# Giardiasis in Bergen. Outbreak and clinical consequences.

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## Scientific environment

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*"There are no truths outside The Gates of Eden"*

Bob Dylan, 1965(1)

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Knut-Arne Wensaas

# Contents

# Abstract

**Background**

*Giardia lamblia* is a common cause of waterborne disease. It is endemic in many parts of the world, especially where sanitation is poor, but in Europe and North America it is most often encountered in outbreaks following contamination of drinking water. The first registered outbreak of giardiasis affecting a large community in Norway happened in Bergen in the autumn of 2004. The reservoir "Svartediket" was the source, and the water probably held *Giardia* cysts for several weeks.

*Giardia* can cause acute and chronic gastroenteritis. Several drugs constitute effective treatment, and metronidazole is the main drug available in Norway. Prior to the outbreak in Bergen there were no published studies on long term effects after eradication of the parasite.

**Aims**

The aim of the studies in this thesis is to investigate the course of giardiasis and its consequences following a large outbreak in an area where *Giardia* is not endemic.

**Methods**

In the first study, we concentrated on patients from general practice. Patients with clinically defined giardiasis were identified through a search in the medical records at two general practice clinics located in the area receiving water from the contaminated reservoir. Of the 7,100 persons registered, 134 fulfilled the inclusion criteria and 119 consented to take part in the study. Data were retrospectively obtained from the medical records. The patients were then requested to complete a mailed questionnaire and submit stool samples six months after the outbreak. A second questionnaire was sent out one year after the outbreak. The main outcome variable was abdominal symptoms that were not present prior to the acute infection.

In the second study, we investigated a historic cohort of 1252 patients with giardiasis verified by detection of *Giardia* in stool samples submitted as part of regular clinical investigations in Bergen during the outbreak. A 2:1 control group matched by age and gender was recruited from the general population of Bergen. This group was later expanded so that the whole control group consisted of 3594 individuals. All participants received a questionnaire by mail three years after the outbreak. Main outcome variables were irritable bowel syndrome (IBS) according to Rome III criteria and "chronic fatigue" as defined by the Fatigue Questionnaire.

**Results**

In the group of patients from general practice the majority was between 20 and 39 years of age (51.4%), and there were more women (69.3%) than men. The diagnosis was supported by a positive test for *Giardia lamblia* in 55% (66/119) of the patients. Treatment with metronidazole was given to 89 (75%), and after initial treatment 36% (32/89) returned to their doctor because symptoms recurred. A second prescription was given to 28% (25/89), after which 16% (14/89) returned once more. 11% (10/89) received a third treatment with metronidazole. Six months after the outbreak stool samples were positive for *Giardia* in three of 82 patients. At this point 37% (44/118) reported gastrointestinal symptoms related to their *Giardia*-infection, and after 12 months this proportion was 19% (19/99).

In the cohort of patients with laboratory verified giardiasis the prevalence of IBS three years after the outbreak was 46% (355/770), compared to 14% in the control group. The adjusted relative risk (RR) was 3.4 (95% confidence interval (CI) 2.9 to 3.8). The prevalence of chronic fatigue was 46% (366/794) among the *Giardia*-patients, and 12% among the controls, giving an adjusted RR of 4.0 (95% CI 3.5 to 4.5). IBS and chronic fatigue were associated, but there was also an increased risk of having IBS only (RR 1.8, 95% CI 1.4 to 2.3) or chronic fatigue only (RR 2.2, 95% CI 1.7 to 2.8).

## Discussion

In the study from general practice we identified patients that would have been missed by a strict laboratory based inclusion criterion, either because stool samples were not submitted or due do misclassification when samples were negative. Several patients did not receive treatment and this could suggest that they did not have giardiasis, but another reason could be that they called at the medical centre before the outbreak was known and recovered spontaneously without treatment. After clearance of the parasite a substantial proportion of the patients had persisting symptoms 6 and 12 months after the outbreak, which shows that potential negative health effects of giardiasis was more extensive than first anticipated.

In the cohort of persons with verified giardiasis the infection was associated with a high prevalence of IBS and chronic fatigue three years after the outbreak, and the risk was significantly higher than in the control group. This supports the findings in the group from general practice, and shows the consequences in a larger population and over a longer period of time. The prevalence of IBS in this study and gastrointestinal symptoms in the first one differs, but cannot be easily compared. The sample sizes vary, the case definitions are different and the questionnaires used to define the outcomes are not the same. Put together the two studies illustrate a wider range of the clinical consequences after the outbreak.

## Conclusions

These studies show that a considerable proportion of patients consistently had persisting symptoms after giardiasis from the time of the acute infection and up to three years after. The association between acute giardiasis and later gastrointestinal symptoms and fatigue is strong. This calls for more research on the mechanisms for both giardiasis and medically unexplained physical symptoms like IBS and chronic fatigue.

# List of publications

I. Wensaas KA, Langeland N, Rortveit G. Prevalence of recurring symptoms after infection with Giardia lamblia in a non-endemic area. Scand J Prim Health Care. 2009;27:12-7.

II. Wensaas KA, Langeland N, Rortveit G. Post-infectious gastrointestinal symptoms after acute Giardiasis. A 1-year follow-up in general practice. Fam Pract. 2010;27:255-9.

III. Wensaas KA, Langeland N, Hanevik K, Mørch K, Eide GE, Rortveit G. Irritable bowel syndrome and chronic fatigue three years after acute giardiasis: historic cohort study. Manuscript accepted for publication.

# Abbreviations

| | | |
|---|---|---|
| CF | - | Chronic fatigue |
| CFS | - | Chronic fatigue syndrome |
| CI | - | Confidence interval |
| CRF | - | Cancer-related fatigue |
| EPQ-N | - | Neuroticism-part of the Eysenck Personality Questionnaire |
| FSS | - | Fatigue Severity Scale |
| FQ | - | Fatigue Questionnaire |
| HPA axis | - | Hypothalamus-pituitary-adrenal axis |
| GP | - | General practitioner |
| IBS | - | Irritable bowel syndrome |
| OR | - | Odds ratio |
| PI-IBS | - | Postinfectious irritable bowel syndrome |
| RR | - | Relative risk |
| WHO | - | World Health Organization |

# 1. Background

## 1.1 *Giardia lamblia*

### 1.1.1 History and nomenclature

*Giardia lamblia* has been considered one of the most ancient and primitive eukaryotic organisms on the planet(2). This view has been challenged by resent research(3), but still this parasite has been around for a long time, and most of it without our awareness. Our knowledge about microorganisms was very limited up to the second half of the 19th century when a range of bacteria and other microbiological pathogens were described and linked to specific infections. However, as early as in 1681 the Dutch pioneer microscopist Antony van Leeuwenhoek observed a small animalcule in great numbers in his own diarrhoeal stools. Based on his notes and drawings it has been concluded that this was *Giardia lamblia*(4). The Czech physician Vilém D. Lambl described it in more detail in 1859, calling it *Cercomonas intestinalis*(5), and in 1888 Blanchard named it *Lamblia intestinalis* in his honour(2). In the same period other organisms later known to have been *Giardia* species were described. The name *Giardia*, given in honour of the French zoologist Alfred M. Giard(2), was used for the first time by Kunstler in 1882 for an organism he found in tadpoles. In 1915 Kofoid and Christensen proposed *Giardia* for the genus and *lamblia* for the species(2), and this is still the official name according to the Integrated Taxonomic Information System(6).

*Giardia lamblia* is the most common name in English literature, but *G. intestinalis* and *G. duodenalis* are also used and some argue that the latter is the most correct form(7). So far there is no agreement to choose one before the others. In medical literature the term "*Giardia*" is often used synonymously with the species *G. lamblia*.

### 1.1.2 Microbiology

*G. lamblia* is a unicellular flagellated motile eukaryotic microorganism. Other *Giardia* species include *G. agilis* found in amphibians and *G. muris* found in rodents, but *G. lamblia* is the only one found in man. Within the species *G. lamblia* there are several genotypes dividing it into different "assemblages", each with preference to different hosts and with possible variations in the clinical manifestations of infection. Assemblages A and B are found in humans. The range of variations has led to the argument for a revision of the whole *Giardia* taxonomy(7).

*G. lamblia* is found in two distinct forms, the cyst and the trophozoite. In the environment it survives as cysts, which to a certain extent is resistant to environmental stress. However, they will not survive cold winters in water(8) or deposited in soil(9). The cyst has a relatively robust wall consisting of 60% carbohydrates and 40% proteins with strong interactions between them(3). Inside the cyst wall electron microscopy has revealed the existence of four nuclei, ribbon-like microtubules and flagella(10). After ingestion the cyst undergoes rapid transformation into the trophozoite stage within 15 minutes. This excystation is triggered by the acids in the stomach, and after passing into the small intestine the cysts rupture and release the excyzoite, an intermediate form of the parasite. The excyzoite then divides twice producing four trophozoites(11).

The trophozoite is the form that causes disease in man. It has a characteristic appearance with a pear-shaped outline, four pairs of flagella and two nuclei symmetric to the long axis. A ventral disc is located on the concave side of the body (Figure 1). *Giardia* differs from other eukaryotes by the absence of peroxisomes and proper mitochondria, but contains mitochondria-like organelles called mitosomes(12). The trophozoites colonize the small intestine of their host, predominately in the mid-jejunum, where they attach to the intestinal wall by their ventral disk(2). They multiply by cell division, but genomic and population genetic studies have shown evidence of heterozygotes indicating some kind of recombination or sexual reproduction as well(13-15). Some trophozoites encyst following nuclear replication.

This occurs in the jejunum, and is triggered by host factors like high levels of bile, low levels of cholesterol and a basic pH(16).

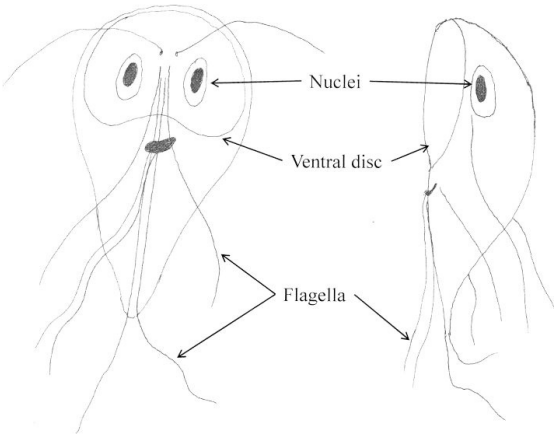


Figure 1. *Giardia lamblia*

### 1.1.3 Clinical features of giardiasis.

The role of *Giardia* in disease was unknown for centuries, well into the 20th century. Its ability to produce enteritis in man was suspected, but not established(17). When the microbiologist Clifford Dobell in 1919 convincingly argued that van Leeuwenhoek had been the first to identify the parasite, he made a point of congratulating the Dutch for not making the wrongful assumption that *Giardia* was the cause of his diarrhoea(4). In the 1950's experimental studies showed that ingestion of *Giardia* cysts in capsules or drinking water led to infection in man, in the sense that cysts would later be found in stool samples. Still it could not be established that this caused clinical illness(18, 19). Clinical accounts suggested that the infection may be followed by diarrhoea and other symptoms, but there was substantial controversy about the pathogenicity of *Giardia*, as summarized by Petersen in 1972(20). The main question was whether the parasite would cause disease, or merely be observed in greater numbers because the diarrhoea would constitute a more favourable environment in which *Giardia* would multiply. During the last 40 years *Giardia* has been isolated in several outbreaks of gastroenteritis in both Europe and

North America(21-28) providing arguments for its pathogenic potential. The experiment that formally established the pathogenicity of *Giardia* in humans by fulfilling the Koch postulates was published by Nash in 1987(29). Following thorough medical investigation 15 healthy volunteers, all men, were given sterile inocula with *Giardia* trophozoites that had been grown from two different strains (GS/M and Isr) of cysts obtained from patients suffering from giardiasis. The trophozoites were administered by a tube into the small intestine. Later cysts would be detected in multiple stool samples from all ten men receiving GS/M *Giardia*, as sign of infection. *Giardia* cysts were not detected in any of the five men receiving the Isr strain. Of the ten men infected with *Giardia* four developed typical diarrhoeal disease, which proved that *Giardia* can lead to both infection and clinical disease.

The disease caused by *G. lamblia* is called giardiasis (or lambliasis/lambliosis). It presents as acute or chronic gastroenteritis, and the clinical manifestations will vary from hardly any symptoms at all to profuse diarrhoea accompanied by severe weight loss and fatigue(30). Both experimental and clinical studies have shown that people may be infected without developing symptomatic giardiasis(18, 22, 29).

The mechanisms by which *Giardia* causes disease are not fully understood. The diarrhoea frequently observed in symptomatic giardiasis is caused by combined malabsorption and hypersecretion that is the result of diffuse shortening of microvilli. These alterations are partly mediated by host T lymphocytes that are activated secondary to disruption of epithelial tight junctions and increased transepithelial permeability(31). Most of this knowledge is based on studies *in vitro* and in animals, but in 2007 Troeger and co-workers described epithelial barrier dysfunction and signs of both malabsorption and hypersecretion in biopsies from the distal duodenum of 13 patients with chronic giardiasis(32).

Acute giardiasis is characterized by diarrhoea, abdominal cramps, flatulence, foul-smelling stools, bloating, nausea, anorexia, weight loss and fatigue(27, 29, 33). The incubation period is just over one week in experimental studies(29, 34), but may be

longer in clinical settings(35-37). Most patients will be treated in the community but an unknown proportion of patients will need hospital care (38).

Some patients will recover spontaneously, and it has been suggested that this will happen in 2 to 6 weeks(30, 39). However, there are no good data on the proportion of patients that will recover without treatment, nor on how long spontaneous recovery may take. This issue is further complicated since different strains of *Giardia* will have different pathogenic properties(29), and also because host factors may play a role.

van Leeuwenhoek is credited with the first description of *Giardia*, but he may also have given the first description of chronic giardiasis. He found the “animalcules” in great numbers in his stools when loose, but very few or none when the stools were normal. He wrote of his experience from the summer of 1681: "I have usually of a morning a well-formed stool; but hitherto I have had sometimes a looseness of the bowels in two, three, or four weeks, so that I went to stool some twice, thrice, or four times a day. But this summer this befell me very often, and especially when I took hot smoked beef, that was a bit fat, or bacon, which food I eat with much enjoyment; indeed, it persisted once for three days, and whatever food I took I retained in my body not much above four hours . .”(4).

In some patients chronic giardiasis may persist for months and even years if left untreated. Features of chronic giardiasis are intermittent diarrhoea with malabsorption resulting in weight loss, fatigue and possibly vitamin deficiencies (30, 40). The symptoms of chronic giardiasis will often resemble and be indistinguishable from irritable bowel syndrome (IBS)(41, 42).

Chronic or repeated infections in children may cause failure to thrive, reduced weight gain and impaired cognitive development(43, 44), which is of great concern in regions where sanitation is poor and *Giardia* endemic.

There is limited knowledge on persisting symptoms and complications of giardiasis after eradication of the parasite. Prior to the outbreak in Bergen in 2004 there were no published studies on this topic.

### 1.1.4 Treatment

Several drugs are effective against *Giardia*, but there is uncertainty about the optimal regimen(45, 46). There is concern about the development of resistance to existing treatment, and research on new therapeutic drugs for giardiasis has been initiated(46). In Norway metronidazole is the only drug approved for treatment of giardiasis, and for refractory cases alternative drugs must be imported(47). In treatment schedules with metronidazole given twice or thrice daily for five to ten days the efficacy ranges from 60 to 100% (median 89%)(45). Shorter courses would be favourable because of side-effects, but are less efficacious.

### 1.1.5 Epidemiology

*Giardia* is mainly spread through contaminated drinking water, but other pathways of transmission are also recognised. The first food-borne cases were reported in 1981, when cysts were detected in home-prepared salmon(48). Later other outbreaks have been linked to noodle salad(49), fruit salad(50) and raw sliced vegetables(51). In the 1980's records of two outbreaks of giardiasis caused by fecal contamination of swimming pools were published(52, 53). A review of all known outbreaks associated with recreational waters in the United States between 1971 and 2000 concluded that in 97 of 259 registered outbreaks (37.5%) protozoa were the etiologic agent(54). Giardiasis has also been to linked to interactive water fountains, the first report came from Florida in 2006(55). In 2003, 30 primary cases of giardiasis during a large outbreak in Boston, Massachusetts, were linked to exposure to a children's pool, but as many as 105 secondary cases probably resulted from person-to-person spread(56). Transmission from person to person is a well-known problem in child day care centres(57-62), and this was the site for the first outbreak of giardiasis in Norway, in Trondheim in 2006(63). Not only the children are at risk, one study showed that nappy handling was associated with a four-fold increased risk of giardiasis(64). *Giardia* may also spread through sexual activity, and increased prevalence among homosexual men has been reported(65-70).

*Giardia* is widespread throughout the world. It is endemic in tropical and subtropical areas where hygienic conditions are poor. The prevalence of infection or symptomatic disease is not well established. The World Health Organization (WHO) has given the highest estimate to date in a 1996 report, stating that 200 million people in Asia, Africa and Latin America had symptoms of giardiasis(71). Numbers frequently quoted in review articles are prevalence rates of 2-5% in the industrialised world and 20-30% in low-income countries(30, 72, 73). None of these estimates are backed with strong evidence.

Studies on prevalence of *Giardia* in Europe and North America are few and yield diverging results. In 1972-73 the prevalence of *Giardia* in Colorado was 3% based on a laboratory survey(74). In contrast, the annual incidence rate of *Giardia*-infection in Vermont between 1983 and 1986 was 46 cases pr. 100,000 population. The incidence was highest among children 1-4 years of age(75). A prevalence study in five Berlin kindergartens in 2006 identified *Giardia* in three of 202 children (1.5%)(76). A meta-analysis on 13 non-heterogeneous studies from the Nordic countries published before 2004 estimated a pooled prevalence of 5.8% among persons with and 3.0% among persons without gastrointestinal symptoms(77).

The high estimates for prevalence of *Giardia* infection in low-income countries are most uncertain. They are partly based on studies with few participants, restricted to patients with gastroenteritis or limited to children. In some instances there is no clear distinction between symptomatic or asymptomatic infection. As a result the reported prevalence varies.

Several studies from South and Central America have estimated prevalence in groups of children. Farthing followed a group of 45 children in rural Guatemala with stool examination every week for three years between 1964 and 1966. All children had at least one episode of *Giardia* positive stools, and the overall prevalence was 20.2%. The ratio between symptomatic and asymptomatic cyst excretion varied between 1:2 and 1:3(43). Forty years later Cook reported a prevalence of 10.9% in a larger study based on single stool samples from children aged 5-15 years visiting for routine

investigation in another part of rural Guatemala. In total there were samples obtained from 5,705 visits during a period of four years(78). In a group of 845 children from marginal urban districts in Peru *Giardia* was found in 23.8% (79). In North-east Brazil one study on children followed from birth and up to the age of four years (mean follow-up 543 days) found a prevalence of *Giardia* infection of 22.8% (80). Another study from a different city reported detection of *Giardia* cysts in single stool samples from 13.7% of 694 pre-school children undergoing routine investigation (81). In Southern Brazil a similar result has been reported, a prevalence of 19.2% was found in 133 children in two day care centres(82). All the referred studies are restricted to children. A population based study on 2,367 individuals of all age groups from three different municipalities in southern Brazil revealed a prevalence of only 1.7%(83).

Giardiasis is also common in Africa. In a study from the rural Nile delta of Egypt stools were analysed once a week as part of a six months investigation of 42 children, and during this period *Giardia* was detected in 41 of the children and in 42% of the specimens analysed(84). In rural Ethiopia a prevalence of 25.8% among children was recorded in the dry season of 2005 and 39.8% in the wet season of 2006(85). A Zambian study on 100 pre-school children followed for one year with analyses of stool samples once a month showed that 75 of the children had been infected with Giardia during that year, but 21 of those had no episodes of diarrhoeal disease(86). From Africa there are also some studies where children with diarrhoeal disease are included. Among 31 Gambian children with diarrhoea and malnutrition giardiasis was diagnosed in 14 (45%)(87). A larger study from a district hospital in Mozambique found that only 2.5% of 529 children with diarrhoea were infected with *Giardia*(88), and similarly Moyo found *Giardia* in 1.9% of 280 children hospitalised with diarrhoea in Tanzania(89).

A review of 33 articles on giardiasis in South Asia, South East Asia and the Far East in the period 2002-2007 revealed that the prevalence of *Giardia* varied markedly between regions and different demographic groups. The prevalence ranged from

around 3% in several South East Asian regions to 23% in the Kathmandu valley, and as high as 73% in Eastern parts of Nepal(90).

Exposure to *Giardia* is common and infection frequent in many areas, but it is challenging to estimate the consequences of giardiasis in developing countries. This is emphasised by the findings in a study from Dhaka, Bangladesh (91). *Giardia* was detected in 205 of 3,646 patients (5.6%) seeking treatment for diarrhoea, but also in 440 of 2,575 controls (17.1%) without diarrhoeal illness in the previous three months. This inverse correlation between infection with *Giardia* and diarrhoeal disease was surprising, but indicates a more complex role of parasitic infection in endemic areas. The aforementioned reports on children with diarrhoea in Mozambique and Tanzania showed very low rates of *Giardia* infection (2.5% and 1.9%), but without a control group it is difficult to interpret the result(88, 89). A study from the Iraqi city of Dohuk also indicated an inverse trend. *Giardia* was detected in 31.3% (134/428) of children with diarrhoea at the local paediatric hospital, but also in 42.8% (305/712) of stool samples collected routinely from children in school and day care centres (134).

It is difficult to reach a conclusion on the health impact of *Giardia* around the world. Nevertheless, diarrhoeal disease as a whole represents a major health problem and is still the second leading cause of death among children under five globally(92). This will have important implications for the management of giardiasis alongside other gastrointestinal infections, and *Giardia* was included in WHO's "Neglected Diseases Initiative" in 2004(93). Giardiasis is not among the 17 listed Neglected Tropical Diseases the WHO has decided to focus on(94), but still receives attention (personal communication, Dr. Antonio Montresor, WHO). Another department of the WHO, the Department of Water and Sanitation Health specifically addresses the aspects of giardiasis linked to drinking-water(95) and safe recreational water(96).

In developed countries the highest numbers of patients with giardiasis is found in outbreaks of waterborne gastroenteritis. In the period 1954 to 2001 there were at least 132 waterborne outbreaks of giardiasis registered in Europe and North America, 104

of them were associated with contaminated or presumably contaminated drinking water(97).

Non-epidemic cases of giardiasis in high-income countries have often been the result of infection abroad. In the 1970's there were several reports on giardiasis among travellers to the Soviet Union(35, 36, 98, 99), and there was also one report of an outbreak among travellers on a Mediterranean cruise in 1973(100). Later case-control studies have also showed that foreign travel is associated with an increased risk of giardiasis(101-103), but now most patients get infected in South-central Asia and South America(104). A case-control study on sporadic giardiasis in England after excluding cases infected abroad and possible secondary cases showed that drinking tap water (dose-response relationship), swimming in pools or fresh water, and eating lettuce was associated with infection(105).

The outbreak of giardiasis in Bergen in 2004 was the first large outbreak in Norway, but two outbreaks had been reported earlier in Scandinavia, both in Sweden. In October 1982 sewage entered the water system of the village Mjövik, and 454 of about 600 inhabitants (76%) fell ill with gastroenteritis. *Giardia* was detected in stools from 56 patients(26). Four years later, during Christmas of 1986, sewage overflow into the drinking water system exposed about 3,000 persons to contaminated water at the ski resort of Sälen. A total of 1,400 had giardiasis diagnosed by microscopy(106). The first known outbreak in Finland occured in 2007, after the municipal drinking water system of Nokia, a city of 30,500 inhabitants, was contaminated by sewer. During a 16 weeks period following the contamination the hospital laboratory detected *Giardia* infection in 97 patients(28).

The first report on giardiasis in Norway was given in 1931, and based on figures from hospitalised patients without gastro-intestinal symptoms the prevalence of giardiasis in the healthy population was estimated to be 7% in 1941(107). In 1953 attention was drawn to the disease by the publication of a case report in the Journal of the Norwegian Medical Association(108). In a study on patients with gastrointestinal complaints referred to Ullevål hospital in Oslo in the period 1966-1970 *Giardia* was

found in duodenal content from 6.5% (19/293) of the patients. *Giardia* cysts was also detected in stool samples from 3.2% (6/190) of unselected hospitalised patients(20). The first known community outbreak of giardiasis in Norway took place in a child day care centre in Trondheim during the winter of 2004, in which *Giardia* cysts were detected in stool samples from 12 individuals(63).

The annual incidence of reported giardiasis in Norway has varied between 200 and 400 cases, with less than 100 patients infected in Norway (Table 1). The unusual high number in 2004 was the result of the outbreak in Bergen, which constitutes the basis for this thesis. Just one year prior to the outbreak, some authors questioned whether giardiasis is underdiagnosed in Norway(109). This concern was supported by findings during an investigation of raw waters in Norway in 1998 and 1999. *Giardia* cysts were found in 11.8% (48/408) of samples and 18.4% (27/147) of sites examined(110).

Table 1. Reported cases of giardiasis in Norway 1992-2010(111).

| Place of infection | 1992 | 1994 | 1996 | 1998 | 2000 | 2002 | 2003 | 2004 | 2005 | 2006 | 2010 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Norway* | 71 | 72 | 41 | 53 | 56 | 67 | 68 | 1327 | 220 | 98 | 57 |
| Abroad | 159 | 178 | 162 | 328 | 287 | 349 | 254 | 253 | 208 | 196 | 205 |
| Total | 230 | 250 | 203 | 381 | 343 | 416 | 322 | 1580 | 428 | 294 | 262 |

*Including unknown place of infection.

## 1.2 Waterborne outbreaks of disease

The WHO states that “access to safe drinking-water is essential to health, a basic human right and a component of effective policy for health protection”(95). This is based on the experience from lessons learned.

The plague of cholera in the early 19$^{th}$ century, and John Snow’s conclusion that the disease must have been spread by drinking water, subsequently leading to the removal of the handle of the water pump on London’s Broad Street, is often used as a prelude

to the importance of both water sanitation and the academic field of epidemiology. Although some aspects of the myth have been questioned, there is still unequivocal support for Snow's prominent place in medical history(112), and the intriguing story is worthy of a novel(113).

Sanitation has improved in high-income countries, but contamination of water still occurs. The focus on safe drinking water has led to surveillance programmes in several countries. In the US data has been collected on the occurrence and causes of waterborne outbreaks of disease since 1920, and has been reported periodically since 1971. For the period 1971-2006 a total of 833 outbreaks were reported, 780 (93.6%) of these were associated with contamination of drinking water (114). An etiologic agent was identified in 467 (56.1%) outbreaks, most frequently parasites (153 outbreaks (18.4%)). *Giardia* was the sole pathogen responsible for the highest number of outbreaks (123 (14.8%)), and linked to 28,127 cases of infection. Other identified microorganisms were *Cryptosporidium*, *Shigella*, *Salmonella*, *Campylobacter* and *E. coli*. The largest registered outbreak was caused by *Cryptosporidium hominis*, and happened in Milwaukee, Wisconsin in 1993. Retrospectively it has been estimated that 403,000 people were ill(115), 4,400 patients were hospitalized(116), and that 50 deaths were associated with the outbreak(117).

In Canada 288 outbreaks of disease in the period 1974-2001were linked to drinking water. *Giardia* was the most frequently reported causative pathogen, found in 51 of the 144 outbreaks where an etiologic agent was identified. The most widely known outbreak occurred in Walkerton, Ontario, in May 2000(118). Following heavy rain fall the municipal drinking water was contaminated with both *Campylobacter* and *E. coli*. The town's inhabitants have been followed with regular medical examinations since 2002, resulting in several research projects(119, 120).

Also in Europe drinking water may constitute a health hazard. In the period 1992-2003 89 outbreaks were reported in England and Wales. *Giardia* was identified in only five outbreaks, and in three of these in combination with *Cryptosporidium*.

*Cryptosporidium* is a major concern in the UK as it alone or combined with other pathogens was implicated in 67 (78%) of the outbreaks(121).

In 2007 Karanis published a review of 325 reported water-associated outbreaks (drinking water, recreational water, travel) of parasitic disease worldwide(97). Most outbreaks, 171 (52.6%), were reported in the US, while Europe accounted for 106 (32.6%). The rest were divided between Canada, Japan, Australia and New Zealand. *Giardia* was identified as the causative agent in 130 outbreaks (40.0%) and *Cryptosporidium* in 165 (50.8%). Of the *Giardia* outbreaks, 104 (80%) were caused by contaminated drinking water compared to 77 (47%) of the *Cryptosporidium* outbreaks.

Norway is not exempted from the threat of waterborne disease, as the Bergen-outbreak of giardiasis in 2004 convincingly illustrates. A recent study describes the situation, summarising 102 waterborne outbreaks and 17,200 cases of disease in Norway during the period 1987-2007. An etiologic agent was identified in 60 (59%) outbreaks, but in contrast to the observations in the US and Great Britain parasites were registered in only two outbreaks. The most prevalent pathogens were *Campylobacter* (26 outbreaks (25%)) and Norovirus (19 outbreaks (19%)).The mean number of persons affected in surface water outbreaks was 253, the median 35(122).

## 1.3 Postinfectious complications

Infections can cause permanent injury and evident sequelae. However, in some cases patients experience loss of function and persisting symptoms that are difficult to define and without explanatory objective findings. Two conditions that are often investigated are irritable bowel syndrome (IBS) and chronic fatigue syndrome (CFS). Both are linked to infections, but are also found in patients without a history of preceding infectious disease.

### 1.3.1 Irritable bowel syndrome

IBS is a common gastrointestinal disorder, both in the general population and in patients seen in clinical practice. The reported prevalence varies between 2.5% and 37%, reflecting differences in settings and diagnostic criteria(123). The Rome process aims to reach consensus on criteria for functional gastrointestinal disorders and develop valid questionnaires to use in research and clinical practice. The criteria have been revised on four occasions, last in 2006 (Rome III)(124).

Diagnosis of IBS is based on the presence of abdominal pain or discomfort for a defined period of time and linked to alterations in bowel habits(125). The practical use of the agreed criteria still faces obstacles, as illustrated by a study comparing Rome II criteria and the clinical judgement of Norwegian general practitioners (GPs)(126). There was poor agreement, both in overall prevalence of IBS and in which patients that were identified. Reasons might be that the GPs also emphasised psychosocial factors and that in clinical practice others symptoms, for instance bloating and loose stools, are often considered essential parts of the syndrome. As a result IBS will be diagnosed more often in patients with co-morbidity, and less often in patients with few symptoms restricted to the alimentary tract.

Increased incidence of IBS has been documented after bacterial gastroenteritis caused by *Salmonella*(127, 128), *Shigella*(129, 130) *Campylobacter*(131, 132) and mixed *Campylobacter*/*E. coli* O157:H7(133), as well as after infection with the roundworm *Trichinella*(134). In one study on IBS following viral gastroenteritis, probably Norovirus-infection, 23% reported IBS after 3 months, but after 6 months the prevalence was no higher than in the control group (135). In a study on hospitalised patients in the UK there was a higher incidence of IBS after gastrointestinal (GI) infections, but unexpectedly also after non-GI infections compared to controls without infection(136). This study was small and the findings should be confirmed in other studies, but it reflects that mechanisms for IBS are complex.

One study found clinical and histological differences between patients with postinfectious IBS (PI-IBS) and IBS without history of precipitating infection(137). This suggests that PI-IBS is a clinically distinct subgroup of IBS.

Several factors have been associated with an increased risk for PI-IBS, both pathogen and patient characteristics(138). In one study the toxicity of the strain was a risk factor for IBS six months after infection with *Campylobacter*(132). Prolonged duration of the acute illness in bacterial gastroenteritis plays a role(129, 139), but it is not clear whether this is a proxy for bacterial toxicity or patient vulnerability. Receiving antibiotic therapy for *Salmonella* infection was associated with increased risk of IBS in an observational study(140). However, since there was no control group interpretation is difficult. Smoking was found to be a risk factor for PI-IBS in one study, but few participants (18 with IBS among a total of 127) and lack of obvious mechanisms for the role of smoking make conclusions uncertain(141). The biopsychosocial model is considered helpful in the understanding of IBS(142), and different psychosocial factors have been investigated in a few studies on PI-IBS. Depression(131), hypochondriasis and adverse life events (143) have been shown to be independently associated with IBS in multivariate analyses. Patients with bacterial dysentery who developed PI-IBS scored higher on anxiety, depression, somatization and neuroticism than did controls in another study(144).

IBS is more common among women with a reported female:male ratio up to 2.5. The association is stronger in clinical studies than in population based studies, and stronger in the Western countries compared to Asia(145). This suggests that both health care seeking behaviour and cultural differences may confound the results. In studies on PI-IBS there are conflicting results on the role of gender, but it seems like the impact of gender is smaller. Following the Walkerton outbreak in 2000 (mixed *Campylobacter*/*E. Coli*) a large number of patients developed IBS and the OR for female gender was 1.5 (95% CI 1.1 – 1.9)(133). Some other studies have demonstrated a higher incidence in women(127, 139), but others have not. No gender difference in development of IBS was found among patients with *Shigella* infection in

China(129)or traveller's diarrhoea in Israel(146), or after outbreaks of *Salmonella* infection in Spain(128) and *Shigella* infection in Korea(130).

### 1.3.2 Chronic fatigue

Fatigue is a common feature of different diseases such as cancer, infections, hypothyroidism, marked anaemia and psychiatric disorders. It is an unspecific symptom, and most often there is little focus on fatigue in the acute phase. As a symptom in prolonged loss of function after apparent recovery it occupies a more prominent place. It is the key symptom in chronic fatigue syndrome (CFS) which during the last years has received more attention as it affects people of young age and in many cases causes profound loss of function. Epidemiological data are uncertain, but estimates converge on a prevalence around 0.4-1.0% (147, 148). There are no specific tests that will confirm CFS. It is a clinical diagnosis based on thorough investigation to exclude other conditions that can explain the fatigue. Several definitions have been proposed for the diagnosis and although they include many of the same criteria they differ to some extent in duration and number of required symptoms (149-151). Presence of persisting severe fatigue of new onset is mandatory in all definitions, and in addition there should be a number of other symptoms, for instance sleep disturbances, muscle or joint pain, headaches, cognitive dysfunction, sore throat or painful lymph nodes.

Risk factors for CFS are often divided into predisposing, precipitating and perpetuating factors(147). Neuroticism and introversion are personality characteristics that have been linked to increased vulnerability to CFS. Around 75% of patients are women, but the reason for this gender difference is not clear(147).

A majority of patients with CFS can relate onset to some sort of infection(152). Studies have shown that several infections can trigger the condition, including Epstein-Barr virus, parvovirus B19, enteroviruses and *Coxiella Burnetti*(148). Adverse life events may also trigger CFS. Case-control studies have shown a higher rate of such events among CFS patients in the period prior to onset(153, 154).

Factors that may influence the course and prognosis in CFS can be divided into two main groups. Patients own ideas about the condition and their level of function, their sense of control over symptoms and how they relate to physical activity are linked to degree of fatigue. Interactions with others, such as partner, family and health personnel, also play a role as this will influence perception and behaviour(147). Treatment studies support that these factors are important as both cognitive behavioural therapy (CBT) and graded exercise therapy (GET) are found to be beneficial(155, 156).

Fatigue will change over time, as observed in patients with CFS. A systematic review concluded that 40% of patients improved over time and 5% eventually recovered fully(157). A more precise account of the time perspective was difficult as the duration of symptoms at inclusion and follow-up period varied between studies.

The degree of perceived fatigue or tiredness will vary along a continuum, with no clear cut-off to differentiate a medical condition from a normal situation in a healthy individual. The degree of fatigue in CFS should be substantial and lead to profound reduction in previous activity-levels. None of the criteria for CFS are based on results from questionnaires that measure fatigue. From this follows that epidemiological studies utilizing questionnaires to assess fatigue cannot identify cases of CFS or "fatigue similar to what is seen in CFS". Caseness will be based on a set cut-off to define "fatigue", "substantial fatigue" or "chronic fatigue" (CF). The value of these studies will depend on using questionnaires that are validated and tested on individuals in different populations.

### 1.3.3 Medically unexplained physical symptoms

CFS and IBS belong to a group of conditions labelled "medically unexplained physical symptoms" (MUPS) that also include fibromyalgia and other pain syndromes. They share the characteristic that the symptoms are not explained by clinical findings or results of laboratory tests. Some investigators argue that these should be considered different manifestations of some common pathologic

mechanism(158). Opposed to this is the view that these conditions are not similar and are better viewed as distinct entities(158). IBS, CFS and other syndromes are most often investigated separately, but when studied together reviews have concluded that overlap in prevalence is substantial (159, 160). On the other hand, a prospective study on patients with *Campylobacter* gastroenteritis and mononucleosis found that the two infections predisposed to different postinfectious conditions; gastroenteritis to IBS and mononucleosis to CF/CFS(161).

# 2. Aims of present study.

The outbreak in Bergen was the starting point for several research projects on different aspects of giardiasis. The aim of the present thesis was to investigate the course of giardiasis and its consequences following a large outbreak in a non-endemic area.

In the first weeks after the contamination of the water reservoir was acknowledged the aim was to describe the outbreak as it unfolded in two general practice clinics in the affected part of the city. When we during clinical practice experienced that a substantial number of patients returned to the physician's office with recurring symptoms we extended the project. We followed the patient histories for four months in the medical records, and then investigated the presence of persisting symptoms six and twelve months after the outbreak.

Three years after the outbreak we studied another and larger group of patients. The aim of this study was to estimate the prevalence and relative risk of IBS and CF in the cohort who had giardiasis verified by detection of *Giardia* in stool samples during the outbreak.

# 3. Materials and methods

## 3.1 Setting

### 3.1.1 Outline of the outbreak in Bergen in the autumn of 2004.

In 2004 there was a striking increase in the number of diarrhea cases during September and October. It was the subject of talk in town, and it was evident at the GPs' offices (figure 2). A noticeable feature that caused concern was the duration; usually people would get over a bout of gastroenteritis in a few days, but this time the symptoms lasted much longer. Still, it took several weeks before the cause of disease was found(162), as physicians were unfamiliar with the clinical picture and investigations for detection of *Giardia* were not performed routinely. The conclusion after the outbreak was that there was no uniform diagnostic approach, but that different clues or coincidences had led to diagnoses and gradual accumulation of identified cases(163).

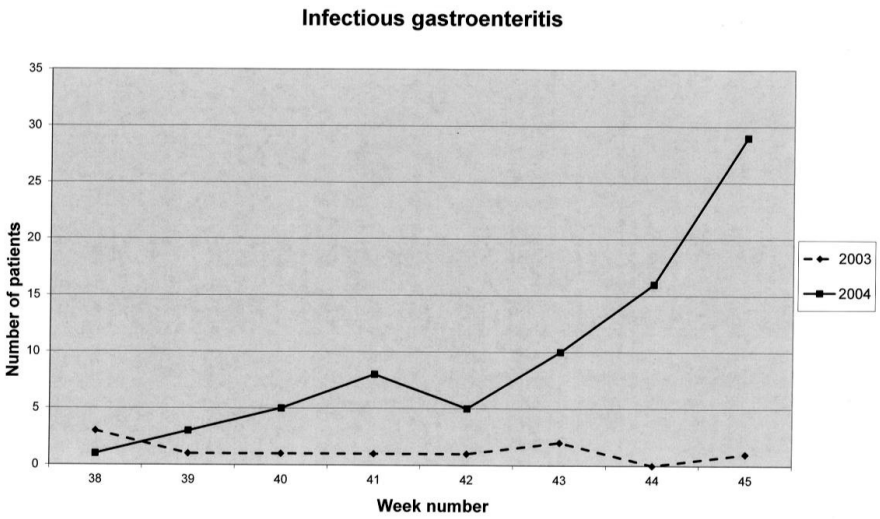


Figure 2. Number of patients diagnosed with infectious gastroenteritis per week at the general practice clinic "Kalfaret legesenter" during the autumn of 2003 and 2004.

Both an internal(164) and an external(165) evaluation committee have investigated the outbreak. On October 29th the municipal health officer was alerted by the local laboratory because in four weeks had identified *Giardia* cysts in stool samples from 27 persons who had not been abroad, more than the total number of positive samples that would normally be registered through a whole year. A task group was formed on the following weekday, November 1st, and information about the outbreak was conveyed to the public. The ensuing reaction showed that many of Bergen's inhabitants were affected, and a steep increase in the number of diagnosed cases followed. Most new cases were registered in November and in the beginning of December, but a few cases linked to the outbreak would be identified each week until spring(165).

A specific investigation into the waterworks and the quality of the drinking water in Bergen concluded that *Giardia* had entered the reservoir "Svartediket" through malfunctioning sewers in the surrounding area. Heavy rain fall in the end of August was considered the triggering factor, and the drinking water was probably contaminated for several weeks (Figure 3)(166).

Svartediket and the central parts of Bergen. (Photo: K-A Wensaas)

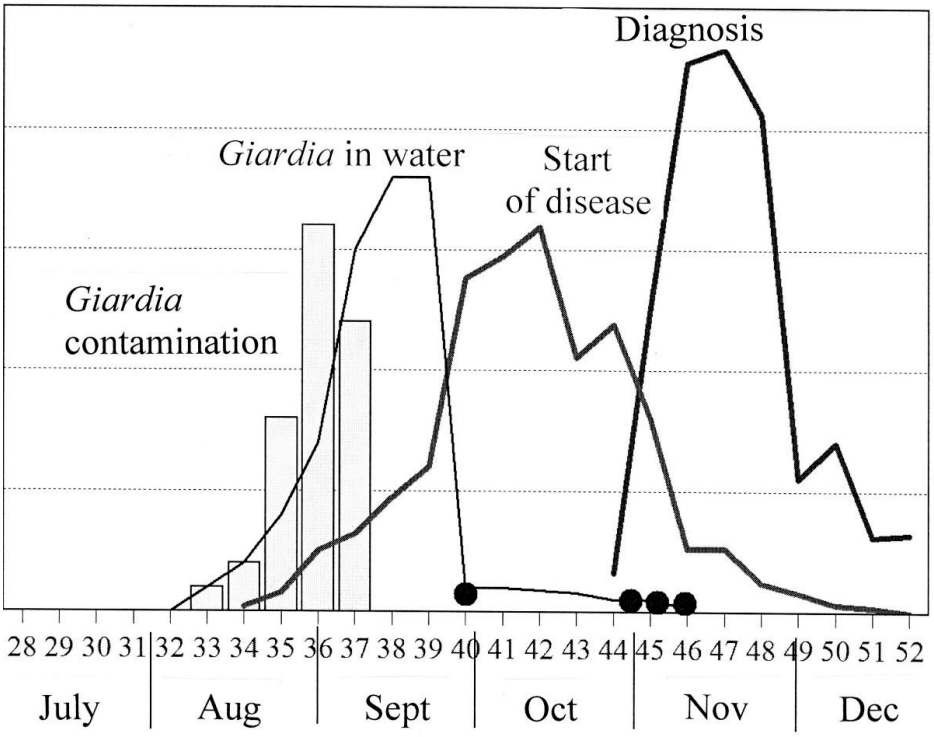


Figure 3. Possible development of the outbreak week by week in the autumn of 2004. *Giardia* contamination and *Giardia* in water are estimates. (Used by permission from Rådgivende Biologer(166).)

*Giardia* was the subject of much local attention for a long time after the outbreak. The municipality posted regular updates on its web-page, and there were several articles in the two major newspapers in the city (Bergens Tidende and Bergensavisen). There was a lot of focus on patients not recovering after the infection, and on the responsibilities of the municipality as supplier of water to the inhabitants. In April 2005 the Municipality of Bergen claimed full responsibility for the outbreak and decided to give compensation for any verified economical loss to those affected, including students' loss of future income due to delayed completion of exams. Table 2 shows how the dates for profiled articles in the media related to key dates in this project.

Table 2. Timetable showing media coverage and development of *Giardia*-projects.

| Date | News-story | Research projects |
|---|---|---|
| November 2nd 2004 | Outbreak of giardiasis detected | |
| January 11th 2005 | Treatment options for resistant giardiasis | |
| February 28th 2005 | | Registration ended for paper I |
| April 4th 2005 | Municipality claim responsibility, will offer compensation. | |
| May 1st 2005 | | First questionnaire (six months) mailed for paper II |
| June 6th 2005 | Mother of two died after *Giardia*-infection | |
| September 24th 2005 | Doctors uncertain about the management of chronic giardiasis | |
| November 1st 2005 | | Second questionnaire (12 months) mailed for paper II |
| Mars 2nd 2006 | Hundreds still sick from giardiasis | |
| June 10th 2006 | Several *Giardia*-victims claim millions of kroner | |
| March 3rd 2007 | 12 persons still sick after *Giardia*-infection | |
| August 5th 2007 | *Giardia*-victims feel they have been forgotten | |
| November 1st 2007 | | Questionnaire mailed for paper III |

### 3.1.2 The registered patient list system

Norway has a registered patient list system for general practice, where every citizen can choose to be on the patient list of one specified GP. As of December 31st 2008 99.6% of the Norwegian population of 4.8 million was part of this system(167). Patients are supposed to seek their GP for all first-time contacts with the health services, regarding both acute and chronic illness.

In addition, there are emergency wards open to the public day and night outside regular opening hours. During the *Giardia* outbreak the municipal emergency ward in

Bergen registered an increased number of patients with gastroenteritis and giardiasis(168).

## 3.2 Design

The outbreak of giardiasis is Bergen constituted a defined and unexpected change in the environment and can be considered a "natural experiment". This gives better control of time order as the *Giardia*-infection preceded the conditions that are investigated. Still it was not an experiment in the sense that it was possible to assign individuals to the exposure, as defined by contaminated water, infection or clinical disease. The design is therefore observational with its problems of bias and confounding. In addition, there is limited control over who was actually exposed, as discussed elsewhere in this thesis.

In the study on patients from general practice (Papers I and II) participants were identified and data were collected retrospectively from the medical records, but the information was registered prospectively as part of regular clinical work. The follow-up 6 and 12 months after the outbreak were done prospectively.

The patients with verified giardiasis during the outbreak (Paper III) were identified retrospectively from the records at the parasitology laboratory at the hospital. They constitute a historic cohort that has been followed prospectively after the outbreak.

## 3.3 Participants

### 3.3.1 Patients with giardiasis

The participants who were sick with giardiasis and investigated in the studies for this thesis constitute two different groups, representing two different patient populations. The two groups partly overlap.

Papers I and II are based on data from 119 patients contacting two general practice clinics located in the area supplied with water from the contaminated reservoir (Figure 4). The patients were identified by a structured and targeted search in the patient records at the clinics, and included based on a clinical case definition, with at least one of the following criteria:

- Two or more of the following symptoms for more than one week: diarrhoea, nausea, distension, abdominal pain, foul-smelling flatulence/belching.
- Positive faecal test for *Giardia lamblia* (microscopy and/or antigen detection).
- The responsible doctor stating that the patient has giardiasis, either in the text or by giving the specific diagnosis.

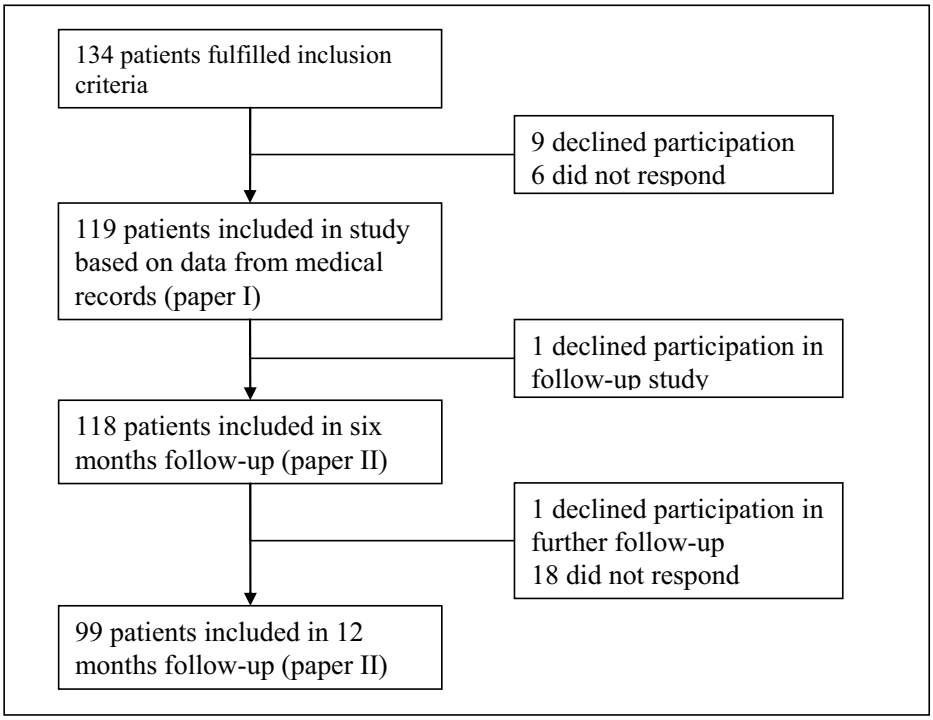


Figure 4. Patients from general practice included in study of giardiasis.

Data from a period of six months around the time of the outbreak were recorded, starting two months before it was publically known. The patients were then mailed questionnaires 6 and 12 months after the outbreak and at six months were also asked

to submit stool samplesPaper III is based on self-reported symptoms among patients three years after they suffered from acute giardiasis during the outbreak. They were identified by detection of *Giardia* in stool samples collected as part of regular clinical practice. 66 of the 119 patients in the first group from general practice had a positive faecal test for *Giardia lamblia*, and were thus included in this study as well. The samples were analysed at the parasitology laboratory at Haukeland University Hospital, the only one of its kind serving the Bergen area. On behalf of the research group the laboratory mailed a questionnaire to all patients registered with a positive test (Figure 5).

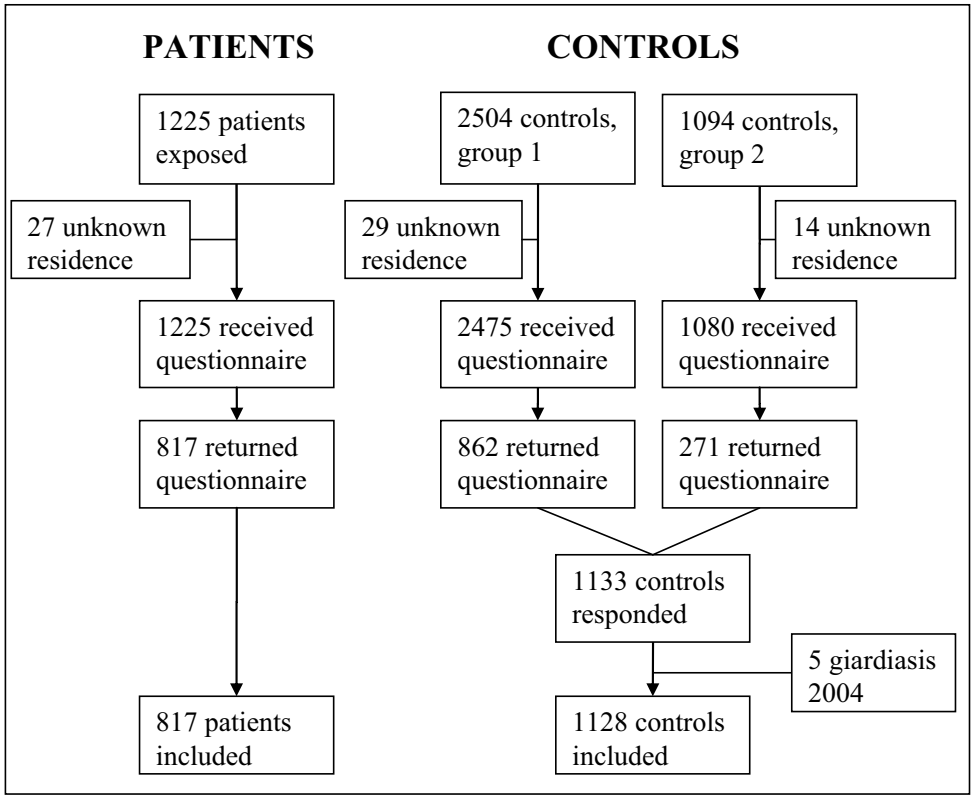


Figure 5. Participants in a study of self-reported symptoms three years after an outbreak of giardiasis. Patients with verified giardiasis during the outbreak, and controls matched by age and gender. (Paper III.)

### 3.3.2 Diagnostic procedures

Detection of cysts or trophozoites in stool samples establishes that there is an infection with *Giardia*. Since asymptomatic infection occurs there should also be clinical symptoms present in order to diagnose giardiasis, the clinical disease.

*Giardia* is commonly demonstrated by two available methods, microscopy and antigen detection. Since shedding of cysts is inconsistent there is a risk that diagnosis is missed if only one single sample is analyzed. It is therefore recommended that regular investigation should include three stool samples from three different days in order to improve sensitivity(30).

When the prevalence of giardiasis is increased, for instance during an outbreak, the probability of giardiasis will be higher when a person has symptoms of gastroenteritis. In this setting the clinical presentation may be considered to be diagnostic, and neither the patient nor the physician would see any added benefit in submitting stool samples for analysis.

The epidemiologist would be interested in complete data on both clinical symptoms and stool examination, but in an outbreak situation this goal will seldom be reached, especially when many people are affected. For instance, for a period after the outbreak was known the municipal health officer requested that physicians did not submit stool samples in obvious cases as the capacity at the laboratory was exceeded. Registration of symptoms may also be less adequate than expected for research when the diagnosis seemed sufficiently clear to warrant treatment.

### 3.3.3 Control group

In the study on which Paper III is based we included a control group. Three main issues had to be resolved before the group could be selected. First, we had to define which criteria the individuals in the group should meet. We knew that among those with verified giardiasis there were more women than men, and nearly 50% were in the age group 20 – 29 years(162). In order to balance this we matched the controls by age

and gender. The second issue was to decide which population the controls should be drawn from. We wanted the controls to differ as little as possible from those exposed, and were concerned about the risk of uncontrollable confounders if the sample was drawn from a population in another city in Norway, for instance Oslo or Trondheim. We ended up recruiting controls from the general population in Bergen, and were aware that some of the controls could have been exposed to *Giardia* and also infected. We considered the risk of such misclassification to be acceptable as controls were sampled from the whole of Bergen, and those who reported giardiasis during the outbreak were excluded. Third, we had to decide on the number of controls. Based on the results from a population based study in Norway(169) we assumed the prevalence for chronic fatigue to be 0.11 among the exposed, and a statistician performed a calculation of statistical power using the software East4. When introducing a 2:1 matched control group of 2,500 individuals we would identify a difference in prevalence of 0.04 between the groups at the 95% power level (two-sided). We expected the actual difference to be larger, but since we could not meet all the requirements in the model (the designed model is a randomized trial) and we wanted to compensate for potential loss of power after adjusting for other variables in the analyses we concluded that this would be an appropriate sample size.

According to this, Statistics Norway mailed the questionnaire to a group of 2504 control persons matched by age and gender and drawn from the general population in Bergen. Of these, 862 responded (34.4%). In an effort to reduce possible bias caused by this low response rate we decided to expand the control group by adding two more controls for each exposed individual when none of the first two controls had responded. As a result the questionnaire was mailed to 1,094 additional controls six months later (Figure 5).

## 3.4 Variables

The variables and research questions were developed based on observations by members of the research group during their clinical work as physicians, and on the findings in the studies as the projects evolved. Prior to the outbreak in 2004 health services in Bergen had limited experience with giardiasis, and very little was published in the literature about the possible long-term consequences following the infection.

### 3.4.1 Outcome variables

*Gastrointestinal symptoms and functional gastrointestinal disorders.*
In Paper I we describe how a proportion of the patients contacted their GP on several occasions because the complaints elicited by the infection persisted in spite of treatment. We did not have a clear understanding of the extent of the problem or how to interpret what we observed, other than that the symptoms resembled those of acute giardiasis. In the questionnaires mailed to the patients 6 and 12 months after the outbreak we simply asked whether they still had specific gastrointestinal symptoms appearing after the acute infection (Appendices).

We found that many patients had persisting symptoms even beyond eradication of the parasite, and that many of them stopped seeing the health services. This made us wonder if a substantial part of all patients in Bergen who got sick during the outbreak might have persisting symptoms without our awareness. To investigate this we addressed another patient population, everybody who had *Giardia* detected in a stool sample submitted as part of clinical investigation during the outbreak. They received similar questions on the presence of specific symptoms two years after the outbreak, and it turned out that a 38% reported persisting gastrointestinal symptoms(170). It was difficult to interpret the result since we didn't know what a population without previous giardiasis would report, and comparison with other studies suffered since the questions were not the same. As a result, when we did a follow-up study one year

later (Paper III) we included a control group and we decided to use a questionnaire that was accepted as a more valid tool to measure and classify gastrointestinal symptoms in the absence of pathological findings. Our main focus was on irritable bowel syndrome (IBS), and we designed a questionnaire based on Rome criteria (Appendix). When we planned our study the latest Rome III criteria had just been published(171). We decided to follow the new criteria; the alternative would have been to use Rome II criteria which had been the standard up to that point. There are differences between Rome II and Rome III criteria for IBS (Table 3) and it is not clear which will most accurately identify the "true" condition. Later studies have shown that Rome III criteria will give a higher prevalence of IBS compared to Rome II criteria(172-174).

As it is difficult to assess the clinical implications of the consensus based diagnosis of IBS, we designed two categories of more serious IBS. "Frequent IBS" was defined as IBS with pain or discomfort more than one day a week. "Severe IBS" was defined as IBS limiting or restricting daily activities at least "often".

Table 3. Comparing Rome II and III criteria for irritable bowel syndrome (IBS).

| | Rome II criteria(175) | Rome III criteria(125) |
|---|---|---|
| Key feature | Abdominal pain or discomfort | Recurrent abdominal pain or discomfort |
| Duration | At least 12 weeks (need not be consecutive) in the preceding 12 months | At least 3 days per month in the last 3 months, with symptom onset at least 6 months ago |
| Additional characteristics (at least two out of three) | • Relieved with defecation<br>• Onset associated with a change in frequency of stool<br>• Onset associated with a change in form of stool | • Improvement with defecation<br>• Onset associated with a change in frequency of stool<br>• Onset associated with a change in form of stool |

### *Fatigue*

When patients did not get well after the *Giardia*-infection we expected gastrointestinal symptoms, and in the clinical setting this is what we looked for. But it became evident that fatigue might also be a problem, as several patients spontaneously complained of this. As a consequence we addressed this through a single question in the study performed two years after the outbreak on the group with verified giardiasis, and found a prevalence of 41%(170). We felt it urgent to elucidate this finding and include a validated set of questions on fatigue in the follow-up study. Several questionnaires are designed to measure fatigue, and we wanted one that had been used in similar setting previously. We considered the scale used by Hickie in an often cited prospective study on postinfectious fatigue following infection with Epstein-Barr virus, *Coxiella Burnetti* (Q fever) or Ross River virus(176), but it was impossible to elicit which scale had actually been used. The article refers to a 12-item "SOMA"-scale, but the reference given discusses a 10-item "SOFA"-scale(177). We ended up assessing two scales that have been widely used in different settings; the Fatigue Severity Scale (FSS)(178) and the Fatigue Questionnaire (FQ)(179) in its revised version(180). Some researchers in the group were familiar with the FSS as it had been used in patients with chronic fatigue syndrome in our area. However, we landed on the FQ, most importantly because it has been used in a study on fatigue in the general population in Norway(169). The FQ has also been widely used to measure cancer-related fatigue (CRF)(181), and has been considered a useful tool for assessing fatigue in a variety of conditions(182). A later review, from 2009, has questioned whether it discriminates cases from non-cases with acceptable sensitivity and specificity, and its ability to act as an outcome measure sensitive to change with disease progression or treatment. In these respects the FSS is considered to perform better(183).

As with IBS we also designed two categories of more serious fatigue. "Severe fatigue" was defined as the combination of chronic fatigue and a total fatigue score of 23 or more, and "consistent fatigue" as chronic fatigue combined with fatigue present at least 75% of the time.

The term “fatigue” when used in conversation or as a symptom is not easily defined. Even though we use the generally accepted Fatigue Questionnaire we decided to elaborate further the aspects of this symptom and included Epworth Sleepiness Scale in the questionnaire(184). These data are not yet published.

### 3.4.2 Explanatory variables

#### *Exposure to Giardia*

In the study reported in Paper III we included a control group, and whether the participants were exposed to infection with *Giardia* was a key variable in this study.

#### *Demographic variables*

Age and gender were recorded for all participants in both studies. The cohort of patients with verified giardiasis and their controls were asked about marital status, level of education, occupation and whether they were students during the outbreak.

#### *Variables to grade exposure*

In the group of patients from general practice, data concerning the acute phase of the infection (result of stool sample analysis, treatment, and time from symptoms until treatment) were obtained from the medical records. At six months after the outbreak we asked them how much tap water they drank prior to the outbreak, as a measure of exposure to the pathogen. A case-control study performed just after the outbreak was acknowledged showed that a daily intake of more than five glasses of tap water was associated with giardiasis(162).

#### *Predisposing or perpetuating factors*

The group of patients from general practice where asked about prior abdominal complaints in the questionnaire mailed six months after the outbreak. There was poor agreement between the answers to that question and what was documented in the medical records during the previous two years. We decided to use the data from the

medical records in the analyses, as they were not influenced by recall bias. Since both IBS and CFS is associated with several psychosocial factors it would be of interest to obtain data on this. Twelve months after the outbreak the patients were asked to complete the neuroticism-part of the short scale Eysenck Personality Questionnaire (EPQ-N).

When we designed the questionnaire for the three year follow-up, we were unsure about introducing questions on psychosocial factors, like anxiety, depression, hypochondriasis, neuroticism or adverse life events. We feared that some patients would find the questions irrelevant and that introducing them could reduce the response rate, even if the response rate among those who received the EPQ-N was 81% (95/118). Another aspect was that the questionnaire should not be too extensive, and the one prepared for the cohort with verified giardiasis (Paper III) already was six pages. As a result we decided not to introduce these variables in the questionnaire.

## 3.5 Analyses and statistical methods

In Papers I and II chi²-test or Fischer's exact test was used to test differences between proportions, and Student's T-test to test differences for continuous variables. In Paper II relative risks (RR) were given for categorical variables and mean differences for continuous variables in the secondary analysis, both with 95% confidence intervals (CI). All tests were two-sided, and level of statistical significance was set at $p<0.05$. All data in Paper I were analysed in SPSS version 14.0, and in Paper II in SPSS for Windows version 15.0.

In Paper III some of the responders did not complete all parts of the questionnaire. Persons with missing data were excluded from the analysis involving that particular variable, unless data were still sufficient to unequivocally define the outcome according to set criteria. For instance, the diagnosis of IBS requires a positive response to two out of three questions in addition to presence of abdominal pain. If response to one of those questions were missing, data would still be sufficient if the

response to the other two were either both positive or both negative, but not if one was positive and the other one negative.

Association in 2×k tables was tested by Pearson's chi²-test, and results reported as RR with 95% CI. IBS and chronic fatigue were analysed separately with respect to the risk factors and possible interactions using multiple logistic regression producing adjusted odds ratios (OR) with 95% CI. Since both outcomes are common, the ORs will be higher than the RRs and the difference bigger than when outcomes are rare. ORs were therefore converted to RRs and corresponding CIs by the method of Zhang an Yu(185) in order to improve clarity and interpretation of results. Other methods are also available, and they will to some degree yield different results(186). Effect modification was tested by the Breslow-Day test for homogeneity of ORs after stratification. Confounding was evaluated by use of the Mantel-Haenszel common OR and multiple logistic regression analyses, and variables found to be confounders were then controlled for by multiple logistic regression. Means of continuous variables were compared between groups using Gosset's unpaired t-test. Internal consistency of the FQ was tested by calculating Cronbach's alpha for all items. Level of statistical significance was set at 0.05, and all tests were two-sided. All analyses were done using SPSS for Windows version 15.0.

## 3.6 Ethics

All studies in this thesis have been approved by the Regional Committee for Medical and Health Research Ethics (projects 060.05, 219.05 and 150.07). All studies are also approved by the Ombudsman for Privacy in Research, Norwegian Social Science Data Services (projects 12518, 13562 and 17014).

The studies were all initiated before the new Act on Medical and Health Research entered into force in 2009. The purpose of the new act is to improve and simplify the regulations in the field by collecting all aspects within the framework of a single act, and make researchers relate to one organization only for notification and approval of

projects. Any research must now obtain advance approval by the Regional committee for medical and health research ethics. The committee's mandate is to conduct an ethical evaluation of the project and to examine that it is in accordance with current laws and regulations.

We have felt an ethical obligation towards the participants in the projects and the community both to secure the integrity of the individuals and to maximize the quality of the research. We highly appreciate the effort the participants have made by supplying the information needed for the studies, and therefore feel it urgent to complete the work made possible by their contributions.

All included individuals have given written consent to participate, for those less than 18 years old consent was given by a guardian. Non-responders where reminded once and requested to participate. The data for Papers I and II will be held for 10 years, for Paper III for 30 years.

The group of patients from general practice were requested to submit stool samples six months after the outbreak. All who complied received a written notification of the result with instructions on further measures to be taken if the sample was positive for *Giardia*.

As the projects evolved we found that a substantial proportion of the patients who suffered from acute giardiasis in 2004 reported long term complications. This was something we had not foreseen. To facilitate further research into mechanisms and associations we asked the participants in the study three years after the outbreak (Paper III) for consent to link data in the current study with information in health registries.

# 4. Summary of results

## 4.1 Paper I

### Prevalence of recurring symptoms after infection with *Giardia lamblia* in a non-endemic area.

In this study we described the course of giardiasis as it was recorded in the patients' medical records, with special attention to prolonged disease and recurring symptoms.

From the population of 7,100 inhabitants assigned to two general practice clinics located in the outbreak area we identified 134 patients who met the criteria for a clinical case definition of giardiasis. Of these, 119 gave consent to take part in the study. We retrospectively registered data entered into the patients' medical records during the outbreak and up to February 28$^{th}$ 2005, four months after the outbreak was publically acknowledged. We also registered any information on gastrointestinal complaints in the period two years prior to the outbreak.

There were more women among the patients (69.3%), and the majority was between 20 and 39 years of age (51.4%). A positive test for *Giardia lamblia* was registered for 55% (66/119) of the patients. Eighty nine (75%) were treated with metronidazole. After initial treatment 36% (32/89) returned to their doctor because symptoms recurred. Compared to those not returning a significantly higher proportion of this group had seen their GP for other GI-complaints in the previous two years (8 of 32 vs. 3 of 54, p=0.009). A second prescription for metronidazole was given to 28% (25/89) of the patients, and most often both the daily dose and the duration were increased. After the second treatment 16% (14/89) returned once more, and 11% (10/89) were treated a third time. Five patients (6%) not getting well after the third treatment were referred to the hospital's outpatient clinic for further investigations and follow-up.

Conclusion: A strict laboratory based diagnosis would have missed a substantial part of patients with clinical giardiasis during the outbreak. One third of the patients experienced recurring symptoms after treatment, and there was an association between previous gastrointestinal complaints and recurrent symptoms.

## 4.2 Paper II

### Post-infectious gastrointestinal symptoms after acute Giardiasis. A 1-year follow up in general practice

The aim of this study was to investigate the presence of gastrointestinal complaints and persistent infection among *Giardia*-patients in a one year period after the initial infection.

From the study population described in Paper I, 118 patients gave consent to take part in a follow-up study, and returned a questionnaire delivered by mail six months after the outbreak. They were also requested to submit stool samples. A second questionnaire mailed 12 months after the outbreak was returned by 99 patients (84%).

Eighty two of the patients (69%) submitted stool samples six months after the outbreak. *Giardia lamblia* was detected in samples from three patients, all of whom were already referred to the out-patient clinic at the hospital and followed up. In the questionnaires returned after six months 37% (44/118) of the patients reported gastrointestinal symptoms related to their *Giardia*-infection. This proportion went down to 19% (19/99) after 12 months. The reported water intake prior to the outbreak was significantly higher in patients with persistent symptoms, but there was no association with gender or neuroticism.

Conclusion: Persistent gastrointestinal symptoms are common complications after giardiasis in a population most likely previously unexposed to *Giardia lamblia*. This study shows an attenuation of symptoms with time.

## 4.3 Paper III

### Irritable bowel syndrome and chronic fatigue three years after acute giardiasis: historic cohort study

The aim of this study was to estimate the relative risk of irritable bowel syndrome (IBS) and chronic fatigue three years after acute giardiasis. Data were collected by a questionnaire mailed to all 1252 patients with giardiasis verified by detection of cysts in stool samples, and to a matched control group of 3598 individuals without previous infection. The response rates were 65.3% (817 of 1252) among the exposed and 31.5% (1133 of 3598) among controls.

The prevalence of IBS in the group with previous giardiasis was 46.1% (355/770) compared to 14.0% (155/1105) in the control group, and the adjusted relative risk was 3.4 (95% CI 2.9 to 3.8). Chronic fatigue was reported by 46.1% (366/794) of the exposed and 12.0% (134/1118) of the controls, the adjusted relative risk was 4.0 (95% CI 3.5 to 4.5). IBS and chronic fatigue were associated and the relative risk for the exposed group of having a combination of the two outcomes was 6.8 (95% CI 5.3 to 8.5). The relative risk was also increased for having just one of the two syndromes only, 1.8 for IBS (95% CI 1.4 to 2.3) and 2.2 for chronic fatigue (95% CI 1.7 to 2.8). The RRs of the secondary outcomes “severe” or “frequent” IBS were 5.1 (95% CI 3.6 to 7.2) and 6.2 (95% CI 4.5 to 8.3), respectively, and for the secondary outcomes, “severe” and “consistent” chronic fatigue, the RRs were 7.4 (95% CI 4.9 to 10.9) and 3.6 (95% CI 2.6 to 4.7).

Conclusions: Infection with *Giardia lamblia* in a non-endemic area was associated with a high prevalence of irritable bowel syndrome and chronic fatigue three years after acute illness, and the risk was significantly higher than in the control group. This indicates that the potential consequences of giardiasis are more serious than previously known.

# 5. Discussion

The main finding in this thesis is that acute giardiasis is associated with long-term postinfectious gastrointestinal disorders and chronic fatigue. This is based on observations during the outbreak and 6 months, one year and three years afterwards.

## 5.1 Strengths and weaknesses

Outbreaks offer good opportunities to investigate clinical aspects of infectious diseases because exposure will be quite clearly defined and the number of patients will often be large. However, there are several limitations inherent in this kind of research and even though they are hard or even impossible to avoid they should be addressed.

### 5.1.1 Research on outbreaks of disease

Large clinical research projects based on outbreaks of infection are rare. Outbreaks will often hit at random and in places far from the expertise on that particular infection. For professionals involved, focus will be on public health issues and patient care. The main tasks will be to determine the source of the outbreak, find the etiologic agent and determine the number of affected patients. This is necessary in order to close the routes of transmission and give proper treatment to those with disease. As a result, most articles describe the issues mentioned above, since this information would have been recorded as part of the regular surveillance of the outbreak. Further research is often limited by restricted resources and limited time to set up a research protocol, apply for necessary approvals and raise the funds needed.

A major strength of this project is that we responded fast, established a research group and managed to investigate several aspects of the outbreak in Bergen. We have described and analysed the clinical manifestations during the acute phase, as they

were recorded by GPs in real time. The data were collected after the outbreak subsided, but we found that the quality of the registration was good, for instance did all GPs consistently record key symptoms and duration of illness for all patients identified. We have also followed patients and registered symptoms several times after the acute disease. The quality of the joint scientific effort in Bergen is enhanced by a continuous collaboration between researchers from both primary and secondary care.

### 5.1.2 Lack of baseline data from the time prior to the outbreak.

Valuable background data about patients prior to outbreaks will often be scarce. We have some data on the patients from general practice (Paper I), but none that is systematically registered. Assessing retrospective information on symptoms or characteristics is very difficult, and should be done with great care. In our studies we have decided not to focus on these factors and accept the limitations this causes.

### 5.1.3 Risk of misclassification

When describing the overall effect of an outbreak of infectious disease there is always an inherent risk of misclassification of patients. This means that a person with disease is classified as without disease, or the other way round. When evaluating this risk several aspects must be considered:

- The definition of disease in contrast to asymptomatic infection.
- The prevalence of the infection, both symptomatic and asymptomatic, in the population.
- The sensitivity and specificity of the diagnostic test. In this setting the word “test” includes both analyses in the laboratory and clinical signs and investigations.

*Persons with disease falsely classified as without disease.*
This may result from a diagnostic test being falsely negative, or the test not being applied (either because the person didn't seek health care or because the test was not performed). There may be systematic differences between patients falsely classified as without disease and those correctly identified.. This can be attributed to both different sensitivity and specificity of the test in the groups, to different clinical presentation and severity, and to difference in patients' vulnerability or threshold to seek health care. The identified patients, the "true positives", may be more susceptible to infection, to complications or both, compared to the unidentified patients, the "false negatives".

*Persons without disease falsely classified as having disease.*
The diagnostic test might not differentiate between persons with the disease in question and other conditions. This pertains to the specificity of the test. Symptoms of gastroenteritis may be considered diagnostic of giardiasis during an outbreak, but it is possible that in some cases there would be another cause. Laboratory tests may also give a positive result even if the pathogen is absent. Another aspect of laboratory tests is that they will not differentiate between persons with disease and asymptomatic carriers of the pathogen. If clinical information is missing it will be difficult to define the population that is being investigated. A person without symptoms who wants an investigation because of exposure (for instance high intake of tap water during the outbreak) belongs to a different population than a person with severe and typical symptoms of giardiasis. The pre-test probability will be different, and the risk of getting a false positive result will differ accordingly. Those individuals without the disease, but receiving the diagnosis and getting misclassified, the "false positives", will differ from the "true positives" in exposure. They may also differ from the "true negatives" in respect to previous health complaints, health care seeking behaviour and susceptibility to persisting symptoms.

When recruiting participants based on health-care seeking behaviour there will be reduced control of what the pre-test probability of disease is, since people's

inclination and reason for seeing their doctor will vary. The population in question will also differ depending on whether the persons are recruited from primary, secondary or tertiary care. It is therefore important to bear in mind which group of patients that have been investigated, especially when discussing to what extent the results will be valid in other populations.

### 5.1.4 Selection bias in relation to identified patients

Participants were included based on attendance at the GPs office or on the investigation of stool samples submitted as part of regular clinical practice. The composition of the study groups will therefore be influenced by health-care seeking behaviour by individuals. We have tried to include all patients in the defined groups, but their identification also relied on the actions or information recorded by the physicians. As diagnosis will more readily be made based on typical symptoms, patients with few or atypical symptoms might be missed. In a cohort of patients with laboratory verified giardiasis the physicians' decision to ask for stool samples was instrumental in defining the patient population. The inclination to do this will most certainly vary over time for any one physician, and also more systematically between different physicians.

In the study referred in Paper III we have included patients with giardiasis based on detection of Giardia in stool samples. This group might differ from patients with giardiasis without positive stool samples, who will either have a negative test result or did not submit a sample. The risk of a false negative result may be higher in patients with fewer parasites or cysts in the stool, which may also lead to milder disease and a lower risk of complications. There may be several reasons why a stool sample was not submitted for investigation, some decisions made by the doctor and some by the patient. Patients with less symptoms and/or spontaneous recovery may not have contacted the health services at all. For patients with more severe and typical symptoms of giardiasis during the outbreak physicians would probably prescribe treatment without waiting for the result of stool analyses. Then stool samples might

not be submitted, either because the doctor didn't find it necessary or because the patient didn't see the benefit. Since treatment failed in some cases this could prompt patients with more complicated infection to submit stool samples.

### 5.1.5 Selection bias in relation to included patients and controls.

In the study from general practice, 89% (119/134) of the identified patients were included, with response rates of 88% (118/134) after 6 months and 74% (99/134) after 12 months. In the study of patients with verified giardiasis the response rate was 65.3% in the exposed group. These rates are acceptable, and high compared to other studies investigating postinfectious complications after bacterial gastroenteritis, based on both mailed questionnaires(128) and consultations(133). Still there is a risk of bias, but it is difficult to estimate how this would influence the results. Non-responders could be recovered patients who felt the questionnaire did not concern them, but also include some with severe symptoms and no wish or energy to complete yet another questionnaire.

The low response rate (31.5%) among the controls in the last paper gives reason to be aware of possible selection bias in this group. We are to a certain extent reassured by the fact that a prevalence of 14% IBS and 12% chronic fatigue is not very different from what has been found in population based studies in Norway(169, 187). Nevertheless, a higher response rate would be desirable. We tried to address this by expanding the control group after we found that the initial response rate was only 34.4%, but we ended up with just 24.8% of the additional controls responding. What we gained in number of cases and better control of random statistical error we may have lost in reduced response rate and greater risk of systematic error.

### 5.1.6 Recall bias and information bias.

The answer to any question relating to the past may be imprecise or incorrect. In the study from general practice on which Paper II is based we asked the patients whether they had abdominal problems or symptoms prior to the outbreak. The answers did not

correlate well with the information in the medical records referred in Paper I, and therefore were difficult to interpret. The main topic of interest in the study was persisting symptoms after giardiasis, and we asked the participants about persisting symptoms that were new after they fell sick with giardiasis. Although this pertained to the current situation, there was also an aspect of recall. They may be inclined to relate chronic recurring symptoms to the *Giardia*-infection and give a positive response suggesting a relationship where in fact it may be weak.

In the larger cohort of patients with verified giardiasis and their controls we asked about current symptoms. The answers would not be dependent on memory, but there might still be some information bias. There is a possibility that those having suffered acute giardiasis would be more aware of symptoms and more likely to find them abnormal than the controls, and thus more often report complaints.

## 5.2 The scale of the outbreak of giardiasis in Bergen 2004

We know that at least 48,000 individuals were potentially exposed to Giardia cysts in the drinking water. This is the number of registered subscribers receiving water from the contaminated reservoir(162). Many people from outside the area were also exposed as they would visit shopping areas, offices, schools and universities in the central part of the city. Intake of tap water, drinking more than 5 glasses daily, was associated with an increased risk of giardiasis(162), but we don't know the number of people with an intake of tap water sufficient for getting the infection.

The exact number of affected patients is unknown, as there were no precise records of all giardiasis cases. It has been suggested that 5 - 6,000 persons might have been infected, with varying degree of illness(165). This estimate is most unsure, as there was no environmental testing of persons receiving water from Svartediket during the outbreak. In view of the high number of people exposed this could be considered a conservative estimate.

The parasitology laboratory at Haukeland University Hospital, the only one of its kind serving the Bergen area, detected *Giardia* in stool samples from 1,253 persons assumed to have been infected during the outbreak. The samples were all submitted as part of regular clinical work.

Twice as many were probably treated for giardiasis. A review of prescriptions for metronidazole at pharmacies in Bergen concluded that an excess of 2,500 persons were treated in the months following the outbreak, compared to the period before(162). In paper I of this thesis we report that 55% of patients with clinical signs of giardiasis had the diagnosis verified by a positive stool sample. A review of medical records at Bergen Accident and Emergency Department revealed the same trend; 420 patients had records were the name "*Giardia*" was entered and for 200 of them (48%) a positive stool sample was registered(168). This supports that the number of affected individuals was at least twice the number of infected patients registered at the laboratory, probably somewhat higher. In our study 89 out of 119 patients (75%) received treatment by their GP. Some of the remaining patients may have been given metronidazole elsewhere without this being registered in the medical records, but it is unlikely that this is the case for all of them. This illustrates that a proportion with symptomatic giardiasis was not treated. One reason could be spontaneous recovery as diagnosis was delayed.

During the outbreak it was feared that a substantial number of asymptomatic carriers might constitute a reservoir for subsequent outbreaks in the years to come. Fortunately, history has so far not confirmed this concern. And further, one year after the outbreak the prevalence of *Giardia* in preschool children living in the exposed area was low (1.7%), and small children are thus unlikely to be a source of continued transmission(188).

Table 4. Number of persons affected during the outbreak of giardiasis in 2004.

| Number of persons affected | Characteristic of group |
|---|---|
| 48,000 | Registered receivers of water from contaminated source ("exposed")(162) |
| 5,000 | Unverified estimate of persons with infection ("infected") |
| 2,500 | Persons treated with metronidazole ("clinical disease")(162) |
| 1,250 | Persons with *Giardia* in stool samples ("verified giardiasis")(170) |

## 5.3 The clinical consequences of giardiasis

Why does a substantial proportion of the patients with acute giardiasis in 2004 report long lasting symptoms?

This thesis, together with other studies after the outbreak, sheds light on the long term effects of giardiasis in a previously unexposed population.

Before 2004 large waterborne outbreaks of giardiasis had never before been registered in Norway. Neither the Water and Sewerage Works of Bergen nor the health services anticipated that *Giardia* could strike here. As a result of the delayed detection many patients had symptoms for several weeks before treatment. In the group of patients from general practice the mean interval from onset of disease was 5.7 weeks (Paper I). Once diagnosis was made, however, there was good reason to believe that the situation would be resolved, as metronidazole treatment was readily available. The literature gave no indication that the infection would cause prolonged problems, with the exception of possible treatment failure in a small proportion of cases. Recurring symptoms were therefore perceived as persisting infection, and new courses of metronidazole were prescribed. A few metronidazole resistant cases would be referred to the hospital's outpatient clinic, and all known patients would eventually have the parasite eradicated(47).

When symptoms continued this was something the patients experienced in contrast to the physicians' expectations. We found that already in the first period after the acute infection the proportion of patients with recurring or persisting symptoms at the GPs offices was higher than expected (paper I). Six months later 37% of the patients still reported gastrointestinal symptoms that they did not have prior to the infection, after 12 months this proportion was 19% (Paper II).

At the hospital's outpatient clinic they found that among 124 patients admitted between January 2005 and March 2006 with persisting symptoms both microscopy and antigen test for *Giardia* were negative in 84(42). Many of these patients also complained of fatigue and some were referred for further investigation (Kurt Hanevik, personal communication).

The first investigation on a larger cohort was done two years after the outbreak. All patients registered with a positive faecal test for *Giardia* received a questionnaire by mail, and 1017 of 1262 responded (81%). The questions were not standardised to set specific diagnoses, but 38% (389/1017) reported abdominal symptoms elicited by the infection, and 41% (419/1017) reported having problems with tiredness.

Three years after the outbreak the same cohort and a matched control group received a new questionnaire designed to diagnose IBS and chronic fatigue. The prevalence of IBS was 46% in the *Giardia*-group, compared to 14% in the control group, and the prevalence of chronic fatigue was 46% and 12%, respectively. The corresponding relative risks were 3.4 and 4.0 (Paper III). In the Giardia-group there was also a higher prevalence of the secondary outcomes "severe" and "frequent" IBS, and also "severe" and "consistent" chronic fatigue, with RRs in the range 3.6 – 7.4. This suggests that the results represent clinically significant symptoms and possible loss of function.

The studies in this thesis show that patients consistently had persisting symptoms after giardiasis from the time the acute infection hit and up to three years after. We have shown that 36% (32/89) of patients treated for giardiasis in general practice

experienced recurring symptoms (Paper I). In some this was caused by treatment failure and persisting infection, but even after eradication of the parasite a substantial proportion experienced prolonged abdominal symptoms; 37% (44/118) after 6 months and 19% (19/99) after 12 months (Paper II). A study that is not part of this thesis found that in a cohort of patients with verified giardiasis 36% reported abdominal symptoms and 41% tiredness two years after the outbreak(170). We have demonstrated a high relative risk of clinically significant IBS and chronic fatigue after three years in the same cohort (Paper III). The prevalence of the outcomes in the papers differ, and is highest after three years. These numbers cannot be directly related to each other since the populations and settings are different, the samples sizes vary and the questionnaires were not the same. Differences in response rates could also play a role. All these factors and the possible biases discussed above illustrates that the accuracy of the numbers to some extent will be uncertain. But the observed associations between acute giardiasis and prolonged postinfectious conditions are so strong that the conclusions are considered to be valid.

## 5.4 Interpretation

The theory that the symptoms were explained by chronic infection not identified by analysis of stool samples was not confirmed. At the university hospital an extended antibiotic regime was given to 25 patients with persisting symptoms and negative stool analysis. They received treatment with either albendazole/metronidazole or tetracycline/folate. Initially four patients reported substantial improvement, but symptoms later recurred in all four. One year later 24 still reported abdominal symptoms(189).

The outbreak in Bergen was caused by *Giardia* assemblage B(190). There are indications that different strains differ in their ability to produce disease in man(29, 191), but there are no conclusive data on the importance of different assemblages.

The mechanisms for prolonged symptoms and loss of function are unclear. In medically unexplained physical symptoms possible important factors have been divided into three groups; premorbid disposition or vulnerability, factors that trigger the condition (for instance an infection) and factors that sustain it.

We have little data on premorbid vulnerability. In Paper I we found that among the patients returning to their GP with recurring symptoms a significantly higher proportion had seen a physician for abdominal complaints the previous two years. This was an objective measure since the data were extracted from the medical records. However, the number of patients was low. The larger cohort was asked two years after the outbreak about abdominal problems prior to their *Giardia*-infection. There was no association between the response to that question and current abdominal symptoms(192).

We were reluctant to retrospectively introduce questions on psychological factors and adverse life events in mailed questionnaires. We feared that participants would find these issues of limited relevance, and possibly lead to lower response rate. The results would also be vulnerable to recall bias as discussed above. However, in paper II we did include the neuroticism-part of the short-scale Eysenck Personality Questionnaire (EPQ-N) at 12 months. This consists of 12 questions to be answered by “yes” or “no”, giving a total score between 0 and 12(193). Of the 118 persons invited to participate, 99 returned the questionnaire and 95 completed the EPQ-N (80.5% response rate). The interpretation of the results will be restricted by the cross-sectional design and the low number of patients, but we did not find any correlation between EPQ-N score and persistent symptoms.

Acute giardiasis is the triggering factor in this group of patients, but it is intriguing that this non-invasive infection of the small intestine causes persisting systemic symptoms and impaired function of the large bowel.

Duodenal inflammation persisted for a period after successful treatment in some patients referred to the hospital(42), and in another group some degree of visceral hypersensitivity was demonstrated(194).

Lactose intolerance has been suggested as a cause of lasting symptoms, but this has not been backed by research. Reduced lactase activity during infection has been shown in experimental studies of giardiasis in gerbils(195), and also in *Giardia*-patients(196). However, lactose intolerance does not seem to play a role in the development of postinfectious IBS after bacterial gastroenteritis(197), nor does subjective milk intolerance predict lactose intolerance in IBS patients(198). In Paper III we found no correlations to support that lactose intolerance is the mechanism for post-giardiasis IBS. Also, lactose intolerance could hardly explain the fatigue. And finally, if this was the explanation, patients would be likely to experience exacerbation of symptoms related to intake and avoid milk. The symptoms should therefore subside.

The mechanisms of parasite pathogenicity and the host response in giardiasis are largely unknown. During the acute infection there are various degrees of inflammation with villous abnormalities, epithelial dysfunction and disruption of tight junctions, but in many patients with symptomatic giardiasis there is no detectable microscopic changes. Both mast cells and T-cells play a role in the immune response, as do B-cells which produce IgA and IgG anti-*Giardia* antibodies. However, most of what is known about disease mechanism is based on studies in animals, and has not been confirmed in humans(199). The results of infection are hypersecretion, malabsorption and reduced disaccharidase activity causing diarrhoea and other gastrointestinal symptoms(200). Fatigue has also been identified as a common feature of acute giardiasis(33). This means that a large proportion of patients suffered from long term symptoms that resemble those of the acute infection. It is not clear why symptoms persist. A broad approach to the pathogenesis is needed, and focus should be on both pathogen and host factors. The immune response may be important, in particular T-lymphocytes. Their role has been linked to acute giardiasis(199). In

postinfectious IBS T-lymphocyte infiltration has been reported(138) and the conclusion of a systematic review was a trend towards increased T-cell activity in patients with CFS(201). Another approach to the understanding of both IBS and chronic fatigue has been to focus on the hypothalamus-pituitary-adrenal axis (HPA axis)(202, 203). There is no obvious one-dimensional connection between giardiasis, or infections in general, and the HPA axis, but this scope opens for a more multi-dimensional understanding of the pathogenesis of unexplained somatic disorders, including immunological, endocrinological, psychological and even cultural influences.

We have not investigated patient factors that might sustain symptoms, like anxiety, depression, hypochondriasis, adverse life events and patients coping ability and strategies.

The municipality of Bergen claimed full responsibility for the outbreak and decided to give compensation to those affected. The conclusion was reached in the beginning of April 2005, after a period when several patients had voiced their discontent in the media and in meetings with politicians. This could possibly lead to aggravation of symptoms, both because the dissatisfaction with the authorities could counteract health promoting mechanisms and because symptoms could have consequences for the claims. On the other hand, having somewhere to place responsibility and blame could move focus from what had happened during the outbreak towards future improvement and coping. Also the benefit of exaggerating the symptoms would be limited as compensation was restricted to any verified economical loss. Although these considerations will apply in all outbreaks of disease caused by contamination of public water we have not seen this addressed in other studies published after waterborne outbreaks.

Finally, in the effort to contribute with novel findings one should bear in mind the possible pitfalls of assumed causality. In order to avoid this several criteria for causality have been proposed. The list presented by Bradford Hill in 1965 is still

accepted as a valid framework(204). There are nine aspects that should be considered before inferring that an association is explained by causation:

1. Strength. The stronger the association the more likely causation will be, but he warns against dismissing causality barely on the grounds of a wearer association.
2. Consistency. An association observed several times by different observers, in different settings and populations, is more likely to be causal.
3. Specificity. If one factor is associated to a specific disease or other restrictions concerning outcome this will be an argument for causality. However, there are also good examples of non-specificity, for instance smoking increasing the risk of several diseases, and several risk factors increasing the risk of coronary heart disease.
4. Temporality. The cause must precede the effect.
5. Biological gradient. When possible one should try to find evidence of a dose-response curve, and if found this suggests causality.
6. Plausibility. Although dependent on the state of current knowledge a proposed causation is more likely if biologically plausible.
7. Coherence. A theory of causal relationship should be coherent with other knowledge of the disease and the potential causal factors.
8. Experiment. Introducing a causal factor would not be ethically possible, but removing the factor could yield support for causality.
9. Analogy. If similar situations or factors have caused serious effects it could be argued that analogy should be deemed sufficient to infer causality, as a precautionary principle.

The criteria relating to specificity or biological gradient are not met. The association between acute giardiasis and postinfectious complications is consistent in several studies following the outbreak in Bergen, but should be confirmed in other settings and populations. Based on studies on postinfectious complications among patients suffering from other infections and possible mechanisms, one could argue that

causality to some extent is supported by analogy, and maybe also plausibility and coherence. The studies in this thesis were based on a “natural experiment” as the outbreak happened by accident, and though this design is weaker than a proper experiment it should be given consideration in the interpretation of the results. At least it suggests that the infection preceded IBS and chronic fatigue. All these indications and the strength of the association constitute substantial support for causality between acute giardiasis and postinfectious IBS and chronic fatigue.

# 6. Conclusion

Infection with *Giardia lamblia* is associated with an increased risk of persisting abdominal complaints like IBS and chronic fatigue. This has been shown in studies performed in the acute phase of the infection and on patients six months, one year, two years and three years after the outbreak.

The association between acute giardiasis and postinfectious IBS and chronic fatigue is strong, and although we have not investigated all possible contributing factors, we conclude that it is valid for a previously unexposed population. This shows that the potential consequences of giardiasis are more serious than previously known.

One should be cautious about applying the results from our studies on patients in areas where *Giardia* is endemic.

There might be important contributing factors to the development and maintenance of postinfectious IBS and chronic fatigue, but the studies in this thesis were not designed to investigate this.

# 7. Further research

There are still many unanswered questions about *Giardia*, giardiasis and postinfectious complications. The following topics should be addressed in future research:

We have found a strong association between acute giardiasis and postinfectious IBS and fatigue, but the design is observational and the results should be confirmed. In case of future outbreaks of giardiasis or other infectious diseases, experienced research groups like ours in Bergen, and the one at McMaster University in charge of the Walkerton investigations, should be considered important resources.

In order to improve the understanding of IBS, chronic fatigue and possibly other medically unexplained physical symptoms, further research into the mechanisms of giardiasis and other infections, as well as the mechanisms of IBS and fatigue should be performed.

The number of chronically affected individuals in Bergen emphasises the need for further research on treatment for chronic fatigue and IBS.

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