additional children aged 0–5 years were surveyed via a postal questionnaire. Respondents reporting new episodes of diarrhoea during the outbreak were defined as cases; those who did not report diarrhoea were defined as non-cases. In a prospective cohort study conducted in 2021, all respondents (n = 1,404, 69.4%) were surveyed about possible long-term symptoms. We used logistic regression analysis to examine a possible association between reported symptoms and case status. The results were published separately for adults (Study I) and children aged 0-5 years (Study II). For adults, we also evaluated the consistency of reported symptoms, including the results of a 5-year follow-up of the same cohort, and its possible association with disease duration during the outbreak. For children, we investigated healthcare utilisation and diagnoses during the follow-up period.

All questionnaire respondents in 2011 were included in a retrospective, incremental cost analysis focusing on production loss and healthcare utilisation during 2011-2019 (Study III). Data were collected on net days of sickness and childcare benefits, outpatient visits, and

inpatient days. Possible differences between cases and non-cases were assessed by logistic and negative binomial regression analyses. Costs were calculated using population data and mean wages.

In an observational study (Study IV), we compared pre-outbreak (2006-2010) and post-outbreak (2011-2019) incidences of IBD and MC in RJH using negative binomial regression analysis.

<u>Results</u>

In 2021, 538 adults (74%) responded to the questionnaire. Cases were more likely to report abdominal (aOR ~3) and joint symptoms (aOR ~2). They were also more likely to report symptoms at both the 5- and 10-year follow-up. Cases with consistent abdominal symptoms had more symptomatic days during the outbreak than cases reporting no or varying symptoms (mean 9.2 days [median 6.5, range 1.0-55.0] vs 6.6 days [median 5.0, range 0.0-33.0]; $p = 0.003$).

Among children aged 0-5 years, 295 (69.7%) responded. Cases were more likely to report joint symptoms (aOR 4.0, 95% CI 1.3–12.0) and fatigue (aOR 1.9, 95% CI 1.1–3.4), although overall numbers were low. No between-group differences were observed in healthcare utilisation or disease diagnoses.

The cost analysis revealed that more cases than non-cases received sickness benefits in the years following the outbreak (142/346 [41.0%] vs 125/399 [31.3%], $p = 0.006$). Cases also claimed more benefit days (8,946 per 100 cases vs 4,538 per 100 non-cases; $p = 0.003$). This corresponds with an incremental cost of ~33.3 million SEK (€2.9 million) for our study population, or ~1.7 billion SEK (€148.5 million) for the entire population of Östersund. No differences were observed in childcare benefits or outpatient visits. Hospital admission rates were comparable between groups, though cases generally had shorter hospital stays, likely reflecting factors unrelated to cryptosporidiosis.

In RJH, 410 patients were diagnosed with IBD and 155 with MC between 2006 and 2019. For the population >40 years old, the incidence of IBD (IRR 1.69, 95% CI 1.13–2.51) and the incidence of Crohn's disease in particular (IRR 2.23, 95% CI 1.08–4.62) increased post-outbreak. The incidence of MC increased sixfold (IRR 6.43, 95% CI 2.78–14.87).

<u>Conclusion</u>

Long-term symptoms commonly occur following a *C. hominis* infection, particularly in adults. The 2010 outbreak generated an estimated 1.7 billion SEK increase in sickness benefits. Furthermore, post-outbreak, the incidence of late-onset IBD and MC increased. These findings underscore the importance of prevention and may guide future public health interventions and water management policies.

List of scientific papers

I. **Boks M**, Lilja M, Widerström M, Karling P, Lindam A, Sjöström M. Persisting symptoms after *Cryptosporidium hominis* outbreak: a 10-year follow-up from Östersund, Sweden. *Parasitol Res* 2023;122:1631–1639. doi: https://doi.org/10.1007/s00436-023-07866-8

II. **Boks M**, Lilja M, Lindam A, Widerström M, Persson A, Karling P, Sjöström M. Long-term symptoms in children after a *Cryptosporidium hominis* outbreak in Sweden: a 10-year follow-up. *Parasitol Res* 2025:124:13. doi: https://doi.org/10.1007/s00436-025-08455-7

III. **Boks M**, Lilja M, Lindam A, Hazara M, Eriksson L, Lindholm L, Sjöström M. Long-term economic impact of a *Cryptosporidium hominis* outbreak: costs of production loss and healthcare utilisation. [*Manuscript*]

IV. **Boks M**, Lilja M, Widerström M, Karling P, Lindam A, Eriksson A, Sjöström M. Increased incidence of late-onset inflammatory bowel disease and microscopic colitis after a *Cryptosporidium hominis* outbreak. *Scand J Gastroenterol* 2022;57:1443–1449. doi: 10.1080/00365521.2022.2094722

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Abbreviations

(a)OR	(adjusted) Odds Ratio
CD	Crohn's Disease
CI	Confidence Interval
ECDC	European Centre for Disease Prevention and Control
FAO	Food and Agriculture Organization
GSRS	Gastrointestinal Symptom Rating Scale
IBD	Inflammatory Bowel Disease
IBS	Irritable Bowel Syndrome
IQR	Interquartile Range
IRR	Incidence Rate Ratio
MC	Microscopic Colitis
MLVA	Multi-locus Variable-number Tandem-repeat Analysis
NBHW	National Board of Health and Welfare (*Socialstyrelsen*)
PCR–RFLP	Polymerase Chain Reaction–Restriction Fragment Length Polymorphism
PI-IBS	Post-Infectious Irritable Bowel Syndrome
RJH	Region Jämtland Härjedalen
SSIA	Swedish Social Insurance Agency (*Försäkringskassan*)
SWIBREG	Swedish Inflammatory Bowel Disease Register
UC	Ulcerative Colitis
WHO	World Health Organization

Sammanfattning på svenska

Infektion med parasiten *Cryptosporidium hominis* är en vanlig orsak till diarré och buksmärtor över hela världen. I november 2010 insjuknade omkring 27.000 personer i Östersund på grund av förorenat dricksvatten.

Tidigare forskning har visat att långvariga magtarmsymtom, ledsymtom, trötthet och huvudvärk kan förekomma upp till 5 år efter infektionen, men det saknas studier på längre sikt. Den akuta fasen av utbrottet har kostat samhället omkring 70 miljoner svenska kronor i form av sjukfrånvaro, men det är oklart vad utbrottet har kostat på längre sikt. Läkare inom Region Jämtland Härjedalen tycker att antalet människor med kroniska inflammatoriska tarmsjukdomar, såsom Crohns sjukdom och ulcerös kolit, samt mikroskopisk kolit har ökat sedan utbrottet. Det här projektet undersöker de långvariga konsekvenserna av *Cryptosporidium*-utbrottet.

Strax efter utbrottet skickades en enkät till 1524 slumpmässigt utvalda invånare av Östersunds kommun, samt till ytterligare 500 barn mellan 0-5 år. De som svarade att de hade haft nytillkommen diarré under utbrottet definierades som fall och de som inte hade varit sjuka som icke-fall. Uppföljningar gjordes vid flera tillfällen, bland annat 5 och 10 år efter utbrottet.

Det visade sig att vuxna som drabbades av *Cryptosporidium*-infektion 2010, oftare rapporterade symtom än de som inte drabbades, framförallt från magen (3 gånger så ofta) och leder (2 gånger så ofta). Fall som rapporterade buksymptom vid både 5- och 10-årsuppföljningen hade i snitt varit sjuka längre (ca 9 dagar) under utbrottet än de som inte gjorde det (6 dagar). Drabbade barn rapporterar lite oftare symtom från leder samt trötthet, men de besöker inte oftare vården än icke-drabbade, och har samma sjukdomar.

För att kunna beräkna vad utbrottet har kostat samhället på längre sikt, har data samlats in för alla som svarade på första enkäten. Här ingick antalet dagar med sjukpenning och/eller vård av barn, antal besök till vårdcentral eller mottagning på sjukhus och antalet dagar med vård inneliggande på sjukhuset i Östersund för åren 2011-2019. Vi jämförde återigen fall med icke-fall och det visade sig att fler fall tog ut sjukpenning från Försäkringskassan jämfört med icke-fall (41% resp 31%). Fallen tog även ut fler dagar med sjukpenning: 8946 per 100 fall,

jämfört med 4538 dagar per 100 icke-fall. Omräknad till hela Östersunds befolkning har utbrottet kostat ca 1,7 miljarder svenska kronor för ökat uttag av sjukpenning. Det fanns ingen skillnad i uttag av vård av barn eller antal mottagningsbesök i primärvården eller på sjukhuset. Antalet sjukhusinläggningar var ungefär lika i båda grupperna, men dem som drabbades av *Cryptosporidium*-infektionen 2010 vårdades oftast kortare tid, troligen på grund av andra orsaker än själva infektionen.

Vi har även undersökt om förekomsten av inflammatorisk tarmsjukdom och mikroskopisk kolit har ökat i Region Jämtland Härjedalen. Data för denna studie kom från kvalitetsregistret SWIBREG och Region Jämtland Härjedalens journalsystem. Vi såg att de som var >40 år vid utbrottet hade en dubbelt så hög risk att få diagnosen Crohns sjukdom efter utbrottet jämfört med före. Även diagnosen mikroskopisk kolit ställs 6 gånger oftare efter utbrottet än innan. Detta kan tyda på att *Cryptosporidium*-infektion ökar risken för dessa sjukdomar.

Projektet har lett till ökad kunskap om långvariga symtom efter ett *Cryptosporidium*-utbrott. Denna kunskap kan bidra till att vården bemöter drabbade personer bättre och med större förståelse. Det har även visat att förekomsten av kroniska inflammatoriska tarmsjukdomar har ökat efter utbrottet, och att ett parasitutbrott kan innebära stora samhällskostnader – inte bara direkt, utan även på längre sikt.

1. Background

1.1. *Cryptosporidium* and cryptosporidiosis

Cryptosporidium is an intestinal protozoan parasite and a common cause of diarrhoea worldwide.[1,2] Edward Tyzzer first discovered the parasite in the gastric glands of a mouse in 1907, but it took until 1976 for the first human case to be identified.[3,4] The current global incidence has been estimated to be approximately 65 million (43-105 million) human cases each year.[5]

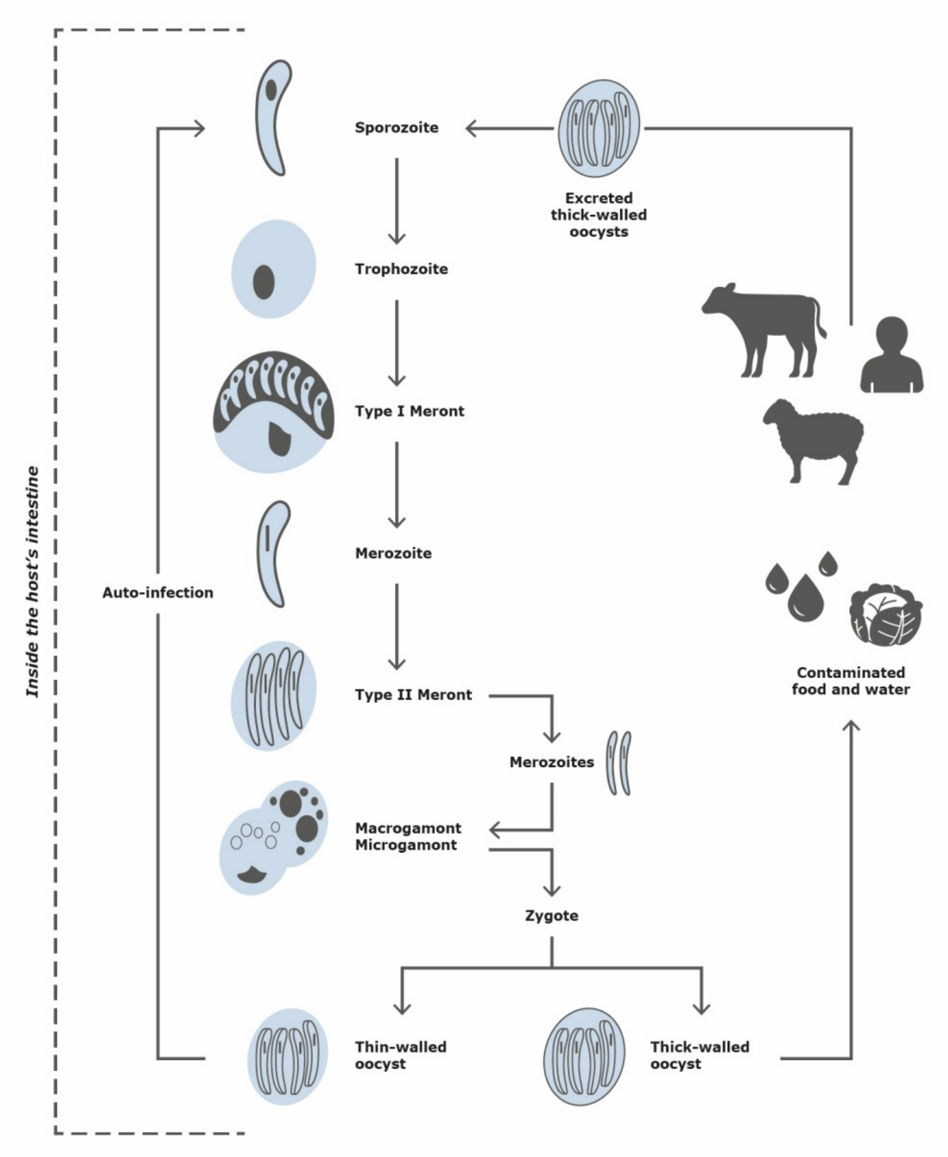
1.1.1. Microbiology

Cryptosporidium is a single-cell prokaryote and member of the phylum Apicomplexa. Apicomplexa have a so-called apical complex, an organelle on their surface that allows them to glide on the surface of the host cell and that plays a role in attachment to and invasion of the host cell.[6-8] The life cycle of *Cryptosporidium* spp. is complex but well described.[6,9-11] It is monoxenous, meaning that the entire life cycle takes place in one host. The life cycle of *Cryptosporidium* spp. can be divided into six stages (Figure 1). Sporozoites, the infective stage of *Cryptosporidium*, are "transported" between hosts in oocysts. The oocysts are capsules that enable the parasite to stay alive even in harsh conditions but will open up under favourable conditions (i.e., in the next host's intestines).

More than 40 species of *Cryptosporidium* have been identified. Most human cases are caused by *Cryptosporidium hominis*, which mainly infects humans and other primates, or the zoonosis *Cryptosporidium parvum*.[6,12,13] Approximately 8% of *Cryptosporidium* cases in Sweden are caused by a non-*hominis* or *parvum* species.[14] *Cryptosporidium mortiferum* is considered to be an emerging zoonosis in Sweden, with increasing numbers of domestic cases.[15]

More than 10 *C. hominis* subtype families have been identified by sequence analysis of the 60-kDa glycoprotein (*gp60*) gene, with Ia, Ib, Id, Ie, and If being the most common.[13] *C. hominis* subtype IbA10G2 is widely distributed around the globe and is the most prevalent subtype in Sweden and Europe, though IfA12G1R5 has taken over the lead as the most common subtype in the USA.[14-17]

Figure 1. *Life cycle of Cryptosporidium spp.*



The life cycle of *Cryptosporidium* explained

After ingestion of the oocyst, sporozoites are excysted in the lumen of the intestine, a process that is triggered by different elements in the gastrointestinal environment. Four sporozoites are released, glide over the intestinal cell using their apical complex, and enter the host cell. They develop into trophozoites that nest in parasitophorous vacuoles close to the surface of the mucosal epithelium. During the stage of merogony, or asexual multiplication, type I meronts containing eight merozoites develop. These merozoites are released and attach to the surface of a nearby host cell to form additional type I meronts or to form type II meronts. Type II meronts release four merozoites that attach to the host cell to continue to sexual replication. Merozoites produce either microgamonts, which develop up to 16 microgametes, or macrogamonts, which develop into a macrogametocyte. During the fertilisation stage, microgametes fertilise a macrogametocyte, creating a zygote. After asexual sporogony and oocyst wall formation, oocysts are released into the lumen of the intestine. Eighty percent of the zygotes become thick-walled oocysts that will be excreted within the faeces to infect another host. The remaining 20% have a thin wall and are intended for auto-infection. These excyst when they enter the lumen and are why many patients experience prolonged or recurring disease.

1.1.2. Epidemiology

Cryptosporidium is a highly infective pathogen usually transmitted via the faecal-oral route.[18,19] Ingestion of only a few oocysts may lead to disease even in healthy individuals.[8,20] During the acute infection and up to a week after recovery, the patient sheds large amounts of infective oocysts.[21] Therefore, household transmission is common, especially when the index patient is under 5 years of age and infected with *C. hominis*.[22] Occupational groups in contact with infected individuals or faeces, as well as young children are at increased risk.[7,22]

Common causes of infection are:

- Intake of contaminated drinking water,
- Intake of contaminated food,
- Contact with an infected human or animal,
- Contact with contaminated recreational water.[6,13,23]

The disease, cryptosporidiosis, is self-limiting in healthy individuals but may lead to severe disease, chronic infection, and even death in immunocompromised individuals and young children.[12] Not surprisingly, low-income countries bear a disproportionate burden of morbidity and mortality caused by *Cryptosporidium* spp.[24 25] The prevalence is estimated to be 5% to >15% in low-income countries, compared to <1% to 3% in high-income countries, mainly due to water quality, sanitary standards, population density, malnutrition, and immune status.[26-28] Between 2007 and 2017, the global prevalence of cryptosporidiosis in HIV/AIDS patients was >10%, and in children under 5 years of age it accounted for an estimated 20.1% of diarrhoea-related deaths, after rotavirus, *Shigella* spp., and adenovirus.[29 30]

Globally, *Cryptosporidium* caused an estimated 118,000 (75,300–178,000) deaths in 2021. It was the third most common cause of diarrhoea-related death in all ages (10.2%) after rotavirus (15.2%) and norovirus (10.6%).[30] However, despite its massive impact on low-income societies, it is not included in the World Health Organization's (WHO's) list of neglected tropical diseases.[31]

Less is known about the impact of *Cryptosporidium* in high-income countries. Several large outbreaks have been reported over the years.[32-34] The largest outbreak was in Milwaukee, Wisconsin, USA, in 1993 due to contaminated drinking water. More than 400,000 inhabitants were infected and 69 died, mainly elderly and immunocompromised patients.[35 36] The second largest outbreak occurred in 2010 in Östersund, Sweden. This outbreak was also caused by contaminated drinking water, resulting in approximately 27,000 cases (45% of the city's population).[34] Only 6 months later, the city of Skellefteå 450 km northeast of Östersund had another outbreak of *C. hominis* IbA10G2, the same subtype as in Östersund. It is most likely that inhabitants of Skellefteå visiting Östersund during the outbreak were infected and spread oocysts upon return to their hometown.[37]

Most of the cases in high-income countries are caused by smaller outbreaks or single/clustered cases, but there are notable differences between countries. In Sweden, the incidence of cryptosporidiosis increases in late summer and early autumn, with no increase during spring as in other European countries.[15 16 38-41] *C. parvum* is the most common species in both domestic and foreign cases reported in Sweden and most *C. hominis* cases are travel-related.[15] In the UK, *C. parvum* is typically associated with rural areas and contact with animals, whereas *C. hominis* is more commonly found in urban environments and among young children.[41] *C. hominis* is also a major cause of outbreaks related to swimming pools because oocysts are resistant to chlorination, and filtration of the water is often insufficient.[42][43]

Since cryptosporidiosis became a notifiable disease in Sweden in July 2004, the incidence increased from 0.8 per 100,000 to 6.0 per 100,000 in 2024 (Table 1).[44] This can be explained by both better diagnostic tools and increased awareness after the outbreak in Östersund. However, it is still an underestimation because protozoan infections are underrecognised.[45] A UK study estimated eight undiagnosed cases for every case reported to the national surveillance system.[46]

1.1.3. Cryptosporidiosis in children

Research on cryptosporidiosis in children has been conducted mainly in low-income countries. In regions with a high prevalence of *Cryptosporidium*, infection in young children is widespread, with a peak infection rate around 9 months, after which the incidence plateaus.[47][48] Young age, low birth weight, and male sex are known risk factors for disease in low-income countries.[48-51] Cryptosporidiosis is associated with an increased risk of death, especially in those under 2 years of age.[52] Furthermore, asymptomatic infection is associated with negative consequences on both growth and cognitive development.[53]

Cryptosporidium is also a common cause of diarrhoea in children in high-income countries: a Danish prospective study using PCR detected *Cryptosporidium* in 4 of 304 children with diarrhoea (1.3%).[54] However, most research conducted in high-income settings dates primarily from the 1990s. Risk factors identified in these studies are immune suppression, HIV, and age ≥1 year.[55][56] Risk factors that were identified in connection to the Östersund outbreak were a high level of water consumption, male sex, and a history of loose stools.[57] Having a diverse microbiome might reduce symptoms.[58]

Table 1. *Number of reported domestic and foreign cryptosporidiosis cases in 2004-2024. Incidence per 100,000 inhabitants per year for total and domestic cases only.*[44]

	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
Cases, total, *n*	47	69	103	110	148	159	392	379	238	224	404
Cases, Sweden, *n* (%)	17 (35.0)	22 (32.0)	31 (30.0)	35 (32.0)	65 (44.0)	59 (37.0)	275 (70.0)	271 (72.0)	114 (48.0)	123 (55.0)	246 (61.0)
Cases, abroad, *n* (%)	10 (21.0)	45 (65.0)	67 (65.0)	72 (65.0)	80 (54.0)	95 (60.0)	93 (24.0)	101 (27.0)	120 (50.0)	100 (45.0)	151 (37.0)
Cases, unknown/ missing data, *n* (%)	20 (43.0)	2 (3.0)	5 (5.0)	3 (3.0)	3 (2.0)	5 (3.0)	24 (6.0)	7 (1.0)	4 (2.0)	1 (0.0)	7 (2.0)
Incidence Sweden /100,000	0.19	0.24	0.34	0.38	0.70	0.63	2.92	2.86	1.19	1.28	2.52
Incidence total /100,000	0.52	0.76	1.13	1.20	1.60	1.70	4.17	4.00	2.49	2.32	4.14

	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024
Cases, total, *n*	527	594	779	715	1088	637	524	716	741	635
Cases, Sweden, *n* (%)	313 (59.0)	341 (57.0)	457 (59.0)	354 (50.0)	771 (71.0)	551 (86.0)	415 (79.0)	550 (77.0)	519 (70.0)	440 (69.0)
Cases, abroad, *n* (%)	207 (39.0)	243 (41.0)	303 (39.0)	345 (48.0)	304 (28.0)	72 (11.0)	83 (16.0)	147 (21.0)	200 (27.0)	183 (29.0)
Cases, unknown/ missing data, *n* (%)	7 (2.0)	10 (2.0)	19 (2.0)	16 (2.0)	13 (1.0)	14 (3.0)	26 (5.0)	19 (2.0)	22 (3.0)	12 (2.0)
Incidence Sweden /100,000	3.18	3.41	4.52	3.46	7.47	5.31	3.97	5.23	4.92	4.16
Incidence total /100,000	5.35	5.94	7.70	6.99	10.53	6.14	5.01	6.81	7.04	6.00

1.1.4. Symptoms of cryptosporidiosis

After a dose-dependent incubation period of 3-12 days, but usually 5-7 days, cryptosporidiosis presents as an acute gastroenteritis, with frequent passing of mostly watery diarrhoea that might also contain mucus or blood.[59-61] Abdominal pain is almost always present.[62] Other common symptoms are nausea, vomiting, fever, fatigue, and weight loss.[2 23] Symptoms are self-resolving in immunocompetent individuals, but some patients experience prolonged (≥7 days) or persistent (≥15 days) diarrhoea.[23 62] Shedding of oocysts continues for a mean period of 7 days after symptoms cease.[23] Relapse of gastrointestinal symptoms is common and was reported by >30% of cases after the outbreak in Milwaukee and by 40% of cases in a UK study.[35 62]

Immunocompromised patients, such as individuals with AIDS, haematological malignancies, or other T-cell deficiencies, are prone to severe disease or chronic infection, with increased morbidity and mortality.[19 63 64] In these patients, involvement of the biliary and respiratory tracts has been reported.[19 65]

Clinical presentation might vary slightly between species. Infection with *C. hominis* might take a little longer to resolve than infection with *C. parvum*, possibly because oocysts are shed for a longer period of time and the numbers detected in stools are higher.[66-68] It has also been suggested that *C. hominis* may cause a more severe infection with more accompanying symptoms, but other studies could not confirm these reported differences.[60 68 69]

It is possible to build immunity against infection given the low incidence rates found in adults in low-income countries and in rural areas, where individuals might be frequently exposed to a low number of oocysts.[70] Longitudinal studies in children and experimental studies on purposely infected adult volunteers indicate the development of protective immunity, as prior exposure reduces parasite burden and symptom severity.[71] Several antigens associated with immunity were identified recently.[72 73]

Asymptomatic carriage has also been reported. Prospective studies conducted in daycare nurseries in Spain and the UK found that between 0.9% and 1.3% of young children were asymptomatic carriers of the parasite.[74 75] In one outbreak at a daycare centre in Belgium, extensive testing revealed that 24% of children infected with *C. hominis* presented no symptoms.[76] Although there is evidence from low-income countries that asymptomatic cryptosporidiosis in young children can negatively impact growth in the months following infection, whether asymptomatic infections can lead to long-term sequelae remains unclear.[77 78] Asymptomatic carriage has also been reported in individuals with an immunodeficiency and in healthy adults.[22 67 79]

1.1.5. Diagnosis of cryptosporidiosis

It is often difficult to differentiate between pathogens based on clinical presentation. Microscopic examination of stools for the presence of oocysts using tinctorial, fluorescent, or immunofluorescent stains has long been used to confirm *Cryptosporidium* infection.[80] Due to varied shedding, detection rates increase significantly when using a triple faeces test.[81]

Alternative diagnostic options are gaining in popularity, and PCR techniques are increasingly available. The increasing use of multiplex assays will probably increase detection rates and, thus, incidence rates.[39 45] This has been the case in Norway since implementing molecular diagnostic methods on a large scale.[82] However, some of the available PCR assays were developed only to detect infection by *C. parvum* and *C. hominis*, which are so alike that they were previously thought to be one species.[9] Species that are too different from these might not be detected, which affects incidence rates and the number of detected species.[83 84]

DNA sequencing of *gp60*, the gene encoding a 60 kDa glycoprotein, has been the standard for subtyping *Cryptosporidium* species. However, a new technique called multi-locus variable-number tandem-repeat analysis (MLVA) has proven to have higher discriminative power than *gp60* sequencing, making it suitable for use in outbreak investigations. However, it is now used only for *C. parvum*.[85 86]

Several studies have shown that the rate of *Cryptosporidium* infection is underestimated due to the clinician's choice of analysis (e.g., to only test for bacterial agents). A Swedish study tested 803 stool samples that had previously only been tested for gastrointestinal bacteria and found 16 (2%) cases of cryptosporidiosis.[45] A prospective Danish study found that, of 304 samples from children up to 16 years of age, 4 (1.3%) tested positive for *Cryptosporidium*.[54]

In the UK, all patients with community-acquired diarrhoea are routinely tested for *Cryptosporidium* spp.[80] Unfortunately, this is not the case in many other countries, where the doctor actively has to request the sample be tested for *Cryptosporidium* oocysts. With the increasing availability of PCR testing, hopefully other countries will eventually include parasites such as *Cryptosporidium* and *Giardia* in routine testing. In Sweden, some healthcare regions have started performing PCR tests including *Cryptosporidium*, which has resulted in higher incidence rates compared to regions that do not test for *Cryptosporidium* by default.[44] In Region Jämtland Härjedalen (RJH), a PCR panel has been introduced for gastrointestinal pathogens, including *Cryptosporidium*, but it is currently mainly used in hospitalised patients.

1.1.6. Treatment of cryptosporidiosis

Despite cryptosporidiosis being a common cause of diarrhoea with significant morbidity and mortality, only one drug is registered for its treatment in the USA: nitazoxanide.[12] It is not formally registered in Europe but might be available on a case-to-case basis.[19] Availability in other parts of the world varies from over-the-counter access in some Latin American countries to increasing access in Africa. Nitazoxanide has broad-spectrum activity against parasites, viruses, and bacteria, but its mechanism of action against *Cryptosporidium* remains unsolved. Unfortunately, it only has a moderate effect on the recovery of immunocompetent adults, and it was not better than placebo in immunocompromised patients.[87,88] Therefore, its efficacy is assumed to be related to the host's immune status. Many other drugs, including paromomycin, macrolides, somatostatin analogues, and serum immune globulins, have been tested without clinically relevant effects. During the last few years, research has focused on phenotypic screening, which has resulted in possible drugs targeting lipid and protein kinases, glycolytic enzymes, mRNA processing, and tRNA synthetases. [87,89]

1.2. Long-term consequences of *Cryptosporidium* infection

1.2.1. Long-term symptoms

Sequalae are common following bacterial and viral gastrointestinal infections and include conditions such as post-infectious irritable bowel syndrome (IBS), functional diarrhoea or constipation, dyspepsia, and extra-intestinal manifestations, such as joint pain.[90] For the diagnosis of post-infectious IBS (PI-IBS), symptoms should be present at least 1 day a week in the last 3 months, with the onset of symptoms at least 6 months prior.[91] However, the definition of "long-term symptoms" varies widely across studies. In this thesis, long-term symptoms are defined as symptoms emerging after the acute phase of infection and may persist for an undefined period of time.

Research on long-term symptoms after infection with parasites has not been extensive. A number of publications have described long-term symptoms up to 5 years after infection with *Cryptosporidium*, such as chronic diarrhoea, headaches, fatigue, joint pain, and eye pain.[2 62 92-96] However, longer-term follow-ups are lacking. Infection with *C. hominis* might cause more long-term symptoms than infection with *C. parvum*.[62]

The Norwegian city of Bergen was affected by a large outbreak with the parasite *Giardia lamblia* in 2004. Chronic fatigue and IBS were reported up to 10 years after the outbreak.[97-100]

Long-term consequences in children might differ from those in adults. Young, malnourished children are particularly at risk for disease. In this group, the prevalence of cryptosporidiosis is estimated at 10-20%, and even asymptomatic or mild cryptosporidiosis has been associated with stunting, poor mental and physical development, and excess death from other causes.[48 52 101-103] Among children living in a Brazilian slum, cryptosporidiosis was shown to impact physical fitness 4-7 years after the initial infection, especially in children under 2 years of age.[104] How children in high-income countries are affected is largely unknown. They were included in several follow-up studies, but the numbers were low and children were not analysed separately.[62 66 92-94]

1.2.2. Irritable bowel syndrome

IBS is a functional gastrointestinal disease with abdominal pain and diarrhoea, constipation, or sometimes both as the primary symptoms. It is a clinical diagnosis, and the diagnosis can be made using the Rome criteria.[91 105] For a diagnosis, the patient should have had recurrent abdominal pain for at least 6 months and on average at least 1 day per week in the last 3 months. This pain should also be associated with at least two of the following criteria:

- Related to defecation,
- Associated with a change in the frequency of stool,
- Associated with a change in the form (appearance) of stool.

The prevalence of IBS in high-income countries is considerable, 6-12%, and women are more often affected than men.[106,107] Those who suffer from anxiety or depression have a doubled risk of experiencing and maintaining IBS symptoms.[108]

Acute gastroenteritis increases the risk of IBS, especially for individuals who suffer from severe infection.[90,109,110] The strongest evidence has been presented for bacterial infections, but it might also apply to infection with parasites such as *G. lamblia* and *C. hominis*.[93,97] How infection contributes to developing and maintaining symptoms of PI-IBS is not completely understood, but dysbiosis in the intestinal flora, activation of the intestinal immune system, and changes in gut-brain axis signalling are thought to contribute.[111]

1.2.3. Inflammatory bowel disease

Inflammatory bowel disease (IBD) is a chronic, immune-modulated, inflammatory disorder of the gastrointestinal tract and includes ulcerative colitis (UC), which mainly affects the colon; Crohn's disease (CD), which can affect any part of the gastrointestinal tract; and IBD-unclassified, which is the term used when a distinction cannot be made between UC and CD. IBD mostly affects people of working age, with a peak incidence in the third decade. The global prevalence is approximately 0.5% and rising, particularly in developing countries where populations are increasingly adopting a Western lifestyle.[112] The prevalence of IBD is somewhat higher in Sweden, approximately 0.65%.[113] It is not entirely clear why individuals develop IBD, but it is assumed to be the result of an interaction between genetic predisposition and environmental triggers, with gut microbiota dysbiosis seeming to serve as a unifying mechanism.[114] The gut microbiota fulfils important roles within its host, from digesting non-absorbable dietary components and the synthesis of essential vitamins, to modulation of the epithelial mucosal barrier and maturation of the intestinal immune system.[114-116] Dysbiosis occurs when the microbiota can no longer fulfil these roles, allowing pathogens to take over. In IBD patients, dysbiosis is characterised by a decline in beneficial bacteria, less microbial diversity, and pathogen proliferation, and a correlation has been demonstrated between intestinal dysbiosis and IBD onset.[114] Studies have suggested that bacterial dysregulation is common in IBD patients, but even mycobiota dysregulation and imbalance in the gut virome have been proposed as potential pathophysiological mechanisms in IBD, causing decreased microbiota diversity and enhanced inflammatory responses.[114,117,118]

It is highly accepted that environmental factors play a key role in the development and maintenance of IBD. A large systematic review of meta-analyses showed that risk factors include smoking, Western-style diets including ultra-processed foods, and antibiotic use early in life, whereas protective factors include breastfeeding, fibre-rich and plant-based diets, fish consumption, and maintaining an active lifestyle.[119] These conclusions have also been supported by migrant studies. For example, a Danish study showed that first-generation migrants have a lower risk of developing IBD compared to the native population, whereas second-generation immigrants have a risk comparable to the native population.[120]

Bacterial gastroenteritis, as well as infection with *Amoeba* spp. and *Toxoplasma gondii*, has been associated with an increased risk of eventually developing IBD.[121 122] Whether this also applies to *Cryptosporidium* infections has not yet been established, but cryptosporidiosis has led to the development of IBD-like symptoms in animal models with defective T-cell receptor-α or -β.[90 123] Cryptosporidiosis has also been suggested to cause relapse of disease in patients with known IBD.[124] Physicians in RJH appreciate that the incidences of IBD and microscopic colitis (MC) increased in RJH after 2010.

1.2.4. Microscopic colitis

MC is a chronic inflammatory condition of the colon that is only visible under a microscope. Although it is considered a form of IBD, it is typically not included in standard discussions of IBD due to its distinct clinical presentation and the population it affects. MC is mainly a disease of the elderly, with a peak incidence in the seventh decade, and it affects more women than men.[125 126] The global incidence of MC is increasing but in Sweden the numbers have been stable for the last two decades, with an estimated lifetime risk of 0.9% in women and 0.4% in men.[126-128] In Denmark, the incidence of MC has remained stable since 2012.[129]

There are two main types of microscopic colitis: lymphocytic colitis, characterised by increased numbers of intraepithelial lymphocytes, and collagenous colitis, in which subepithelial collagen band thickening is seen.[114] The pathophysiology of MC is largely unknown, but an altered interaction between the immune system, intestinal flora, and other environmental factors is suspected.[114 130] Smoking, alcohol consumption, and autoimmune disease increase the risk of developing MC, whereas obesity or adult weight gain might have a protective effect in women.[125]

Some studies have shown an elevated risk of developing MC after gastrointestinal infections.[131,132]

1.2.5. Other long-term conditions reported

Reactive arthritis is a peripheral mono- or oligoarthritis that occurs as a reaction to infection elsewhere in the body. In a majority of cases, the patient has the HLA-B27 gene that is associated with autoimmune diseases, including rheumatic arthritis and IBD.[133] Reactive arthritis might be self-limiting, lasting months, or it may lead to chronic symptoms lasting for years. Primarily, bacteria have been associated with reactive arthritis, but some case studies have reported reactive arthritis after cryptosporidiosis in both adults and children.[134-136]

Guillain-Barré syndrome is a demyelinating polyneuropathy that might occur after gastrointestinal infection with bacteria, such as *Campylobacter jejuni*, or viruses, such as cytomegalovirus and Epstein-Barr virus, amongst others. There is no compelling evidence for a connection between cryptosporidiosis and Guillain-Barré syndrome, but a case report has been published on recurring Guillain-Barré syndrome 2 weeks after laboratory-verified infection with *Cryptosporidium*.[137]

An estimated 20% of all cancers can be attributed to infection.[138] Some of these associations are well established, such as those between hepatitis B and C viruses and hepatocellular carcinoma, and between *Helicobacter pylori* and gastric cancers. Several studies have reported a higher incidence of cryptosporidiosis in cancer patients, but it is unclear if cryptosporidiosis could increase the risk of cancer.[138-141]

1.3. Östersund and the *Cryptosporidium* outbreak

Östersund is the only city in RJH, a sparsely populated area of nearly 50,000 km^2 with 130,000 inhabitants, and half of them live in the city (Figure 2). RJH refers both to the geographic area and to the regional health authority that is the principal provider of healthcare services within this area. Before the COVID-19 pandemic, the numbers of individuals moving to and from the region were relatively low. Commuting from Östersund to other regions is limited due to its central location within the region and long distances to other cities. Residents of RJH that need secondary care are all referred to the region’s only hospital in Östersund.

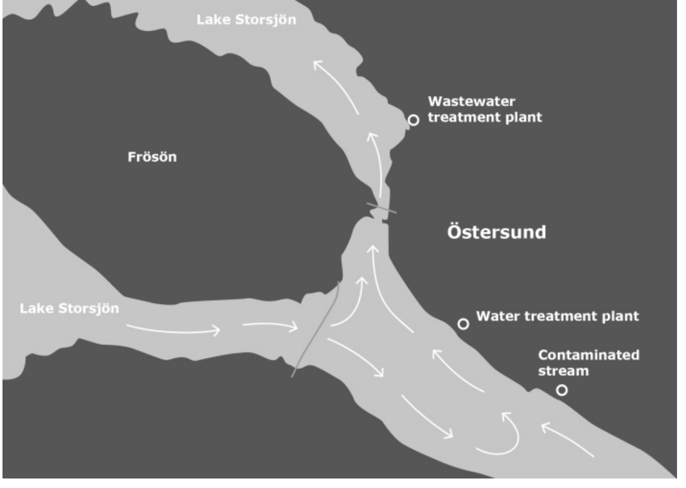
__Figure 2.__ Map of Sweden, Region Jämtland Härjedalen, and Östersund. Printed with permission from Region Jämtland Härjedalen.

1.3.1. Drinking water supply

Water treatment plant *Minnesgärde* in Östersund supplies around 51,000 of the city's inhabitants with drinking water (Figure 3). It was built in the 1970s, 4 km upstream from the wastewater plant to avoid contamination. Each day, ~16 million litres of surface water is cleaned and distributed. The water taken from Lake Storsjön is of good quality from the start. First, it is alkalised to neutralise acids in the water and to maintain a stable pH, and then it is purified using flocculation, sedimentation, rapid sand filtering, chloramination, and ozonation. Twice a week, raw water (at the intake from the lake, prior to any treatment) and consumption-ready water are sampled and checked for pathogens and chemicals according to the guidelines of the Swedish

Food Agency.[142] In 2010, this process followed the Swedish guidelines for water treatment plants but was not sufficient to destroy oocysts, which are very environment-resistant.[34]

Figure 3. *Map of Östersund and Lake Storsjön, showing Minnesgärde water treatment plant, the wastewater treatment plant, water currents, and a contaminating stream.*



1.3.2. The outbreak

An increase in cryptosporidiosis cases in Östersund was noticed in November 2010. Initially, a relation to food was suspected because of several small (independent) outbreaks elsewhere in the country in October 2010.[143] However, several employers in the city reported to the County Medical Office that 10-20% of their employees were suffering from gastroenteritis. At the same time, the local health advice line received several calls from persons with gastroenteritis, many of them living in Östersund municipality.[144] Contamination of the drinking water was then assumed by the authorities and a boil water advisory issued for Östersund municipality. Östersund municipality took samples from raw water and drinking water and sent them to the Swedish Institute for Communicable Disease Control, which confirmed the presence of *Cryptosporidium* in both raw and drinking water on 29 November.[143] The Swedish Institute for Communicable Disease Control also received faecal samples related to the outbreak and was able to confirm the presence of *C. hominis* using the PCR-restriction fragment-length polymorphism (RFLP) technique, excluding a zoonotic source. Further subtyping confirmed an identical subtype, IbA10G2, in all tested samples, confirming a single source of contamination.[143]

It has never become entirely clear how Lake Storsjön was contaminated, although higher densities of *Escherichia coli* measured in raw water in the weeks before the outbreak suggest that faecal contamination from a human source is a probable explanation.[34] A small water stream that runs into Lake Storsjön upstream of the raw water intake was found to be contaminated with wastewater. The levels of *C. hominis* in these samples were higher than in other raw water samples, making it a plausible, but not definite source of the outbreak.[143] Based on the questionnaire described in the methods section of this thesis, approximately 27,000 individuals, or 45% of Östersund's population, is estimated to have contracted clinical cryptosporidiosis during the outbreak.

1.3.3. Investigations

<u>Faecal samples</u>
Faecal samples from inhabitants with acute gastroenteritis from 1 November 2010 through 31 January 2011, were tested for bacteria, viruses (PCR), *Entamoeba* spp. and *Giardia lamblia* (conventional light microscopy) and *Cryptosporidium* oocysts (standard concentration techniques and modified Ziehl-Neelsen staining). 38 faecal samples were further analysed, determining the *Cryptosporidium* species using PCR-RFLP analysis of the 18S rRNA gene and sequence analysis of the *gp60* (60-kDa glycoprotein) gene.[34]

In total, 186 laboratory-confirmed cases related to the outbreak were reported to the national surveillance system, of which 149 cases were diagnosed in Region Jämtland Härjedalen. These 149 samples all tested negative for other gastrointestinal pathogens.[34]

<u>Water samples</u>
A total of 163 samples were taken from raw water, drinking water, and wastewater in an attempt to trace the source of the outbreak and to monitor the number of circulating oocysts. Most samples were taken at or near *Minnesgärde* and the main wastewater plant.

C. hominis IbA10G2 was identified in two samples from the stream closest to the main water treatment plant, in five samples from untreated wastewater, and in four samples from other wastewater treatment plants in Jämtland County. No other *Cryptosporidium* species or subtypes were detected in any of the analysed samples, indicating that the outbreak was the result of a single source.[34]

The highest number of oocysts in the drinking water was detected on 12 December 2010 (1.4 presumptive oocysts/10 L), but the average density in drinking water was low (0.32/10 L in samples from the water treatment plant and 0.20/10 L in samples from the distribution network). How these low densities led to the high attack rate is unclear. Suggested reasons were a short peak of oocyst density before the outbreak and widespread secondary transmission within households.[34]

The number of oocysts in wastewater samples peaked on 29 November 2010 (270,000/10 L) and then declined within 4 weeks to pre-outbreak levels (≤200/10 L).[34]

Coincidentally, the Swedish Institute for Communicable Disease Control had an ongoing project on the prevalence of parasites in wastewater, including the wastewater plant in Östersund. The sample taken on 8 November 2010 contained 20,000 oocysts/10 L, but the sample from 16 November 2010 contained 180,000/10 L, indicating that the outbreak had started before it was noticed through other channels; however, these samples were not analysed until after the outbreak started.[143]

1.3.4. Confirmed cases

Only a minority of patients had their diagnosis confirmed by faecal microscopy. A small unpublished study was performed using data from all confirmed cases from the outbreaks in Östersund and Skellefteå; 285 individuals were identified, with 247 (87%) consenting to participate in the study. A total of 160 women and 87 men were included, comprising 126 participants from Östersund and 121 from Skellefteå. Ninety-eight individuals were hospitalised (40%): 52 (53%) women and 46 (47%) men, with an average age of 46 years. Logistic regression analysis was performed to identify risk factors for admission to the hospital due to *Cryptosporidium* infection, which were the presence of a stoma, IBD, immunosuppression, and the presence of malignant disease. Furthermore, age >60 years and male sex were associated with hospitalisation. However, the results should be interpreted with caution, as the number of patients was small, but the findings indicate which patient categories are at highest risk when infected with *C. hominis*.[145]

1.3.5. The end of the outbreak

Östersund municipality was able to buy a UV system suitable for neutralising oocysts from Katrineholm municipality, and it was effective as of 23 December 2010.

The distribution system was flushed several times, and samples from outgoing water and tap water were controlled on multiple occasions before the water was considered safe for consumption. The boil water advisory was withdrawn on 18 February 2011.[143]

1.3.6. After the outbreak

After the outbreak, awareness of parasitic infections and water safety in general increased tremendously. Many other municipalities evaluated their level of efficiency, and UV-filtration became a standard procedure throughout Sweden. Östersund municipality took it even further and decided that the latest nanofiltration membranes for purifying water should be added to the process.[146] However, it later became clear that it was not possible to install the filter in the old treatment plant without risking the continuity of the production and distribution of drinking water. This was one of the reasons for the decision to build a new water treatment plant next to the current one.[147] The new water treatment plant is expected to be ready in 2029.

In 2018, a national surveillance system for *Cryptosporidium* was implemented by the Public Health Agency of Sweden (*Folkhälsomyndigheten*, formerly *Smittskyddsinstitutet*). During the first 5 years, 3,684 cases of cryptosporidiosis were reported to SmiNet, the national system for reporting communicable diseases, of which 2,639 (72%) were domestic. Most infections were caused by *C. parvum* (n = 1,412, 91%), followed by *C. mortiferum* (previously known as chipmunk genotype I) (n = 59, 4%) and *C. hominis* (n = 56, 3.7%). *C. parvum* was more commonly reported in women (58%), whereas *C. hominis* and *C. mortiferum* were more common in men (56% and 53%, respectively). *C. hominis* was responsible for >20% of cases in children aged 0-9 years but for <5% of cases in those aged ≥10 years. The most commonly reported subtype of *C. hominis* was IbA10G2 (56% of *C. hominis* cases). Several smaller outbreaks were detected during this time frame, mostly foodborne, but some were due to contact with infected animals; however, none were due to contaminated drinking water or recreational water.[15]

1.4. Health economics

Health economics is the discipline of applying the methods of economic analysis to the healthcare sector. It provides a framework for evaluating the costs and consequences of health interventions, supporting decision-making in both health policy and clinical practice.[148,149]

1.4.1. Health-economic evaluations and perspectives

There are several methods of health-economic evaluation:

- Cost-minimisation analysis: compares costs only, assumes equal effect on outcome.
- Cost-effectiveness analysis: compares the costs for each life year gained. Threshold values are used to assess whether the cost per life year gained indicates a cost-effective intervention.
- Cost-utility analysis: adjusts outcomes for quality. Threshold values are used to assess whether the cost per quality-adjusted life year indicates a cost-effective intervention.
- Cost-benefit analysis: compares costs with benefits, including health improvement (in monetary values). The value of health improvements is commonly assessed through studies that estimate an individual's willingness to pay for gains in health outcomes.

In addition to the type of health-economic evaluation, the perspective used is also a key element within health economics. Analyses can be carried out from a societal perspective, including costs and benefits regardless of who experiences them; a healthcare perspective, including direct medical costs only; or a patient perspective, including their expenses and the impact on quality of life.[148,149]

By combining methods and perspectives, health economics provides insights into how the health of a population can be maximised using the available, limited resources.

1.4.2. Health-economic evaluations of *Cryptosporidium* outbreaks

The cost to employers directly connected to the Östersund outbreak was approximately 70 million SEK (€ 7 million).[150] Economic evaluations of several other *Cryptosporidium* outbreaks have been published and also focused on the costs during and/or immediately after the outbreak.[151-153] Long-term symptoms might generate costs for the individual and society but have been sparsely investigated, despite productivity loss representing one of the most substantial economic consequences of outbreaks, particularly in high-income countries.[154] A Dutch study estimated cost-of-illness for *Cryptosporidium* in The Netherlands to be €19.2 million for 50,000 cases in 2017. They found that productivity losses were the most important costs, contributing to approximately 90% of the total costs, and estimated that long-term manifestations accounted for 7% of the total costs.[155] An earlier report from The Netherlands estimated that the costs of acute infection were €5 million, which increased to €7 million when including complications afterwards.[156] To date, no long-term economic evaluations have been published after parasite outbreaks. However, if long-term post-infectious symptoms are expected, it is essential to incorporate a health-economic evaluation of the years post-outbreak to fully assess the overall impact.

1.5. *Cryptosporidium* from a One Health perspective

Since the start of this century, the wellbeing of people, animals, and ecosystems have been increasingly recognised as being deeply interconnected. Approximately 75% of the diseases that have emerged in the last few decades originated in animals and were heavily affected by humanity, with contributing factors including livestock farming and other agriculture, animal trade, climate change, and urbanisation.[157] The WHO, the World Organisation for Animal Health, and the Food and Agriculture Organization (FAO), as well as the United Nations Environment Programme since 2022, cooperate in the One Health initiative. This initiative appreciates that integration of human, animal, and environmental health is essential for improving global health and pursues initiatives to do so.

Key areas for the application of a One Health approach include:
- Antimicrobial resistance,
- Food safety and foodborne diseases,
- Environmental health, including water and air pollution and climate change,
- Vector-borne diseases,
- Zoonotic diseases.

A more holistic approach is important, as >95% of 500,000 diarrhoea-related deaths each year are estimated to be preventable.[157]

1.5.1. *Cryptosporidium* in drinking water

Most waterborne pathogens end up in drinking water due to faecal contamination from either a human or animal source.[158] Therefore, the consumption of contaminated surface water and groundwater is strongly linked to waterborne outbreaks, especially those related to *Cryptosporidium* species.[1] [159] In addition, oocysts are able to survive in the water for months, as was seen in Östersund.[34]

The detection of parasites such as *Cryptosporidium* in water sources is often a lengthy and costly process due to small numbers of organisms in large water volumes. New technologies, such as biosensors, have shown promising results and provide rapid, good-quality, on-site analysis for lower prices, which improves speedy detection. As biosensors are easy and cheap to produce, they would be implementable even in low-income countries.[160] [161]

Treatment of drinking water is necessary to eliminate pathogens and other contamination. Treatment can be achieved by separation (infiltration, filtration, or nanofiltration) or inactivation (chlorination, ozonation, UV irradiation). Chemical treatment is not always successful, as some pathogens, such as viruses and oocysts, are resistant. Therefore, other techniques, such as reverse osmosis and nanofiltration, are increasing in popularity for drinking water treatment.[162] UV irradiation or nanofiltration is required to eliminate *Cryptosporidium* oocysts.[162] [163]

If and how drinking water is treated differs between countries, and even between treatment plants. In Sweden, the Swedish Food Agency has published guidelines on the treatment and control of drinking water, with ample opportunities for water treatment plants to choose what is most suitable for them while having clear minimum requirements to which they have to conform.[142]

1.5.2. *Cryptosporidium* as a foodborne pathogen

Cryptosporidium is considered a significant foodborne pathogen and was ranked second after *Echinococcus multilocularis* in a risk analysis of foodborne parasites in Northern Europe by the WHO and FAO, and ranked fifth in Europe after *E. multilocularis, T. gondii, Trichinella spiralis,* and *Echinococcus granulosus*. The ranking was based on different factors and weighting, including but not limited to the number of cases, severity of morbidity, geographic distribution, and case-fatality ratio.[164] Despite this, surveillance varies widely for the different pathogens and between countries.[165] Within Northern Europe, cryptosporidiosis is not a notifiable disease in Denmark but is notifiable for human cases in Sweden, Norway, Finland, and Iceland. Surveillance is limited to passive surveillance (clinical case reporting) in the human population, except in Denmark, which has active surveillance (sampling according to a specified plan) in cervids.[165]

In 2023, 18 of 35 parasite-related foodborne outbreaks reported in the EU were caused by *Cryptosporidium* spp.[166] It is often difficult to find the source of a suspected foodborne *Cryptosporidium* outbreak, primarily because of the longer incubation period compared to other foodborne pathogens.[167 168] The Nordic countries are overrepresented when it comes to reports on foodborne outbreaks. There is no obvious explanation as to why this is the case, but it has been suggested to potentially be due to a better infrastructure and more directed investigation and reporting.[168]

In recent years, a substantial increase in the incidence of foodborne diseases has been linked to the consumption of fresh vegetables, which are often imported from tropical areas with suboptimal hygiene.[15 166 169] The increasing demand for raw fruits and vegetables out of season leads to increased global transportation and a risk of foodborne disease outbreaks in several places simultaneously.[170] Even inadequate pasteurisation of milk has been the cause of outbreaks.[171 172] The increased popularity of raw food consumption has also increased the risk of exposure to foodborne parasites.[173]

1.5.3. *Cryptosporidium* in animals

Cryptosporidium is a zoonotic pathogen that infects both animals and humans and can be transmitted between species. The most common zoonotic species is *C. parvum*, which is also the most common cause of diarrhoea in calves. The infection causes damage to the intestinal epithelium, even in subclinical cases, and reduces growth rates, leading to increased costs and decreased production for the farmer.[18 174 175] Interestingly, the number of reports of cattle infected with *C. hominis* is increasing.[176]

A study found that 96% of dairy herds in Sweden were infected with *Cryptosporidium*, and 52% of the investigated calves were shedding oocysts. Prevalence decreased with increasing age, from 29% in young stock to 5.6% in cows.[175] Even though the prevalence worldwide varies between countries and studies, it is likely that almost all calves are infected in the first weeks of life.[174] As oocysts are tolerant to most disinfectants, it is nearly impossible to eliminate *Cryptosporidium* from the herd, but hygienic measures can be taken to reduce its spread. Some drugs have proven to be able to reduce oocyst shedding and to reduce the severity/duration of diarrhoea. Most often used is halofuginone lactate, but it has a risk of toxicity in dehydrated animals. Therefore, it is mainly used to prevent infection in the youngest of calves.[87] Recently, the vaccine Bovilis Cryptium® (MSD Animal Health) was approved by the European Medicines Agency for active immunisation of pregnant cows. It was developed to reduce diarrhoea and improve weight gain in calves via passive immunisation through the colostrum.[177] The first field studies showed that it reduced the duration of diarrhoea in calves from vaccinated cows, but it did not reduce the incidence and severity of disease.[178]

C. parvum is also common in lambs and goat kids, making petting zoos a potential source of contamination.[174 179-182] People in contact with agricultural animals, such as farmers and veterinarians, are also at high risk of contracting cryptosporidiosis.[183-186] *C. parvum*, as well as other subspecies with zoonotic potential, also infects ruminants, pigs, birds, equine animals, primates, rodents, and even fish.[13 183 187] Even common pets such as dogs and cats can be infected by *Cryptosporidium* spp., mainly *Cryptosporidium canis* in dogs, *Cryptosporidium felis* in cats, and *C. parvum*. However, *C. canis* and *C. felis* cause only incidental cases in humans and the risk of transmission is considered to be low, despite some studies reporting a significant prevalence in domestic cats and dogs. [188-191] The increasing popularity of feeding raw meat to domestic animals has been suggested to increase the risk of infection with parasites.[192]

The risk of *Cryptosporidium* outbreaks is considered to be increasing. One of the reasons is the increased use of wastewater and agricultural water for the irrigation of fields with produce.[183] Several measures can be implemented to reduce the risk of zoonotic cryptosporidiosis through livestock:

- Manure treatment through composting (heating, >60°C) and slurry storage (ammonia and low pH) to reduce oocyst viability,
- Protection of water catchments from contamination with livestock faeces,
- Prevention and control of infection in livestock through clean pens, hygiene measures, and treatment of infected animals.[18]

1.5.4. Effects of climate change and weather

Climate change resulting in more episodes of extreme weather increases the risk of *Cryptosporidium* outbreaks.[27, 193-195] In the summer of 2023, a rapid increase in *Cryptosporidium* cases was seen in several European countries at the same time. The European Centre for Disease Prevention and Control (ECDC) deemed it most likely to be caused by the extreme weather the weeks prior, even though others found multifactorial causes more likely.[196, 197]

The effects of heavy rainfall and rising temperatures might be direct, by supporting the spread and survival of oocysts; or indirect, by improving the chances of transmission. Heavy rainfall and flooding increase the risk of faecal particles entering drinking water sources, possibly contaminating them with *Cryptosporidium* oocysts, but they might also affect the accessibility of drinking water. Warmer weather promotes water recreation and water consumption, increasing the risk of exposure to oocysts, but it might also affect the availability of drinking water.[183, 194] Higher humidity levels increase oocyst survival.[193] Rising temperatures also lead to increased winter precipitation in some parts of the world, which is also a predictor of contamination.[183, 195] Peaks can also be seen during the dry season in Africa, which are caused by poor hygiene due to a shortage of water or the use of less preferable water sources for the same reason.[27] Studies on the seasonality of cryptosporidiosis have been performed around the world. An association between the different weather elements (high temperature, heavy rainfall, high humidity) could not be proven in all studies due, for example, to differences in climate, socioeconomic factors, and how the studies were executed, but the overall association between extreme weather and cryptosporidiosis incidence is compelling and cannot be ignored.[194]

2. Aims

2.1. Overall aim of the thesis

The overall aim of this project was to study the long-term effects of the outbreak of *C. hominis* that affected the inhabitants of Östersund in 2010. The focus was on the occurrence of persistent gastrointestinal and other symptoms in both adults and children during a 10-year follow-up. The health-economic consequences of the outbreak were also explored, as well as the incidence of IBD and MC after the outbreak. Our goal was to better understand the long-term consequences for both individuals and society, which could ultimately result in better communication with patients and accumulating evidence for investing in water safety, more frequent laboratory testing of patients with diarrhoea, and the development of treatment options.

2.2. Specific aims of the included studies

Study I: to evaluate the prevalence of long-term symptoms in adults 10 years after the *Cryptosporidium* outbreak and how these symptoms presented over time. Secondary aims were to evaluate whether persisting symptoms were associated with prolonged acute infection and if they could be related to self-reported health concerns during the outbreak.

Study II: to evaluate the prevalence of long-term symptoms 10 years after the outbreak in children aged 0-5 years at the time of the outbreak. Secondary aims were to examine whether children who reported symptoms of cryptosporidiosis during the outbreak had higher healthcare utilisation, if they more often reported autoimmune diseases, and if they were more susceptible to common childhood conditions, as compared to children who did not report cryptosporidiosis symptoms during the outbreak.

Study III: to investigate the long-term health-economic consequences of the outbreak for society, focusing on productivity losses and healthcare utilisation.

Study IV: to investigate whether the incidence of IBD and MC increased in RJH after the outbreak in 2010.

3. Materials and Methods

3.1. Overview of the thesis

This thesis is based on four studies that explored different potential long-term consequences of the 2010 outbreak (Table 2) during slightly different time periods (Figure 4). An overview of the included participants can be found in Figure 5.

Table 2. *Overview of the studies included in this thesis.*

	Study I *10-year follow-up, adults*	**Study II** *10-year follow-up, children*	**Study III** *Health-economic evaluation*	**Study IV** *IBD incidence*
Design	Prospective cohort study	Prospective cohort study	Retrospective, incremental cost analysis	Ecological study
Study period	2011-2021	2011-2021	2011-2019	2006-2019
Participants	Randomly selected inhabitants of Östersund municipality ≥18 years old at time of outbreak *n* = 493	Randomly selected inhabitants of Östersund municipality 0-5 years old at time of outbreak *n* = 295	Randomly selected inhabitants of Östersund municipality All ages *n* = 1,404	New IBD or MC diagnosis in RJH age ≥18 years old at time of diagnosis *n* = 565
Data	Questionnaire 2011, 2016, 2021	Questionnaire 2011, 2021 Healthcare utilisation, diagnoses (RJH)	Questionnaire 2011 Sickness/childcare benefit (SSIA), outpatient visits, days inpatient care (NBHW), primary care visit (RJH)	SWIBREG IBD diagnoses, medical records (RJH)
Statistical analysis	Logistic regression	Logistic regression	Logistic regression, negative binomial regression	Negative binomial regression
Exposure variable	Clinical cryptosporidiosis (cases vs non-cases)	Clinical cryptosporidiosis (cases vs non-cases)	Clinical cryptosporidiosis (cases vs non-cases)	*Cryptosporidium* outbreak (before vs after outbreak)
Outcome variables	Long-term symptoms, consistency of symptoms	Long-term symptoms, healthcare utilisation, diagnoses	Claimed sickness benefit (18-67 yr) or childcare benefit (0-11 yr). Number of outpatient visits, days of inpatient care	IBD and MC incidence

SSIA: Swedish Social Insurance Agency; NBHW: National Board of Health and Welfare; RJH: Region Jämtland Härjedalen; SWIBREG: Swedish Inflammatory Bowel Disease Register; IBD: inflammatory bowel disease; MC: microscopic colitis.

Figure 4. *Timeline and study periods.*

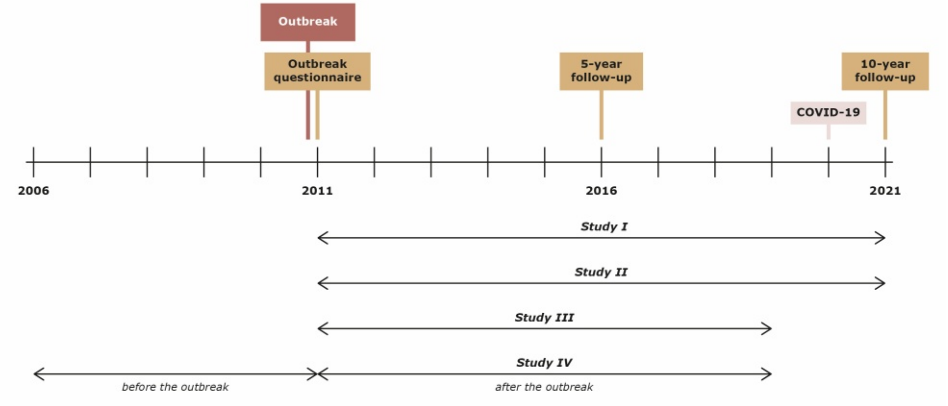
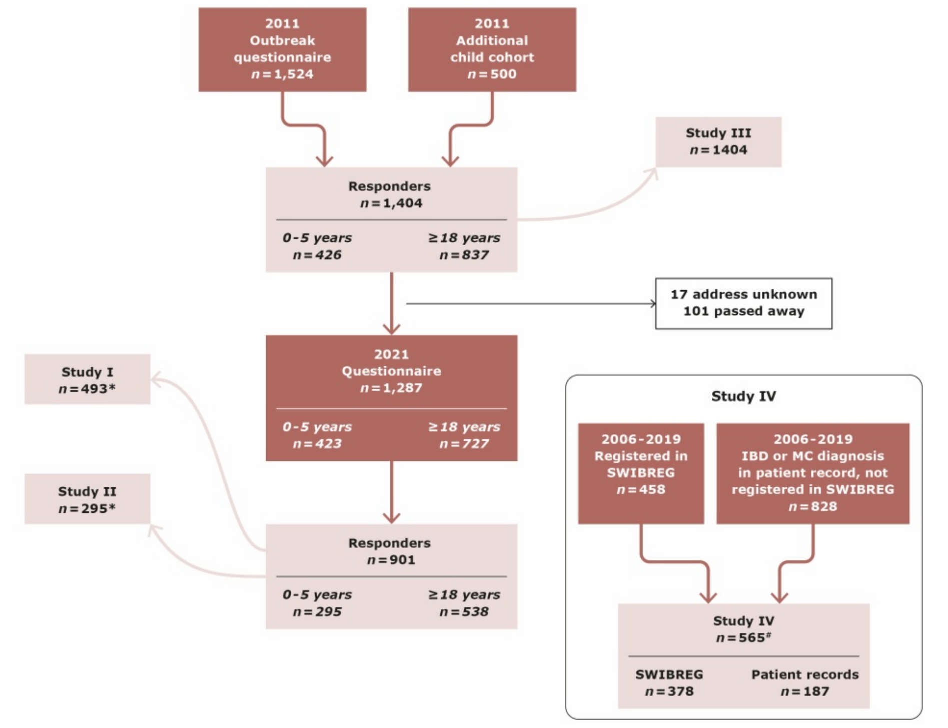


Figure 5. *Overview of participants included in the four studies.*



** Exclusion criteria were self-reported diagnosis of IBD, IBS (Study I and II) or other long-term bowel issues (Study I). # Exclusion criteria were diagnosis in another region, before 2006 or age <18 years, uncertain (date of) or wrong diagnosis.*
IBD: inflammatory bowel disease; IBS: irritable bowel syndrome; MC: microscopic colitis; RJH: Region Jämtland Härjedalen; SWIBREG: Swedish Inflammatory Bowel Disease Register.

3.2. Questionnaires

3.2.1. The outbreak questionnaire

Late January 2011, a questionnaire with a pre-stamped reply envelope was sent by post to 1,524 randomly selected inhabitants of Östersund municipality and an additional cohort of 500 children aged 0-5 years. Guardians were asked to answer for children <15 years old, and a reminder was sent after 2 weeks. The completed questionnaires were scanned and converted into a data file.

The survey included questions on demographic characteristics, water supply, symptoms of cryptosporidiosis, comorbidities including gastrointestinal and joint symptoms, and health concerns in relation to the outbreak. The respondents were asked to report symptoms they had experienced during the last 3 months (i.e. 1 November 2010 – 31 January 2011). Validated questionnaires for diagnosing IBS are extensive and were not used in order to improve the response rate. However, the questions about gastrointestinal symptoms were based on the Rome criteria.[91]

3.2.2. Case definition

The WHO definition of diarrhoea was used to define cases: anyone who reported new episodes of three or more loose stools a day or watery stools between 1 November 2010 and 31 January 2011 was considered to have had clinical cryptosporidiosis.[198]

3.2.3. Follow-up questionnaires

Follow-up questionnaires were sent after 6 months, 2 years, and 5 years. The same questions were asked as in the outbreak questionnaire except that items on acute illness and water supply were removed. Participants were asked to report symptoms they had experienced during the last 3 months. The completed questionnaires were scanned and converted into a data file.

A 10-year follow-up questionnaire (Appendix I-IV) was carried out in February 2021. This follow-up questionnaire was sent by post to all outbreak questionnaire respondents who were alive and for whom a current address was known (n = 1,287). Guardians were asked to answer for children <15 years old. A stamped reply envelope was included, and a reminder was sent after 4 weeks. Those who had not responded 7 weeks after the reminder were considered non-responders. The survey was the same as the questionnaire sent out during previous follow-ups. Once again, participants were asked to report symptoms they had experienced during the last 3 months.

3.3. Study-specific materials and methods

3.3.1. Study I

Study I was a prospective cohort study based on data collected through the questionnaires sent out to adults in 2011 and 2021. Respondents with a self-reported diagnosis of IBD, IBS, or other long-term bowel issues pre-outbreak were excluded in order to avoid bias. Remaining individuals were defined as cases or non-cases based on their response in 2011.

We separately analysed reported symptoms and their associations with case status; when there was considerable overlap in symptoms, we created composite outcomes to increase power. The composite outcomes were abdominal symptoms, including loose stools, watery and bloody diarrhoea, abdominal pain, constipation, changing bowel habits, and bloating; diarrhoea, including loose stools, watery diarrhoea, and bloody diarrhoea; and joint symptoms, including joint discomfort, stiff joints, joint pain, and swollen joints.

To explore whether reported symptoms were consistent over time, we used data from the 5-year follow-up from 2016. We merged the files from 2011, 2016, and 2021 and analysed the composite outcomes abdominal symptoms and joint symptoms, headache and fatigue. These outcomes were chosen because they stood out the most in the 10-year follow-up and/or are frequently reported as sequelae in the literature. All individuals responding to both follow-ups were divided into three categories: consistent symptoms (symptom reported in both 2016 and 2021), varying symptoms (symptom reported once, in either 2016 or 2021), or no symptoms at either of the follow-ups.

To determine whether a longer duration of the acute disease was associated with an individual reporting abdominal symptoms 10 years later, we defined prolonged disease as ≥8 days of abdominal symptoms during the outbreak, as reported in the outbreak questionnaire.

In the outbreak questionnaire, participants rated their level of concern about their own health in relation to the outbreak on a scale from 0 to 10, with 0 indicating no concerns and 10 indicating extreme worry. We converted it to a dichotomous variable with ≥7 as the cut-off point for significant symptoms. This choice was based on the Hospital Anxiety and Depression Scale (HADS) and similar assessment tools, where significant symptoms are typically identified by scores of at least 50–75% of the maximum score.[199]

3.3.2. Study II

Study II was also a prospective cohort study based on the questionnaires sent out in 2011 and 2021 but included children aged 0-5 years during the outbreak who responded to the 10-year follow-up. Participants were defined as cases or non-cases based on their response in 2011. As in Study I, we explored whether outbreak case status was associated with reporting symptoms after 10 years.

In addition, for the individuals still living in RJH, we searched the region's healthcare records database to obtain the following information for each year during 2011-2020:

- Number of visits to primary care and outpatient visits to the Departments of Paediatrics and Child Psychiatry,
- Common diagnoses in childhood (e.g., respiratory infections, acute media otitis, tonsillitis, gastroenteritis),
- Diagnosis of autoimmune diseases (i.e., IBD, celiac disease, type 1 diabetes, and juvenile rheumatic arthritis).

3.3.3. Study III

Study III was a retrospective incremental cost analysis with a societal perspective. The focus was on production loss (i.e., costs for sick leave and care for a sick child) and healthcare utilisation for the years 2011-2019. This time frame was chosen to avoid interference from the COVID-19 pandemic. Data were collected for all individuals responding to the outbreak questionnaire, and the results for cases and non-cases were compared. Data were collected from the following sources for each year during 2011-2019:

- Swedish Social Insurance Agency (SSIA, *Försäkringskassan*): net days of sick leave (>14 days), number of periods with sick leave >14 days, and number of days of care for a sick child. The first 14 days of

sick leave are covered by the employer. These days are not registered centrally and, therefore, reliable data for the first 2 weeks are not available.
- National Board of Health and Welfare (NBHW, *Socialstyrelsen*) patient register: number of outpatient visits in secondary care and number of days with inpatient care.
- RJH database for primary care visits: primary care contacts, divided by type of contact (physical/telephone) and staff category (doctor/nurse).

Population data for 2010 and salary data for central Norrland for 2017 were collected from Statistics Sweden.

3.3.4. Study IV

Study IV was an ecological study based on data from the national quality register SWIBREG and the electronic patient records Cosmic® and VAS®. The study compared IBD and MC incidences and demographic variables before and after the *Cryptosporidium* outbreak in RJH.

SWIBREG is the Swedish quality register for IBD.[200] In RJH, adult individuals with a pathology-confirmed IBD or MC diagnosis are registered by the Department of Gastroenterology at Östersund Hospital after giving their informed consent. Data are entered by the patient's physician or a dedicated nurse upon diagnosis and during follow-up visits. The collected data include details on diagnoses, laboratory results, endoscopies, treatments, and patient-reported outcomes. Currently, more than 800 of the region's 130,000 inhabitants (0.6%) are registered in SWIBREG.

The study included all patients who received a new, pathology-confirmed IBD or MC diagnosis in RJH between 1 January 2006 and 31 December 2019. Variables collected for this study were diagnosis, date of diagnosis, age at diagnosis, and sex. Patients with an unknown date of diagnosis and those who received their diagnosis when <18 years old were excluded.

First, data were collected and validated for all patients registered in SWIBREG. This was later complemented by a search in RJHs former and current patient record systems using ICD codes for IBD and MC (K50.0–52.9) to find patients who were not registered in SWIBREG; these data were also validated. Population data were collected from Statistics Sweden.

The year 2006 was used as a starting point because electronic patient records were in place at both the medical and surgical departments, both of which are involved in the diagnosis of IBD in RJH. Data were collected up to 2019 so they would not be influenced by the COVID-19 pandemic. Included patients were divided into pre- and post-outbreak groups, with the post-outbreak period starting 1 January 2011. This date was chosen in order not to miss cases in which *Cryptosporidium* sped up the process of developing IBD. Patients were also divided into age categories (18-39 years or ≥40 years) based on the Montreal classification system for IBD.[201]

3.4. Statistics

3.4.1. Sample size and selection

The sample size for the questionnaire was calculated by assuming a 50% attack rate and a 70% response rate with a 3% margin of error (95% confidence interval [CI]). An additional child cohort (n = 500, age 0-5 years) was selected to increase power for this specific age group. The addressees were selected from the population register with the help of a random number generator.

3.4.2. Analysis Study I

The study population was stratified according to case status, sex, and age at the time of the outbreak (age categories 18–40 years, 41–65 years, and ≥66 years). Chi-squared and Mann–Whitney U tests were used to evaluate differences in demographic characteristics and the mean number of symptoms between cases and non-cases. Logistic regression was used to evaluate possible associations between case status and symptoms reported during follow-up. The results were adjusted for age (category) and sex and presented as adjusted odds ratios (aORs) with 95% CIs. A possible association between prolonged disease and reported symptoms after 10 years was evaluated by chi-squared test, as was the consistency of symptoms and their association with case status. The Mann–Whitney U test was used to examine an association between consistent symptoms and the mean number of days with symptoms during the outbreak. Logistic regression was used to evaluate a possible association between health concerns and reporting symptoms during follow-up.

3.4.3. Analysis Study II

We analysed the outcome measures by case status, sex, and age at the time of the outbreak, using age categories of 0-2 years and 3-5 years, since previous research indicates that children up to 2 years of age are most vulnerable to infection. We used chi-squared and Mann–Whitney U tests to assess differences in demographics and the mean number of symptoms between cases and non-cases. Logistic regressions adjusted for age (category), sex, and previous history of loose stools were used to examine associations between case status and symptoms reported during follow-up. The results were presented as aORs with 95% CIs. The number of healthcare visits was evaluated by Mann-Whitney U tests. Diagnoses were described and possible between-group differences compared using chi-squared and Mann-Whitney U tests.

3.4.4. Analysis Study III

For sickness benefit, individuals aged 18-67 years were included in the analysis; for childcare benefit, children up to 11 years of age were included. Data for sickness benefit and childcare benefit were presented per year and for the total period for cases and non-cases. Possible differences in the number of individuals claiming benefits and the number of days claimed were analysed by chi-squared or Mann-Whitney U tests. We used a logistic regression model adjusted for sex and age to estimate the odds of cases claiming sickness benefit. Participants were divided into two age categories: 18-29 years and ≥30 years during the outbreak. We chose this cut-off because of an exponential increase in sickness benefit claims after 30 years of age.[202] Sensitivity analyses were performed by varying the upper age limit for sickness benefit (65 and 70 years instead of 67 years). Using chi-squared test, we also analysed the number of periods of received sickness benefit per year.

We used logistic regression models to analyse the number of cases and non-cases visiting primary care, visiting outpatient secondary care, and receiving inpatient care, adjusted for sex and age (category). To compare the number of visits in primary and secondary care and the number of days with inpatient care, we used negative binomial regression analysis adjusted for sex and age (category). The age categories were 0-17 years, 18-67 years, and ≥68 years.

The difference in the mean number of benefit days between cases and non-cases was used to calculate the costs of production loss. The monthly wage was divided by 21 and 46.50% employer costs added to calculate a daily cost.

The results were extrapolated to the total population of Östersund in 2010 to estimate the costs of production loss.

3.4.5. Analysis Study IV

Data were analysed separately for MC and IBD, UC, and CD. Between-group differences (pre- vs post-outbreak) were assessed using the independent samples T-test and Mann-Whitney U test.

The IBD incidence was calculated by dividing the number of new patients by the number of adult inhabitants on the last day of the respective year and was presented as the incidence per 100,000 individuals. This incidence was evaluated in a negative binomial regression analysis using the number of adult inhabitants as the offset variable and the following independent variables: period of diagnosis (pre- or post-outbreak), sex, and age category. The results were presented as incidence rate ratios (IRRs) with 95% CIs.

3.4.6. Statistics software

Statistical calculations were performed using IBM SPSS Statistics for Windows (versions 26, 28, 29, IBM Corp., Armonk, NY, USA). Missing values were excluded from analyses. The significance level was set at 0.05.

4. Ethical considerations

The studies in this thesis were ethically approved by the regional research ethics review board in Umeå, Sweden, and/or its successor, the Swedish Ethical Review Authority (Table 3). All studies were performed in accordance with the Declaration of Helsinki.

Table 3. *Overview of the studies and their ethical approval.*

	Regional Ethical Review Board	Swedish Ethical Review Authority
Study I and II *10-year follow-up, adults and children*	2010/392-31M, 2015/495-31Ö	2020-05554
Study III *Health-economic evaluation*	2010/392-31M	2020-05554
Study IV *IBD incidence*	2018/168-31	2019-05841

RJH was the principal investigator for the outbreak questionnaire, the 5-year follow-up, and the 10-year follow-up. The Public Health Agency of Sweden was the principal investigator for the follow-ups at 6 months and 2 years (not included in this thesis).

Participation in the questionnaire studies was voluntary. Addressees received an accompanying letter explaining the purpose of the study and were informed that data were stored and published anonymously. Participants gave their informed consent by returning the questionnaire. They were not informed about the possibility that data from patient records and/or registers could be collected later. However, this could be considered a minimal intrusion into the patient's privacy; the data were requested for all respondents at once and was returned either completely anonymised or with only the questionnaire number as means of identification. This is not uncommon in research and was done with permission from the Swedish Ethical Review Authority.

Registration in SWIBREG is voluntary and participants are informed that data might be used for research purposes. SWIBREG data were complemented by requesting an overview of patients with an IBD diagnosis in their patient records from RJH. For all patients, a short, directed patient record review was performed to validate the diagnosis. A note was left in the patient record that it had been reviewed for research purposes. Even though this is an intrusion into patients' privacy, it could be considered a minimal burden on the affected individuals.

Data were presented at group level; no individual participants are identifiable in the published data.

The project received funding from RJH and Visare Norr and was also supported by healthcare centres *Svegs hälsocentral* and *Fjällhälsan Härjedalen*. None of these organisations had any influence on any phase of this project. There was no economic compensation for participation in any of the studies.

5. Results

5.1. Outbreak questionnaire

A total of 1,404 (69.4%) individuals responded to the outbreak questionnaire: 600 were defined as cases (42.7%) and 804 as non-cases (57.3%) (Table 4). Cases had a mean age of 28.7 years (median 26 years, range 0-94 years) and non-cases 35.4 years (median 34.5 years, range 0-97 years) ($p < 0.001$). There were no sex differences between cases and non-cases and no differences in age distribution between sexes. Compared with non-cases, cases more often reported suffering from IBS ($p = 0.014$) and gluten intolerance ($p = 0.043$) prior to the outbreak. There were no differences in other comorbidities between the groups.

Table 4. *Baseline characteristics of the study population by outbreak case status.*

	Cases *n* (%)	**Non-cases *n* (%)**
Total	600 (42.7)	804 (57.3)
Sex		
Male	294 (49.0)	374 (46.5)
Female	306 (51.0)	430 (53.5)
Age at outbreak, years		
≤11	221 (36.8)	276 (34.3)
12-17	33 (5.5)	38 (4.7)
18-67	305 (50.8)	354 (44.0)
≥68	41 (6.8)	136 (16.9)
Comorbidity		
IBD	4 (0.7)	2 (0.3)
IBS	28 (4.8)	18 (2.3)
Gluten intolerance	12 (2.1)	6 (0.8)
Lactose intolerance	35 (6.0)	33 (4.3)

IBD: inflammatory bowel disease; IBS: irritable bowel syndrome

The non-response rate was highest among individuals aged 18-67 years (34.4%) and lowest among those aged ≥68 years (15.3%). The distribution of sex was similar for both responders and non-responders. However, when analysing the age groups separately, we found that among 18- to 67-year-olds, women were more likely to respond than men (30.5% and 38.4%, respectively; $p = 0.008$).

5.2. Study I

A total of 538 adults (74.0%) responded to the 10-year follow-up questionnaire. Response rates were lowest among individuals aged 18–40 years during the outbreak (60.1%) and highest among those aged 41–65 years during the outbreak (81.1%). There were no differences in case status or sex between responders and non-responders.

Forty-five respondents were excluded due to symptoms prior to the outbreak caused by IBD ($n = 3$), IBS ($n = 26$), IBD and IBS ($n = 1$), or other long-term bowel issues ($n = 15$).

5.2.1. Symptoms after 10 years

Study I included 203 cases and 290 non-cases (Table 5). Cases were younger than non-cases, with a mean age of 47.8 years (median 47.0 years, interquartile range [IQR] 36.0-60.0 years) and 52.7 years (median 55.0 years, IQR 41.8-65.0 years), respectively ($p \leq 0.001$).

At least one symptom was reported by 59.6% of cases, and the mean number of symptoms among cases was 3.0 (median 2.0, range 0.0–15.0). Among non-cases, 45.1% reported at least one symptom, with a mean of 1.8 symptoms (median 0.0, range 0.0–16.0) ($p \leq 0.001$).

Table 5. *Baseline characteristics of the study population by outbreak case status.*

	Cases *n* (%)	**Non-cases *n* (%)**
Total	203 (41.2)	290 (58.8)
Sex		
Male	91 (44.8)	131 (45.2)
Female	112 (55.2)	159 (54.8)
Age at outbreak, years		
18-40	67 (33.0)	70 (24.1)
41-65	106 (52.2)	156 (53.8)
≥66	30 (14.8)	64 (22.1)
Comorbidity		
Lactose intolerance	11 (5.5)	14 (5.0)
Gluten intolerance	6 (3.0)	2 (0.7)
Previous ulcer	0 (0.0)	10 (3.6)
Diabetes	7 (3.5)	21 (7.5)
COPD / asthma	8 (4.0)	12 (4.3)
Heart failure	1 (0.5)	2 (0.7)
Rheumatic joint disease	4 (2.0)	4 (1.4)
Malignancy	2 (1.0)	0 (0.0)
Medication		
Ulcer prevention	7 (3.5)	12 (4.2)
Cortisone, oral	4 (2.0)	1 (0.4)
Smoking	26 (12.8)	36 (12.5)

COPD: chronic obstructive pulmonary disease

Compared to non-cases and after adjusting for age and sex, cases were more likely to report abdominal symptoms (diarrhoea, changes in bowel habits, abdominal pain, nausea, bloating, and/or heartburn, aOR ≈ 3) and joint symptoms (including joint pain, joint stiffness, and/or joint discomfort, aOR ≈ 2). Headache, fatigue, and loss of appetite were also reported more often (Table 6).

Table 6. *Symptoms reported 10 years after the outbreak by outbreak case status.*

	Cases *n* (%)	Non-cases *n* (%)	OR (95%CI)	aOR (95%CI)
Diarrhoea[a]	36 (18.1)	20 (6.9)	**3.0 (1.7-5.3)**	**2.9 (1.6-5.2)**
Loose stools	27 (13.3)	14 (4.8)	**3.1 (1.6-6.0)**	**3.1 (1.6-6.0)**
Watery diarrhoea	21 (10.3)	13 (4.5)	**2.5 (1.2-5.1)**	**2.4 (1.1-4.9)**
Bloody diarrhoea	8 (3.9)	2 (0.7)	-	-
Constipation	28 (13.8)	28 (9.7)	1.6 (0.9-2.7)	1.5 (0.9-2.7)
Changes in bowel habits	42 (20.7)	24 (8.3)	**2.9 (1.7-5.0)**	**2.9 (1.7-5.0)**
Abdominal pain	38 (18.7)	20 (6.9)	**3.1 (1.8-5.6)**	**3.0 (1.7-5.4)**
Bloating	79 (38.9)	54 (18.6)	**2.8 (1.9-4.3)**	**2.8 (1.8-4.2)**
Heartburn	41 (20.2)	34 (11.7)	**2.0 (1.2-3.3)**	**2.0 (1.2-3.2)**
Nausea	29 (14.3)	20 (6.9)	**2.3 (1.3-4.2)**	**2.1 (1.1-3.9)**
Vomiting	7 (3.4)	5 (1.7)	2.0 (0.6-6.5)	1.8 (0.6-5.9)
Joint symptoms[a]	73 (36.5)	68 (23.6)	**1.9 (1.3-2.8)**	**1.9 (1.3-2.9)**
Joint discomfort	59 (29.1)	45 (15.5)	**2.3 (1.5-3.5)**	**2.3 (1.5-3.6)**
Stiff joints	54 (26.6)	43 (14.8)	**2.1 (1.3-3.2)**	**2.2 (1.4-3.4)**
Joint pain	54 (26.6)	54 (18.6)	1.6 (1.0-2.5)	**1.6 (1.1-2.5)**
Swollen joints	23 (11.3)	26 (9.0)	1.3 (0.7-2.3)	1.4 (0.8-2.6)
Headache	64 (31.5)	57 (19.7)	**1.9 (1.3-2.9)**	**1.7 (1.1-2.7)**
Ocular pain	28 (13.8)	22 (7.6)	**2.0 (1.1-3.6)**	1.9 (1.0-3.5)
Fatigue	58 (28.6)	50 (17.2)	**1.9 (1.3-3.0)**	**1.8 (1.2-2.8)**
Fever	12 (5.9)	8 (2.8)	2.2 (0.9-5.6)	2.1 (0.9-5.4)
Loss of appetite	13 (6.4)	5 (1.7)	**4.0 (1.4-11.3)**	**4.2 (1.5-12.0)**
Weight loss	7 (3.4)	9 (3.1)	1.1 (0.4-3.1)	1.5 (0.5-4.2)

[a] Composite outcome: individuals reporting at least one of the included symptoms. Logistic regression analyses, unadjusted and adjusted for sex, age, and previous long-term abdominal issues. (a)OR: (adjusted) odds ratio; CI: confidence interval. Bolded values are statistically significant.

Cases with abdominal symptoms for ≥8 days during the outbreak were more likely to report abdominal symptoms after 10 years (52.2%) compared with cases who reported symptoms lasting up to 7 days (39.7%) (p = 0.022).

During the 10-year follow-up, cases more often reported suffering from IBS compared with non-cases (18/194 [9.3%] vs 8/277 [2.9%], p = 0.003). For all included participants, this should be a new diagnosis, as those with a (self-reported) diagnosis prior to the outbreak had been excluded. Cases also more frequently reported lactose intolerance (22/196 [11.2%] vs 17/277 [6.1%], p = 0.048). There were no other differences in comorbidities or medications.

5.2.2. Health concerns

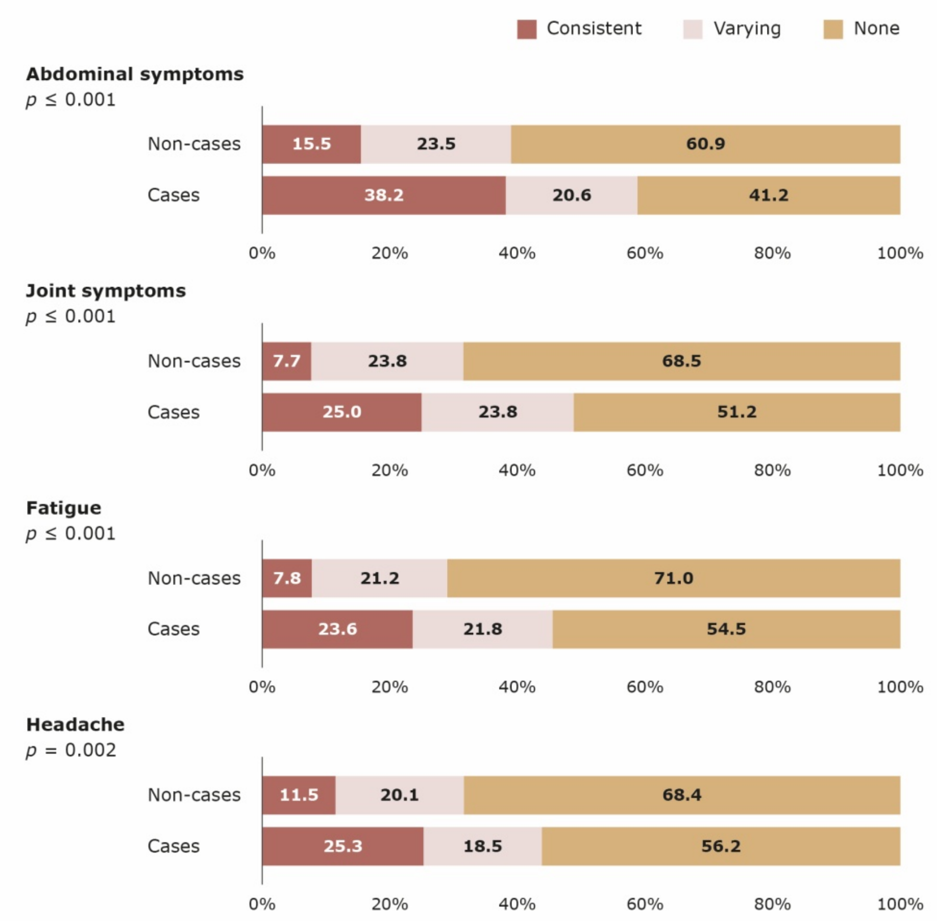
As expected, during the outbreak, cases more often expressed significant concerns about their health compared with non-cases (13.3% vs 2.5%, $p \leq 0.001$). Cases with significant health concerns reported an average of 12.3 days (median 11.0 days, range 3.0-35.0 days) with initial abdominal symptoms, compared to 7.0 days (median 5.0 days, range 0.0-55.0 days) for cases without significant health concerns ($p \leq 0.001$). However, at the 10-year follow-up, cases with significant health concerns during the outbreak did not report having symptoms more often and did not have more concerns about their health compared with cases without significant health concerns.

5.2.3. Symptoms over time

A total of 173 cases and 241 non-cases replied to both the 5-year and 10-year follow-up questionnaires. Consistent abdominal and joint symptoms, as well as headaches and fatigue, were more often reported by cases (Figure 6).

For cases with consistent abdominal symptoms, the average duration of symptoms during the outbreak was 9.2 days (median 6.5 days, range 1.0-55.0 days), compared to 6.6 days (median 5.0 days, range 0.0-33.0 days) among cases reporting varying or no symptoms at follow-up ($p = 0.003$). In addition, cases with consistent abdominal symptoms more often reported a relapse of symptoms (recurrence of diarrhoea after >2 days without symptoms) during the outbreak compared with cases reporting varying or no symptoms at follow-up (36/65 [55.4%] vs 38/102 [37.3%], $p = 0.021$).

Figure 6. *Consistency of symptoms over time by outbreak case status. Percentages of participants reporting consistent, varying, or no symptoms.*



Consistent: symptom reported after both 5 and 10 years. Varying: symptom reported only after 5 or 10 years. None: symptom not reported.
Abdominal symptoms: loose stools, watery or bloody diarrhoea, abdominal pain, constipation, changing bowel habits, and/or bloating. Joint symptoms: joint discomfort, joint pain, stiff joints, and/or swollen joints.

5.3. Study II

A total of 295 children (69.7%) responded to the 10-year follow-up questionnaire. Non-responders were more often defined as cases than responders were (71/128 [55.5%] vs 121/295 [41.0%], $p = 0.006$), but there were no differences in age or sex.

We included 121 cases and 174 non-cases in Study II (Table 7). There were no between-group differences in terms of sex or age. However, on the outbreak questionnaire, cases more often reported long-term or recurrent loose stools during the 2 years before the outbreak compared with non-cases. No differences were noted in the pre-outbreak prevalence of (self-reported) bloating, abdominal pain, cow's milk allergy, lactose intolerance, gluten intolerance, or other food intolerances. At baseline and follow-up, cases and non-cases showed no differences in medication use or the presence of other comorbidities.

Table 7. *Baseline characteristics of the study population by outbreak case status.*

	Cases *n* (%)	**Non-cases *n* (%)**
Total	121 (41.0)	174 (59.0)
Sex		
Male	64 (52.9)	81 (46.6)
Female	57 (47.1)	93 (53.4)
Age at outbreak, years		
0-2	72 (59.5)	84 (48.3)
3-5	49 (40.5)	90 (51.7)
Previous abdominal issues[a]	23 (19.0)	13 (7.5)
Loose stools	16 (13.2)	11 (6.3)
Abdominal pain	9 (7.4)	8 (4.6)
Bloating	4 (3.3)	7 (4.0)
Previous food allergy or intolerance[b]	9 (7.4)	9 (5.2)

[a] Respondents reporting lasting or recurrent episodes of loose stools, abdominal pain, and/or bloating in the 2 years prior to the outbreak.
[b] Respondents reporting cow milk allergy, lactose intolerance, gluten intolerance, and/or other food-related allergies.

5.3.1. Symptoms after 10 years

The number of cases reporting at least one symptom was higher (64/112, 57.1%) than the number of non-cases reporting symptoms at the 10-year follow-up (67/155, 43.2%) ($p = 0.025$). Cases reported a mean number of 1.7 symptoms (median 1.0, range 0.0–14.0), whereas non-cases reported a mean 1.4 symptoms (median 0.0, range 0.0–11.0) ($p = 0.029$). Logistic regression analyses adjusted for sex, age, and long-term or recurrent issues with loose stools prior to the outbreak showed increased ORs for joint symptoms and fatigue (Table 8).

Table 8. *Symptoms reported 10 years after the outbreak by outbreak case status.*

	Cases *n* (%)	Non-cases *n* (%)	OR (95%CI)	aOR (95%CI)
Diarrhoea[a]	9 (7.4)	9 (5.2)	1.5 (0.6-3.8)	1.4 (0.5-3.6)
Constipation	10 (8.3)	19 (10.9)	0.7 (0.3-1.6)	0.6 (0.3-1.4)
Changes in bowel habits	13 (10.7)	14 (8.0)	1.3 (0.6-3.0)	1.2 (0.5-2.8)
Abdominal pain	22 (18.2)	22 (12.6)	1.5 (0.8-2.9)	1.4 (0.7-2.7)
Bloating	15 (12.4)	13 (7.5)	1.7 (0.8-3.7)	1.7 (0.8-3.7)
Heartburn	6 (5.0)	11 (6.3)	0.8 (0.3-2.1)	0.8 (0.3-2.2)
Nausea	20 (16.5)	25 (14.4)	1.2 (0.6-2.2)	1.1 (0.6-2.2)
Joint symptoms[b]	11 (9.1)	5 (2.9)	**3.4 (1.1-9.9)**	**4.0 (1.3-12.0)**
Headache	54 (44.6)	57 (32.8)	1.6 (1.0-2.6)	1.6 (1.0-2.7)
Ocular pain	9 (7.4)	13 (7.5)	1.0 (0.4-2.4)	1.1 (0.4-2.6)
Fatigue	32 (26.4)	27 (15.5)	**1.9 (1.1-3.4)**	**1.9 (1.1-3.4)**
Fever	13 (10.7)	11 (6.3)	1.8 (0.8-4.1)	1.9 (0.8-4.3)
Loss of appetite	5 (4.1)	10 (5.7)	0.7 (0.2-2.1)	0.7 (0.2-2.2)

[a] Composite outcome including all individuals reporting loose stools, watery diarrhoea, and/or bloody diarrhoea.
[b] Composite outcome including all individuals reporting stiff joints, joint pain, swollen joints, and/or joint discomfort.
Logistic regression analyses, unadjusted and adjusted for sex, age, and long-term or recurrent issues with loose stools pre-outbreak. (a)OR: (adjusted) odds ratio; CI: confidence interval. Bolded values are statistically significant. Symptoms reported by fewer than five individuals per group, as well as subtypes of diarrhoea and joint symptoms, are not presented due to the high level of uncertainty in these estimates.

Excluding patients with long-term or recurrent issues with loose stools prior to the outbreak resulted in small changes for bloating, which became borderline significant (aOR 2.7, 95% CI 1.1-6.5; adjusted for age and sex), and fatigue, which was no longer significant (aOR 1.9, 95% CI 1.0-3.5).

At follow-up, there were no differences between cases and non-cases in reported food allergies or intolerances. IBS was reported by two individuals in both groups and diabetes by two non-cases ($p > 0.05$). There were no reports of IBD or rheumatic joint disease.

Univariate analyses showed no significant differences in symptoms between cases and non-cases aged 0-2 years during the outbreak. In children aged 3-5 years, cases more often reported loose stools (4/49 vs 1/89, $p = 0.034$), headaches (27/49 vs 27/88, $p = 0.005$), and stiff joints (4/49 vs 1/88, $p = 0.036$). In logistic regression analyses, only headaches remained more frequently reported in this age category (OR 2.8, 95% CI 1.3-5.7).

5.3.2. Healthcare utilisation

Data from the electronic patient records were available for 119 cases and 169 non-cases. A total of 3,191 visits were recorded. Throughout the follow-up period, cases had 970 visits per 100 participants (median number of visits per case 7.0, range 1.0–45.0), whereas non-cases had 1,205 visits per 100 participants (median number of visits per non-case 7.0, range 1.0–185.0). The majority of visits were to primary care, with 571 visits per 100 cases (median 5.0, range 0.0–21.0) and 527 visits per 100 non-cases (median 5.0, range 1.0–19.0). No differences were observed between cases and non-cases in the number of visits over the total study period, per year, or visits by department.

5.3.3. Diagnoses

During the follow-up period, no participants were diagnosed with CD, UC, or chronic juvenile arthritis. The occurrence of the remaining chronic conditions (type 1 diabetes, celiac disease, lactose intolerance, pollen allergy, and asthma) was generally low, with no differences observed between cases and non-cases. We also found no differences between the groups in the diagnosis of common childhood infections (gastroenteritis, acute otitis media, upper airway infections), abdominal pain, IBS, or joint pain.

5.4. Study III

5.4.1. Sickness benefit

The number of individuals collecting sickness benefit during 2011-2019 was greater among cases than among non-cases, especially in the first years after the outbreak. After 2016, no between-group differences were found. Among those who received sickness benefit, only a few claimed benefits more than one period per year, with no difference found between cases and non-cases.

A logistic regression analysis adjusted for sex and age category (18-29 years or ≥30 years of age during the outbreak) showed an increased OR of 1.53 (95% CI 1.13-2.08) for cases to receive sickness benefit during 2011-2019 (Table 9).

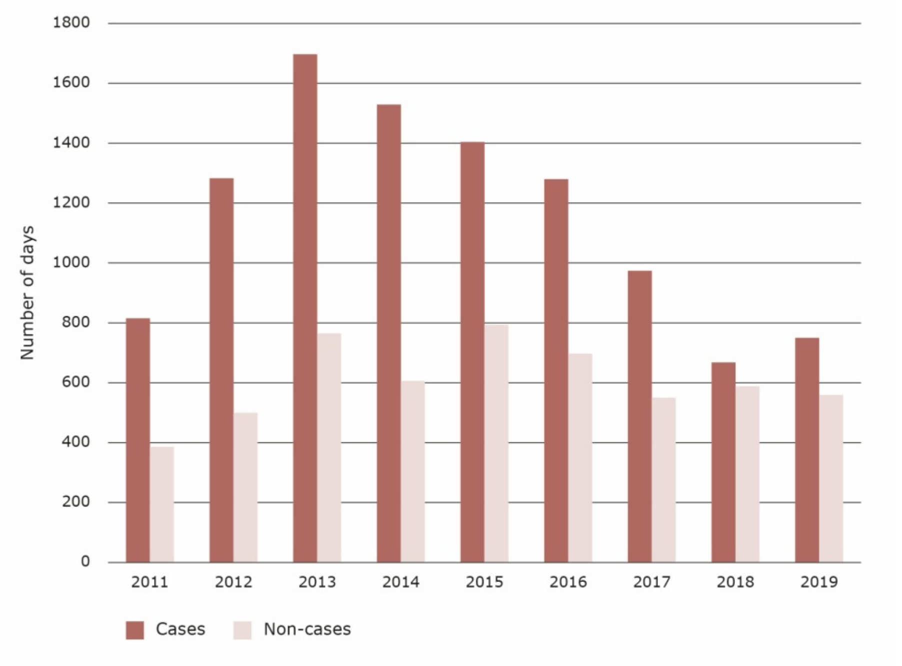
Table 9. *Sickness benefit. Number of individuals aged 18-67 years with sickness benefit and odds of cases claiming sickness benefit.*

	Cases *n* / total (%)	Non-cases *n* / total (%)	OR (95%CI)	aOR (95%CI)
2011	34/307 (11.1)	23/350 (6.6)	**1.77 (1.02-3.08)**	**1.81 (1.04-3.16)**
2012	36/306 (11.8)	29/349 (8.3)	1.47 (0.88-2.46)	1.52 (0.90-2.58)
2013	46/306 (15.0)	31/338 (9.2)	**1.75 (1.08-2.84)**	**1.79 (1.09-2.92)**
2014	44/308 (14.3)	28/335 (8.4)	**1.83 (1.11-3.02)**	**1.85 (1.11-3.08)**
2015	41/306 (13.4)	29/330 (8.8)	1.61 (0.97-2.66)	1.61 (0.97-2.67)
2016	50/310 (16.1)	27/327 (8.3)	**2.14 (1.30-3.51)**	**2.15 (1.31-3.54)**
2017	33/309 (10.7)	29/325 (8.9)	1.22 (0.72-2.06)	1.21 (0.71-2.05)
2018	30/304 (9.9)	30/319 (9.4)	1.06 (0.62-1.80)	1.04 (0.61-1.78)
2019	25/306 (8.2)	24/317 (7.6)	1.09 (0.61-1.95)	1.07 (0.60-1.93)
Total	142/346 (41.0)	125/399 (31.3)	**1.53 (1.13-2.06)**	**1.53 (1.13-2.08)**

Logistic regression analyses, unadjusted and adjusted for sex and age (18-29y/≥30y). (a)OR: (adjusted) odds ratio; CI: confidence interval. Bolded values are statistically significant.

Cases also claimed more sickness benefit days. For the whole study period, they received 8,946 days per 100 participants, whereas non-cases received 4,538 days per 100 participants (p = 0.003) (Figure 7).

Figure 7. *Number of sickness benefit days per 100 participants.*



5.4.2. Childcare benefit

There was no difference between cases and non-cases in the numbers of individuals collecting childcare benefit (cases: 205/240 [85.2%], non-cases: 247/299 [82.6%]). There was no significant between-group difference in the number of collected childcare benefit days.

5.4.3. Healthcare utilisation

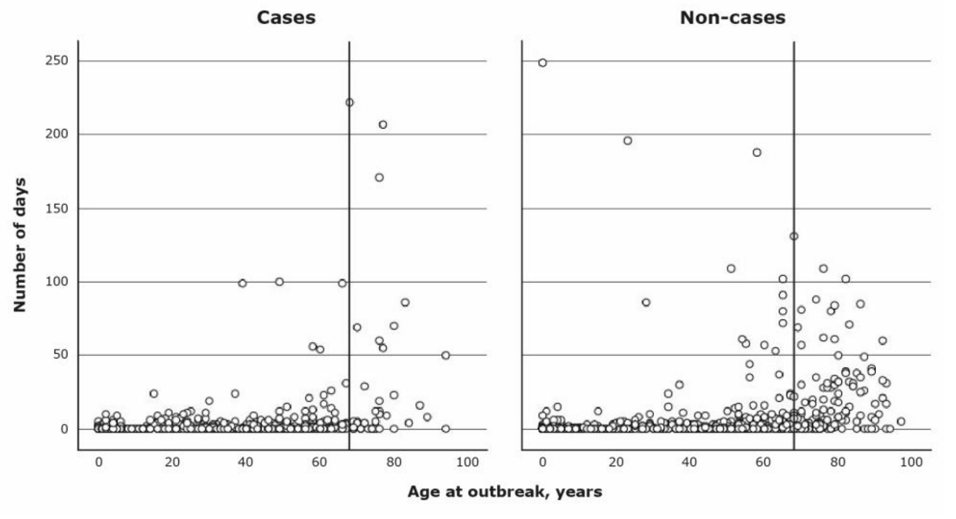
The number of individuals visiting primary care or outpatient secondary care did not differ between cases and non-cases, and we did not find differences in the number of visits to primary care or outpatient secondary care (total number of visits or doctor visits only, all visits or physical visits only).

In total, 33.8% of cases and 38.7% of non-cases received inpatient care during the study period. Logistic regression analysis adjusted for age category and sex did not show a significant between-group difference when compared per year or for the entire period.

Negative binomial regression analysis adjusting for age category and sex showed significantly fewer admission days in the case group compared with the non-case group (IRR 0.71, 95% CI 0.62-0.81) from 2015 onward. However, this significance disappeared after excluding outliers with ≥50 days of inpatient care during 2011-2019 (IRR 0.95, 95% CI 0.82-1.10). Considering this, we deemed the observed difference in the number of in-hospital days too uncertain and likely attributable to factors other than *Cryptosporidium* infection. Consequently, we did not perform cost calculations for inpatient care.

Figure 8 shows the relationship between age at outbreak and the total number of days of inpatient care.

Figure 8. *Relationship between age at outbreak and total days of inpatient care during 2011-2019 by case status. Reference line is at 68 years.*



5.4.4. Cost calculations

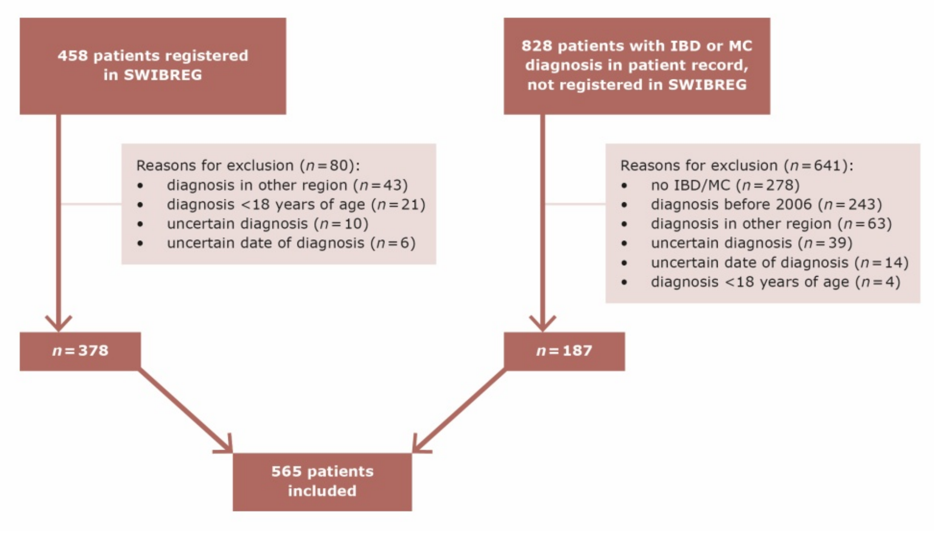
In 2017, the monthly average wage for an adult citizen in central Norrland, was 31,400 SEK, or 2,190 SEK per day including employer social security contributions.[203] During the years 2011-2019, cases aged 18 - 67 years collected 4,408 more sickness benefit days per 100 persons, or 44 days per case. The incremental cost for the study population was ~33.3 million SEK (€2.9 million) (346 cases × 44 days × 2,190 SEK).

By the time of the outbreak, Östersund municipality had 59,416 inhabitants, of which 39,087 individuals were between 18 and 67 years old.[203] Considering a 45% attack rate for the entire working population of the city, this would result in an ~1.7 billion SEK (€148.5 million) ([39,087 × 0.45] × 44 × 2,190) increase in costs for sickness benefit for 2011-2019.

5.5. Study IV

After validation of the SWIBREG data and patient records, 565 patients were included in Study IV (Figure 9). The median age at diagnosis was 52 years (mean 49.7 years, IQR 31-66.5 years), 253 (45%) were male, and a majority (*n* = 287) were diagnosed with UC (50.8%).

Figure 9. *Cohort selection. Patients diagnosed with inflammatory bowel disease (IBD) or microscopic colitis (MC) in Region Jämtland Härjedalen during 2006-2019. SWIBREG: Swedish Inflammatory Bowel Disease Register.*



A total of 410 adults were diagnosed with IBD in an adult population of approximately 105,000 individuals (Table 10). Their median age was 43 years (mean 44.2 years, IQR 26-60 years), and 214 (52.2%) were male. A total of 155 individuals were diagnosed with MC (Table 10). Their median age was 67 years (mean 64.3 years, IQR 56-74 years), and 116 (74.8%) were female. Seventy-two patients had collagenous colitis (46.5%), 74 had lymphocytic colitis (47.7%), and in 9 individuals the diagnosis was not further specified (5.8%).

When stratified for type of diagnosis, no age or sex differences were found between the pre- and post-outbreak groups. Mean annual incidences increased for all diagnoses after the outbreak.

Negative binomial regression analysis showed a trend towards an increased incidence of IBD post-outbreak (IRR 1.39, 95% CI 0.99–1.94), but not when stratified for UC or CD (Table 11). However, in the ≥40 years age group, incidence increased post-outbreak for both IBD (IRR 1.69, 95% CI 1.13–2.51) and CD (IRR 2.23, 95% CI 1.08–4.62).

The incidence of MC increased sixfold post-outbreak (IRR 6.43, 95% CI 2.78–14.87). There were no differences between collagenous and lymphocytic colitis.

Table 10. *Baseline characteristics of the study population, pre- and post-outbreak.*

	IBD[a]		**Ulcerative colitis**		**Crohn's disease**		**IBD - unclassified**		**Microscopic colitis**	
Diagnosis, *n* (%)	410 (72.6)		287 (50.8)		100 (17.7)		23 (4.1)		155 (27.4)	
	2006-2010	**2011-2019**	**2006-2010**	**2011-2019**	**2006-2010**	**2011-2019**	**2006-2010**	**2011-2019**	**2006-2010**	**2011-2019**
Sex, *n* (%)										
Male	64 (52.0)	150 (52.3)	53 (57.6)	101 (51.8)	9 (34.6)	41 (55.4)	2 (40.0)	8 (44.4)	3 (14.3)	36 (26.9)
Female	59 (48.0)	137 (47.7)	39 (42.4)	94 (48.2)	17 (65.4)	33 (44.6)	3 (60.0)	10 (55.6)	18 (85.7)	98 (73.1)
Age at diagnosis, years (median, IQR)	40.00 (25.00-57.00)	43.00 (27.00-61.00)	35.00 (24.25-54.00)	38.00 (25.00-59.00)	46.00 (29.25-63.00)	50.50 (30.75-62.50)	63.00 (44.50-74.00)	57.00 (42.00-68.25)	64.00 (55.50-74.00)	68.00 (56.00-74.00)
Mean annual incidence per 100,000 (95%CI)	24.15 (11.70-36.61)	30.90 (24.67-37.13)	18.07 (7.82-28.31)	21.00 (15.97-26.02)	5.11 (2.93-7.29)	7.97 (5.22-10.71)	0.98 (-0.24-2.19)	1.94 (0.96-2.91)	4.12 (-0.61-8.84)	14.40 (9.57-19.24)

[a] Ulcerative colitis, Crohn's disease, and IBD-unclassified
CI: confidence interval, IQR: interquartile range

Table 11. *Negative binomial regression analysis of inflammatory bowel disease and microscopic colitis.*

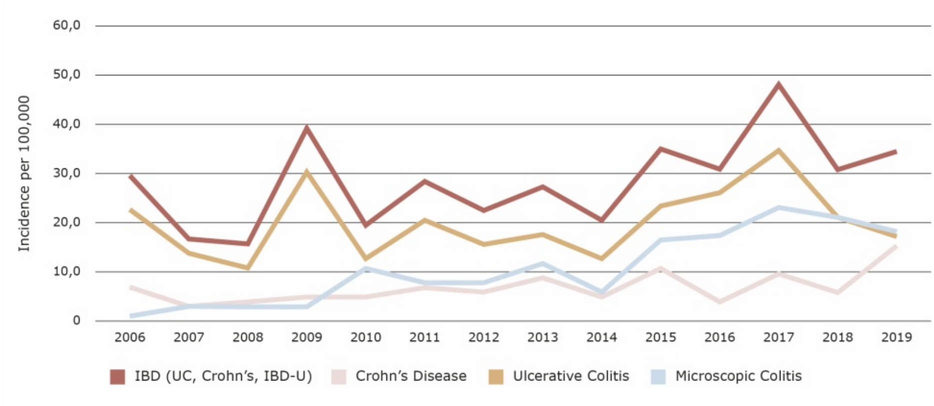
	IBD		**Ulcerative colitis**		**Crohn's disease**		**Microscopic colitis**	
	IRR	95%CI	IRR	95%CI	IRR	95%CI	IRR	95%CI
All ages (≥ 18 years)	1.39	0.99-1.94	1.20	0.84-1.72	1.61	0.95-2.71	**6.43**	**2.78-14.87**
≥ 40 years	**1.69**	**1.13-2.51**	1.49	0.96-2.31	**2.23**	**1.08-4.62**	**6.8**	**2.90-15.92**

IBD includes UC, Crohn's, IBD-U. UC: ulcerative colitis. IBD-U: IBD-unclassified. IRR: incidence risk ratio. CI: confidence interval.
Offset variable: adult inhabitants Region Jämtland Härjedalen. Model adjusted for sex and age category (All ages only). Bolded values are statistically significant.

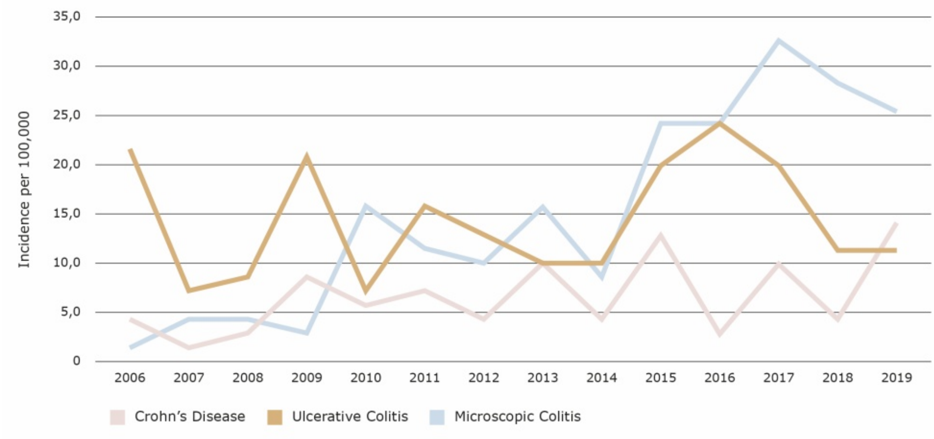
Figure 10 shows the annual incidence of IBD and MC for all adults and the ≥40 years age group.

Figure 10. *Annual incidence of inflammatory bowel disease and microscopic colitis in Region Jämtland Härjedalen. UC: ulcerative colitis; IBD-U: inflammatory bowel disease-unclassified.*

a. *All adults*



b. *Individuals ≥40 years of age*



6. Discussion

6.1. Main findings

The studies included in this thesis show that infection with *Cryptosporidium* might have long-term consequences. Cryptosporidiosis is associated with up to threefold increased odds of adult cases reporting symptoms 10 years after the acute infection has subsided, especially abdominal and joint symptoms, but even headache and fatigue. Consistent symptoms are more likely to occur after prolonged infection. Even children might report long-term symptoms after cryptosporidiosis, but their symptoms seem not as pronounced as in adults or as in their peers living in low-income countries. Long-term symptoms might not generate increased healthcare utilisation but are associated with an increased number of sickness benefit days, leading to high costs for society. Furthermore, cryptosporidiosis is associated with an increased incidence of late-onset IBD and MC.

6.2. Long-term symptoms

6.2.1. Long-term symptoms in adults

Studies I and II on long-term symptoms 10 years after cryptosporidiosis were the first to report persisting symptoms over such a long period of time. Similar outcomes were observed in prior studies, but with shorter time spans.[2 66 92 95]

A 2020 systematic review based on eight studies with a follow-up of 2-36 months found that the most common long-term symptoms after cryptosporidiosis were diarrhoea (25%), abdominal pain (25%), nausea (24%), fatigue (25%), and headache (21%). Abdominal pain, loss of appetite, fatigue, vomiting, joint pain, headache, and eye pain were 2 to 3 times more prevalent following *Cryptosporidium* infection than in controls, which is in line with our findings.[2] A Swedish study including 271 laboratory-confirmed cases of *Cryptosporidium* infection demonstrated that, after up to 36 months, 15% still experienced episodes of diarrhoea and 9% had abdominal pain.[66] The results are also in concurrence with a Norwegian study that reported an association between infection with *G. lamblia* and long-term symptoms 10 years later. In the laboratory-confirmed cases included in that study, the IBS prevalence was 43%, compared to 14% among controls (OR 4.7, 95% CI

3.6–6.2), and chronic fatigue was present in 26% of cases vs 11% of controls (OR 3.0, 95% CI 2.2–4.1).[97]

The mechanisms driving the persistence of post-infectious long-term symptoms are only partially understood. Infections can disrupt the intestinal microbiota, leading to impaired intestinal barrier function, visceral hypersensitivity, and imbalance in the intestinal immune system. This can then, in turn, affect communication along the gut-brain axis and provoke low-grade inflammation, contributing to IBS-like symptoms, fatigue, and other symptoms outside the gut.[109 204 205]

Among adults who reported symptoms at either the 5- or 10-year follow-up, a greater proportion reported symptoms 5 years after the outbreak. Even though this difference was not significant, the pattern may indicate that some individuals experience gradual recovery, even several years after the initial disease.

6.2.2. Post-infectious IBS

Acute gastroenteritis is associated with an increased risk of IBS. This relationship has been established for bacterial infections, such as *Campylobacter* spp., *Salmonella* spp., and *E. coli*, but also for norovirus and the parasite *G. lamblia*.[206] The authors of a comprehensive meta-analysis identified a general PI-IBS prevalence of 11%, with the greatest risk observed following protozoal infection (40%). However, this number was based on the Norwegian *Giardia* cohort only.[206]

It is likely that many of the adult cases reporting abdominal symptoms in the 10-year follow-up suffer from PI-IBS, but we are unable to diagnose them based on our data. However, the results are strengthened by an (unpublished) validation of the questionnaire and the increased number of post-outbreak IBS diagnoses among cases compared to non-cases (9.3% vs 2.9%, $p = 0.003$).[207] Cases also more commonly reported lactose intolerance during the follow-up, aligning with a meta-analysis reporting an OR of 3.5 (95% CI 1.6–7.6) for IBS patients compared to healthy controls.[208]

In our follow-up of adults, cases who experienced abdominal symptoms lasting more than 1 week during the outbreak were more likely to report abdominal symptoms at follow-up than those whose acute symptoms resolved within a week (52.2% vs 39.7%). Furthermore, cases who reported abdominal symptoms at both the 5- and 10-year follow-ups had experienced a greater number of symptomatic days during the acute phase of infection compared to those without consistent long-term symptoms (9.2 vs 6.6 days). This aligns well with a meta-analysis from 2007 in which diarrhoea persisting for longer than 1 week was associated with increased odds of PI-IBS (OR 2.6, 95% CI 1.5–4.6).[110]

6.2.3. Anxiety, depression and health concerns

Anxiety and depression double the risk of developing and maintaining IBS symptoms.[108] In order to improve the response rate, our questionnaire did not include these diagnoses in the comorbidity question or validated questions to diagnose these conditions. Health concerns during the outbreak were not an independent predictive factor for reporting long-term symptoms. It is possible that the public considered the outbreak and the many cases of cryptosporidiosis as annoying but rather harmless.

6.2.4. Long-term symptoms in children

Cases aged 0-5 years reported more joint symptoms, headache, and fatigue during follow-up than non-cases, but the differences were not as marked as in adults or children from low-income countries.[30 48 77 103 104] The latter is hardly surprising, as well-nourished, healthy children from high-income countries should be less affected by a gastrointestinal infection than malnourished children.[6 209] It is also likely that children in low-income countries are generally exposed to higher numbers of oocysts, while the concentration of oocysts in the drinking water during the Östersund outbreak was relatively low.[34] The ingestion of a greater number of oocysts is associated with more severe illness, which may contribute to the development of long-term symptoms.[210 211]

In the 2-year follow-up from Östersund, cases up to 5 years of age did not experience long-term symptoms to a greater extent than non-cases, but only 48 children were included.[95] In the 5-year follow-up, the youngest children were not analysed separately.[92] The results from this 10-year follow-up are in line with the findings in adults, as well as with the systematic review from 2020.[2] However, due to low numbers and wide confidence intervals, it is not possible to draw firm conclusions based on the results.

6.2.5. Healthcare visits and diagnoses in children

Cases and non-cases did not differ when looking at chronic diagnoses and common childhood conditions. The number of diagnoses were too low to find significant differences. No IBD diagnoses were registered for participants during the follow-up period. This is not surprising given a prevalence of paediatric IBD in Sweden of 75 per 100,000 inhabitants, and the median age of childhood-onset IBD of 14 years.[212 213]

Cases and non-cases did not differ in the total number of healthcare visits. This indicates that the long-term symptoms some might experience did not prompt more healthcare visits. However, the number of visits to the Department of Child Psychiatry differed between the groups due to a small number of patients with a very high number of visits. The Mann-Whitney U test is less sensitive to outliers compared to parametric tests, reducing the risk of distorted results. Consequently, excluding the outliers from analysis did not result in significantly different results.

6.3. Health-economic evaluation

Prior to the outbreak, the collection of sickness benefits in Östersund municipality was close to the national average, but between 2011 and 2015 it increased markedly, exceeding the nationwide trend that also increased but to a lesser extent. From 2017 onwards, the figures declined and the collection of sickness benefits in Östersund fell below the national average.[202] We observed a notable difference in sickness benefit claims between cases and non-cases, particularly during the first years following the outbreak, with cases having higher claims that gradually converged toward more comparable levels. This suggests a slow and gradual recovery, which concurs with the trend we found in the 10-year follow-up of adults.

Prior, during, and after the study period, the collection of childcare benefits in Östersund municipality was slightly higher than the national average but followed the same trends. This provides further support for our finding that childcare benefit collection did not differ between cases and non-cases.[214]

Health-economic evaluations were published following other *Cryptosporidium* outbreaks but focused primarily on the costs associated with the acute phase.[150-153 215] A Dutch study found that productivity losses represented 90% of the total costs related to *Cryptosporidium* infection, whereas long-term manifestations were estimated to contribute approximately 7%.[155] Our results indicate that long-term costs may account for a much larger share of total costs than previously estimated. A PubMed search in October 2025 did not identify any other studies reporting on the long-term costs of infection with *Cryptosporidium* or *Giardia*.

6.4. IBD and MC incidence

The incidence of IBD differs around the globe and is increasing in low-income countries adapting a Western lifestyle while stabilising in many high-income countries.[112 216] Incidence rates vary significantly even within Europe, with the incidence of CD ranging from 0.4 to 22.8 per 100,000 and the incidence of UC from 2.4 to 44.0 per 100,000.[216]

It is somewhat difficult to compare the incidence rates found in our study (8 per 100,000 for CD, 21 per 100,000 for UC) to other studies because they are based only on the adult population, whereas most incidence numbers are based on the entire population. The post-outbreak CD incidence in RJH seems to be in line with other Swedish cities (8–10 per 100,000), whereas the post-outbreak incidence of UC in RJH seems to be slightly higher than in other cities (16-20 per 100,000).[216-218] In our study, the incidences of late-onset IBD and MC increased, supporting the theory that the influence of environmental factors on IBD development is stronger in older adults than in those who are diagnosed at a younger age and in whom genetics play a significant role.[219] These findings provide further support for the hypothesis that the *Cryptosporidium* outbreak caused the observed increase in IBD incidence.

Whether these results support or advocate against the hygiene hypothesis in IBD can be discussed. The hygiene theory proposes that reduced microbial exposure at an early age due to improved hygiene results in a lack of exposure to microorganisms. This has a wide range of consequences from less stimulated regulatory T cells to a less robust epithelial barrier due to a lack of stimuli, which in turn might result in an overreaction to new microbes. Inflammation might become chronic due to weak regulatory control, leading to tissue damage, further barrier damage, an altered microbiome, and eventually to the development of IBD.[119 220] The latest Global Burden of Disease study on IBD demonstrated a negative correlation between all included unhygienic environmental factors (e.g., unsafe sanitation, unsafe water source, and not having access to handwashing facilities) and the incidence of IBD, supporting this theory.[221] It also corresponds with identified protective factors, such as increased exposure to microorganisms through living on a farm, having pets, being part of a large household, or consuming unpasteurised milk.[119 222] However, results have not been conclusive. This theory might only be relevant for low-income countries or first-generation migrants from low-income countries.[223] Furthermore, a twin study from the UK demonstrated that fewer self-reported episodes of gastroenteritis were protective against future onset of UC (OR 0.33, 95% CI 0.15-0.74).[224]

Gastrointestinal infections have been associated with an increased risk of developing IBD later in life. A large cohort study revealed an increased risk of IBD following *Campylobacter* and *Salmonella* infection (OR 2.9, 95% CI 2.2-3.9).[225] The numbers of parasitic infections in published studies are low. One case-control study included 3,105 individuals, 39 of whom were affected by a parasite, but none of them with *Cryptosporidium*. Despite low numbers, a pronounced risk of post-infectious CD was found for this group, which is in agreement with our results.[226] However, infection with helminths, parasitic worms, seems to be protective against autoinflammatory disease.[220]

The incidence of MC increased from 4 to >14 per 100,000, which is high compared to reported numbers from Uppsala (12 per 100,000), and the reported overall incidence in Sweden (10.5 per 100,000 in 2006-2015), even when considering that RJH incidence rates are based on the adult population only.[128 227] The observed increase in incidence contrasts the findings of a meta-analysis that reported stable incidence rates in Sweden during 2000-2010.[126] Possible contributing factors to increasing incidence are an extended use of faeces calprotectin as an inflammatory marker and an increased awareness of MC, but the effects of these factors on the incidence are considered to be small given the stable incidences in Northern Europe.[132 216]

Little is known about the role of microorganisms in the development of MC, though some recent studies have demonstrated a high risk of MC after infection with *Campylobacter consisus* and *Clostridioides difficile*.[131 132] These results are consistent with the findings in classical IBD and may explain why the incidence of MC in RJH increased after the *Cryptosporidium* outbreak. Even though we cannot claim a causal connection between *Cryptosporidium* and the increased incidences, the results add evidence to these theories.

6.5. Strengths and limitations

6.5.1. Strengths of questionnaire-based Studies I-III

A key strength of Studies I and II is their prospective design, which was based on a cohort randomly selected shortly after the outbreak. Response rates for the outbreak and follow-up questionnaires were high, presumably due to the rather condensed questionnaire and the immense impact the outbreak had on all inhabitants of Östersund. Another strength is the isolated location of RJH, and Östersund in particular. Relatively few people move to or from the area, and commuting to other regions is very limited. Therefore, external factors are expected to have a limited effect on the outcomes on a population level. This also ensured that we had access to healthcare data for most children included in Study II and participants in the health-economic evaluation in Study III. Another strength of the health-economic evaluation is the high quality of the collected data, as they came from reliable sources and covered a long period of time.

In 2010, the decision was made to send the outbreak questionnaire to inhabitants of Östersund municipality rather than to inhabitants of the city only. Outside the city centre, drinking water comes, to a greater extent, from other sources (i.e., other drinking water plants or private wells), and this ensured that enough non-cases could be included in the study.

6.5.2. Validation of the questionnaire

For the 2-year follow-up in 2013, participants were asked if they were willing to answer an additional, detailed questionnaire. It included the Gastrointestinal Symptom Rating Scale adapted to IBS (GSRS-IBS) and was aimed to validate the questionnaire used after the outbreak and during the follow-ups. GSRS-IBS is a 22-item questionnaire developed to evaluate the severity of symptoms in patients with IBS-like symptoms on a 7-point Likert scale.[228] It was complemented by additional questions about gastroesophageal reflux, dyspepsia, fatigue, and anxiety, amongst others. Differences in scores were compared using non-parametric tests. Simultaneous quantile regression analysis and median regression analysis were performed to adjust for age and sex.

A total of 50 cases and 60 non-cases completed the GSRS-IBS questionnaire. Cases reported a median total GSRS score of 16.5

(IQR 0-19) compared with 3 (IQR 0-12.5) for non-cases ($p < 0.001$). After adjusting for age and sex, cases experienced more gastrointestinal symptoms, such as abdominal pain ($p < 0.001$), bloating ($p < 0.001$), diarrhoea ($p < 0.001$), and dyspepsia ($p = 0.0026$), compared with non-cases. Cases also reported fatigue and anxiety more often than non-cases, though female sex was a confounding factor.[207]

These results confirm the outcomes of the much shorter outbreak and follow-up questionnaires and also support the connection between cryptosporidiosis and IBS-like symptoms.

6.5.3. Strengths of Study IV

The isolated location of RJH and Östersund is also an asset for the ecological study of IBD and MC incidence (Study IV). Other strengths of this study are that the colonoscopies were performed at the region's only hospital and that we used validated data. Furthermore, we did not rely only on register data but complemented it with validated data from medical records for all patients over 18 years of age with an IBD diagnosis in RJH. Therefore, we can be assured that most adult individuals from RJH with a pathology-confirmed diagnosis were included.

6.5.4. General limitations of the questionnaire studies

The major limitation of the questionnaire-based studies is that the case definition was based on self-reported symptoms rather than laboratory-confirmed diagnoses. Only 149 inhabitants of RJH had their infection confirmed by faecal microscopy. Therefore, laboratory confirmation could not be used for the case definition. Genotyping identified *C. hominis* subtype IbA10G2 in all 37 tested faecal samples, as well as all water samples, and no other pathogens were found. Therefore, it is likely that *Cryptosporidium* was the cause of the symptoms for the vast majority of the patients. Misclassification might have occurred, but this would affect both cases and non-cases.

To reduce the risk of overestimating an association between case status and abdominal symptoms, individuals with long-term abdominal symptoms or disease prior to the outbreak were excluded from the questionnaire studies.

Using self-reported data also has potential for recall bias. The short interval between the boil water advisory and the outbreak questionnaire helped minimise recall bias. It is possible that individuals who had been ill were more inclined to respond to the follow-up questionnaire. However, the proportion of cases included in study I (41.2%) aligns well with previously reported attack rates, indicating that cases are unlikely to be overrepresented in this analysis.

Even though the use of a large random sample from the general population strengthens the external validity of the questionnaire-based studies, the population of Östersund in 2010 might not be representative of larger cities, even in Sweden today, potentially limiting generalisability. Symptom-reporting and healthcare-seeking behaviour might be different elsewhere. In addition, the response rates were lower among younger adults and higher in older adults with generally fewer symptoms, potentially underrepresenting long-term symptoms in younger individuals. Furthermore, men aged 18–67 years were less likely to respond to the questionnaires than women in the same age group. Although we cannot completely rule out the potential impact of this difference on our results, the difference is relatively small, and any resulting effect is expected to be minor.

We lacked information on the participants' current infection status at the time of follow-up. As the 10-year follow-up took place in early 2021, it is possible that some reported symptoms may have been related to infection with SARS-CoV-2 rather than being long-term symptoms of *Cryptosporidium* infection. However, cases and non-cases should be equally affected by other infections, and as such this is not expected to account for the differences observed between these groups. No significant outbreaks with other gastrointestinal pathogens were reported in RJH during the follow-up period.

As the key factors involved in the development of long-term symptoms following cryptosporidiosis are not fully known, whether findings from these studies can be extrapolated to other high-income settings is not entirely clear.

Attrition bias is common in questionnaire studies, with responders often having a higher socioeconomic status.[229] We lack data on the socioeconomic status for the addressees and cannot be certain that this has not affected our results.

Studies I and II included a relatively high number of statistical analyses. The risk of producing false-positive results increases with an increasing number of analyses. However, the repeated consistency of our results supports the reliability of the outcomes.

6.5.5. Limitations of Study I

As the validated questionnaire for diagnosing IBS using the Rome criteria was not included in the follow-up questionnaires in order to increase response rates, it is not possible to conclude that cases reporting long-term abdominal symptoms suffer from PI-IBS, even though many of them probably would meet the criteria. A study examining the prevalence of PI-IBS following *Campylobacter* infection found that 21% of participants met the diagnostic criteria for PI-IBS, whereas an additional 9% reported persistent, new-onset abdominal symptoms that did not fulfil the Rome criteria.[230] This indicates that relying solely on the strict definition of PI-IBS might lead to overlooking a substantial group of individuals with relevant symptoms, reinforcing the decision to prioritise response rates over formal IBS diagnoses in our study.

Anxiety and depression double the risk of experiencing and maintaining IBS symptoms.[108] In hindsight, it would have been beneficial to include these diagnoses in the comorbidity question in the outbreak questionnaire, as it would have allowed us to adjust for this possible confounder.

6.5.6. Limitations of Study II

Among children, non-responders were more often classified as cases (55.5%) compared with responders (41.0%), potentially weakening the results. We lack a plausible explanation for this finding.
Paediatric cases did not report more abdominal symptoms compared with non-cases. It is possible that children are affected less by cryptosporidiosis and do not present these kinds of sequelae, but insufficient statistical power due to a higher incidence of abdominal issues in the general paediatric population should be considered as another explanation. A recent meta-analysis reported that functional abdominal pain disorders, including IBS, affect 8.3% (95% CI 6.4%-10.7) of children aged 4-18 years in Europe.[231] Long-term symptoms after asymptomatic carriage might also dilute the results due to misclassification as non-cases. A Welsh prospective cohort study reported that, 1 year after cryptosporidiosis, the share of individuals experiencing IBS-like symptoms was greater in children than adults, indicating that children may also develop long-term abdominal symptoms.[93]

Healthcare data could only be collected from RJH and not from other healthcare regions. However, it is unlikely that this affected our outcomes, as only one child moved from the region during the study period and it is uncommon to seek routine care outside the region.

It would have been of interest to include height measurements in our analysis, as cryptosporidiosis is associated with faltering growth in children in low-income countries. However, growth data are primarily recorded by school nurses in decentralised records, to which we did not have access. Nevertheless, we do not expect that a single episode of gastroenteritis will significantly affect long-term growth or cognitive development in otherwise healthy children living in a high-income country.

6.5.7. Limitations of Study III

A main limitation of the incremental cost analysis is that we lacked data on sick leaves ≤14 days. These days are paid for by the employer and are not reported to the SSIA; therefore, no reliable data are available for sick leave up to 2 weeks. Thus, the data were strongly skewed. However, for the calculations, we had to use the difference in the mean number of received sickness benefit days, and this mean difference may have been different if data for the first 2 weeks of sick leave were included. It is also possible that cases not only claim more sick leave benefit but also have more sick leave episodes <2 weeks. Therefore, the real costs of production loss due to sick leave may be higher than our estimates.

We acknowledge that missed workdays are not the only measure of productivity loss. Reduced efficiency at work due to illness or potential long-term health complications may also decrease productivity. However, as this is more difficult to quantify, it was not included in our analyses.

We found no significant differences between cases and non-cases in the number of healthcare visits or the number of individuals being admitted to the hospital. However, the non-case group had a significantly higher number of inpatient care days in the later part of the study period, even after adjusting for age and sex. Non-cases were older, and this age difference could plausibly explain the differences we found. Nevertheless, a difference in the number of inpatient days remained even after stratifying by age category and adjusting only for sex. However, this difference disappeared after excluding outliers. As we lacked data on the reasons for hospital admission, the underlying cause of this finding remains uncertain. Given that no other differences in healthcare utilisation were observed and that the effect vanished after removing outliers, we consider it more likely that an unmeasured factor, such as differences in morbidity, rather than *Cryptosporidium* infection itself, accounted for the observed variation; therefore, we did not include these results in the cost analysis.

The generalisability of our cost calculation may be limited due to substantial variation in salary levels, even within Sweden.

6.5.8. Limitations of Study IV

The main limitation of the IBD incidence study is that we did not know whether patients diagnosed with IBD or MC following the outbreak actually contracted cryptosporidiosis in 2010. As a result, a causal relationship between the outbreak and the observed increase in IBD and MC incidence cannot be determined.

In addition, this observational study was based on data for RJH rather than Östersund only, as it was not possible to select inhabitants from Östersund specifically. As the majority of affected individuals lived in the city, this may have reduced the study's statistical power. Furthermore, known risk factors for developing IBD, such as smoking (for CD and MC) and the use of non-steroidal anti-inflammatory drugs (for IBD) or proton pump inhibitors, serotonin reuptake inhibitors, statins, and beta-blockers (for MC), were not available for analysis.[116 127 232 233] Therefore, we cannot rule out the possibility that changes in smoking habits or medication use may have influenced the outcomes.

After the outbreak, physicians may have become more vigilant regarding gastrointestinal diseases, leading to an increase in investigations and potential surveillance bias. The number of colonoscopies with biopsies increased in the years following the outbreak, reaching a peak in 2014 according to official statistics from the NBHW.[234] However, these figures are incomplete, as colonoscopies performed by nurses are not reported to the NBHW. In addition, reporting to the NBHW has improved over the years, and the data should be interpreted with caution. Consequently, the true extent of this increase remains uncertain, as whether it reflects improved reporting, increased clinical awareness, a greater number of patients presenting with symptoms, or a combination of these factors is unclear.

7. Clinical implications and future research

7.1. Clinical insights and research opportunities

This thesis shows that long-term symptoms are common after infection with *C. hominis* and implies that cryptosporidiosis should not be taken lightly. The work also suggests that cryptosporidiosis is associated with an increased risk of IBD and significant production loss on a societal level in the years after the outbreak. Early recognition, validation, and management of long-term symptoms are the first steps in improving patient outcomes and may reduce healthcare costs. The high costs of production loss should also be an economic incentive to prioritise prevention of *Cryptosporidium* outbreaks by taking measures toward adequate water treatment worldwide.

Even though many adult cases in our 10-year follow-up reported long-term symptoms, the actual impact on their day-to-day lives is unclear and should be explored in future research. Further research is also needed to better understand the association between *Cryptosporidium* infection and the potential development of IBD and MC, as well as the risk of developing colon cancer. A future follow-up of children aged 0–5 years during the outbreak could offer additional insights into long-term post-infectious symptoms and the possible onset of autoimmune diseases that may not have appeared within the 10-year follow-up period. Such a study could also include education level and socioeconomic status. A qualitative study could provide deeper insights into how long-term symptoms impact patients' daily lives and how patients experience contacts with healthcare providers.

The mechanisms underlying the persistence of symptoms need to be explored in order to develop more effective treatment options for affected individuals. The development of effective preventive measures, such as vaccines, for both humans and livestock, should be a priority. This is particularly important considering the expected increase in *Cryptosporidium* outbreaks as a result of climate change. A One Health approach is vital to successfully reducing disease burden. Therefore, farmers should be another group of interest in future research to gain insight into their knowledge of cryptosporidiosis and which actions they take to minimise risk of contamination, especially for those with farms open to the public or that grow produce for human consumption.

Enhancing healthcare staff knowledge and expanding testing could be important initial steps toward improving care. Despite the significant burden of cryptosporidiosis on both individuals and society, physicians often do not test for it. The reasons for not testing for *Cryptosporidium* are unclear; it may reflect limited awareness or knowledge, economic constraints, or the perception that testing is unnecessary due to the lack of specific treatment options. It would be valuable to investigate physicians' attitudes and knowledge on this topic in order to enhance testing and improve care.

It is also worth considering whether nitazoxanide should be made available for treatment within Europe, as it reduces the duration of illness and, therefore, could potentially reduce the risk of long-term symptoms.

7.2. The important role of primary care

This thesis is meaningful for primary care in several ways. First, it recognises that cryptosporidiosis is not always an annoying but short-term illness. Primary care physicians take care of most of these patients and should be aware that long-term symptoms, including abdominal and joint symptoms, headaches, and fatigue, can persist for years even if the initial infection was self-limiting. Asking about past gastroenteritis when evaluating chronic symptoms might help identify a plausible explanation as to why these symptoms occur.

As a primary care physician, having knowledge of the acute symptoms of cryptosporidiosis increases the chance of correctly identifying the causative pathogen when *Cryptosporidium* is not part of routine testing. Identifying *Cryptosporidium* as the pathogen in a patient with gastroenteritis gives the physician an opportunity to adequately educate the patient about the possible prolonged disease or recurrence of acute symptoms, as well as the risk of long-term sequelae. It is our responsibility to recognise and validate these symptoms, reduce possible worries or anxiety, rule out new-onset lactose intolerance as the cause of persisting symptoms, and provide symptomatic treatment and referrals when necessary. All of these measures are key to improving patient outcomes.

Apart from directly managing patients with acute or chronic symptoms, primary care physicians also have a role in educating the public. We could raise awareness of the risk of the zoonotic spread of diseases among the general public, but also farmers, food handlers, and commercial swimming pool owners in particular. We should educate immunocompromised patients, who should be especially cautious, on how to prevent waterborne gastrointestinal infections by avoiding swimming pools, boiling water when abroad, being careful with raw produce, and thinking twice before petting livestock on open farms.

Last but not least, by requesting appropriate diagnostic tests, primary care can play a key role in highlighting cryptosporidiosis and other underdiagnosed gastrointestinal infections. After all, you won't find what you're not looking for.

8. Conclusions

8.1. General conclusion

Cryptosporidiosis in adults is associated with increased reporting of symptoms, including abdominal and joint symptoms, headache, and fatigue, 10 years later. Children in high-income countries do report possible long-term symptoms but are not as affected as their peers in low-income countries and might not be as affected as adults in high-income countries. Long-term symptoms might not generate increased healthcare utilisation but are associated with an increased number of sick days, leading to costs for both the individual and society. The incidence of IBD and MC in adults increased in RJH after the *Cryptosporidium* outbreak in 2010.

8.2. Specific conclusions

8.2.1. Study I

In adults, clinical cryptosporidiosis was associated with a threefold risk of reporting abdominal symptoms and a twofold risk of joint symptoms a decade post-infection. A symptom duration of 8 or more days during the acute infection was associated with an increased prevalence of long-term symptoms. Consistently reporting abdominal symptoms was associated with a longer period of disease during the outbreak. Health concerns during the outbreak were not an independent risk factor for reporting symptoms 10 years later.

8.2.2. Study II

In children up to 5 years of age from a high-income country, clinical cryptosporidiosis was associated with increased reporting of fatigue and joint symptoms after 10 years, but these results should be interpreted with caution. There were no differences in healthcare utilisation, frequency of autoimmune diseases, or common childhood conditions between cases and non-cases.

8.2.3. Study III

Clinical cryptosporidiosis is associated with increased collection of sickness benefit in the first years after infection. There were no differences in the collection of childcare benefit or healthcare utilisation.

The long-term costs after a *Cryptosporidium* outbreak can be considerable but are challenging to evaluate because they are less direct than acute costs.

8.2.4. Study IV

The incidence of late-onset IBD increased in RJH after the *Cryptosporidium* outbreak in 2010, supporting the hypothesis that cryptosporidiosis can trigger IBD. The incidence of MC increased markedly and could be indicative of cryptosporidiosis as an environmental risk factor.

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Appendices

I. 10-year follow-up questionnaire adults, Swedish

II. 10-year follow-up questionnaire adults, English

III. 10-year follow-up questionnaire children, Swedish

IV. 10-year follow-up questionnaire children, English