Giardia-utbruddet i Bergen – Hva vi vet om parasittene og hvor de stammer fra?

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Sammendrag

I dette innlegget gis først en kort oversikt over noe av vår kunnskap om parasitten *Giardia*, inkludert taksonomi, livssyklus og vertspekter. Spesielt er deling av *G.duodenalis* i ulike Assemblages eller genotyper, hvorav noen åpenbart er vertsspesifikke, beskrevet. Forekomsten av denne parasitten i forskjellige vertsarter her i Norge, og en del tilleggsinformasjon vi har om *Giardia* i Norge, fra tidligere og igangværende forskning, er beskrevet.

Disse opplysingene er benyttet som bakgrunn for å vurdere mulige kilder for kontaminering av vannkilder ved vannbårne utbrudd, særlig utbruddet av vannbåren giardiasis i Bergen høsten/vinteren 2004/2005. Selv om kontaminering fra dyr har vært nevnt som en mulig kilde, har smittekilden som oftest vist seg å være kloakk, i de tilfeller der kilden er identifisert. I Bergen ser det også ut til at kloakk var smittekilden. I innlegget vises det også at bruk av genotyping kan benyttes til å undersøke mulige kilder for kontaminering.

Avslutningsvis er noen av erfaringene fra utbruddet i Bergen brukt til å stille spørsmål og komme med forslag til hva som kan gjøres for å redusere mulighetene for at det samme skal skje igjen i Norge. Spesielt har vi reist forslag og problemstillinger knyttet til rutineundersøkelser og risikoanalyser og det understrekes at selv om mye av oppmerksomheten nå rettes mot *Giardia*, må vi ikke glemme parasitten *Cryptosporidium*.

Abstract

In this presentation some of our knowledge of the parasite *Giardia*, its taxonomy, hosts, and lifecycle is summarised. In particular the division of the *G.duodenalis* species into a number of Assemblages or genotypes, some of which are apparently host-specific, is outlined. The occurrence of this parasite in different host species within Norway and some

additional information we have about *Giardia* in Norway, both from ongoing and previous research, is also briefly described. This information is used as background against which to consider the possible sources of initial contamination of water sources during waterborne outbreaks, and in particular the outbreak of waterborne giardiasis which occurred in Bergen during autumn/winter 2004/2005. Additionally the use of genotyping for exploring a potential contamination source is noted. Finally, some of the experiences from this outbreak are used to raise questions and suggestions about how we may minimise the possibility of a similar situation occurring in Norway again. In particular, suggestions and questions are raised concerning monitoring and risk assessment and it is emphasised that in the furore over *Giardia*, the parasite *Cryptosporidium* should not be overlooked.

Background

In autumn/winter 2004/2005 an outbreak of waterborne giardiasis occurred in Bergen, Norway. Over the course of the outbreak over 1300 patients were laboratory-diagnosed with giardiasis, either by detection of cysts in faecal samples or by antigen Elisa test. The initial source of the outbreak was a municipal drinking water supply.

The parasitology laboratory at Norges veterinærhøgskole (NVH) was involved in the outbreak from the start. The first water samples (raw and treated water) in which *Giardia* cysts were detected were analysed at NVH. Environmental samples, including soil, sewage and dog faeces, were also analysed at NVH. Additionally, almost all the patient samples associated with this outbreak and previously diagnosed at Haukeland Sykehus in Bergen were sent to NVH for further analysis.

Giardia – the parasite and its hosts

The taxonomy and nomenclature of the *Giardia* genus has been controversial for many years. However, at present it is generally agreed that there are six distinct species (Table 1), of which *Giardia duodenalis* (also frequently referred to as *Giardia intestinalis* or *Giardia lamblia*) is the only one of public health significance, as it can be infective to humans, as well as to a range of other mammals. Mammals in which *G. duodenalis* has been detected include: humans, non-human primates, cattle, pigs, horses, cats, dogs, beavers, guinea pigs, chinchillas, rats, sheep, pigs, alpaca, goats, rats, muskrats, voles, deer (various species), and ferrets. Norwegian mammals in which *G.duodenalis* has been detected include: humans, cows, sheep, deer (various species), pigs, cats, dogs, ferrets, goats, and reindeer.

Table 1: Currently accepted *Giardia* species	
***Giardia* species**	**Host species**
G. agilis	Amphibians
G. muris	Rodents
G. ardeae	Birds
G. psittaci	Birds
G. microti	Muskrats, voles
G. duodenalis	Wide range of mammals

The lifecycle of *G.duodenalis* is relatively simple. A viable cyst ingested by a suitable host species excysts in the intestine to the trophozoite stage. This stage replicates asexually within the host, leading to considerable infestation. Encystation of the trophozoites occurs further down the intestine in response to various host triggers, and the robust cyst is excreted in the faeces. The cyst is immediately infectious, requiring no period of maturation in the environment. Nevertheless it can survive in some environments for considerable periods, and thus may contaminate food or water, leading to the potential for community-wide outbreaks.

In recent years it has become increasingly apparent that the *G.duodenalis* species is actually a species-complex, which comprises of various sub-groups. Whilst these sub-groups (referred to as Assemblages or genotypes) are morphologically identical, they appear to have particular host-adaptations, such that some genotypes are only associated with particular groups of host species. The groups can be distinguished between by genetic analysis. Currently 7 Assemblages or genotypes are recognised, and are generally referred to as Assemblages A-G, although often frequently referred to by the host group (e.g. Assemblage C is also known as the dog genotype, and Assemblage E as the hoofed livestock genotype etc.).

Of these genotypes, only two of them, Assemblage A and Assemblage B (also often referred to as Polish and Belgian genotype, after earlier studies), appear to be infectious to humans. They are not, however, solely infectious to humans, and can also infect a range of mammals including livestock and companion animals. Thus despite the occurrence of Assemblages which are specific for animals within the *G.duodenalis* species-complex, giardiasis is also a zoonotic infection with potential for animal to human, and human to animal, transmission. Current data indicate that Assemblage A seems to have a wider host range than Assemblage B. Also, some evidence suggests that Assemblage B is more associated with acute symptoms in human infection than Assemblage A.

Further genetic analysis has divided the Assemblages further into smaller sub-groups (e.g. A1, A2, A3, B1, B2, B3 etc.). The validity of all these sub-genotypes has yet to be ascertained.

Genotyping of *G.duodenalis* cysts in Norwegian animals and Norwegian sewage

Some genotyping of animal isolates of *G.duodenalis* has been conducted at NVH. To date, *G.duodenalis* cysts isolated from dog, calf, cat and sheep faeces have been genotyped. So far, all have been found to belong to the 'animal genotypes' (i.e. Assemblages C-G). This would indicate that these isolates are not infectious to humans.

Until the Bergen outbreak, the only genotyping of human isolates of *G.duodenalis* in Norway had been done on cysts isolated from sewage. As part of an epidemiological study, sewage influent from 38 sewage treatment works (STW) from every area of Norway has been analysed for *Giardia* cysts. Of the STW included in the survey, 90% of them have been positive for *Giardia* cysts, with a mean cyst concentration of over 2500 cysts/litre sewage influent. Of the *G.duodenalis* cysts isolated from sewage, most were from Assemblage A, although 2 isolates from different STW were from Assemblage B. A broad heterogeneity has been noted, with isolates from A1, A2, A3 and 4 previously unreported A-assemblages. None of the *G.duodenalis* cysts isolated from sewage in Norway have been identified as belonging to the animal genotypes (Assemblages C-G).

Knowledge of the concentrations of cysts in sewage influent and the volume of sewage processed daily by a STW allows the calculation of the number of *G.duodenalis* cysts entering a STW within a defined time period. Using this information, in combination with an estimate of the numbers of cysts excreted by an infected individual, enables the calculation of the approximate number of persons infected with *G.duodenalis*, and excreting cysts, in a community served by that STW. Using this calculation (see Table 2) it can be shown that in a Norwegian community served by a medium-sized STW, approximately 10 individuals will be infected with *G.duodenalis*, mostly of Assemblage A. Data from records of diagnosis of human *Giardia* infection in Norway indicates that the majority of these infections will be undiagnosed.

Table 2: Calculation of approximate number of infected individuals in a community using STW data

G. duodenalis cysts per litre	A	2500 cysts/litre
Volume of sewage entering medium-sized STW daily	B	43.2×10^6 litres/day
Estimated number of cysts excreted daily in a severe giardiasis infection	C	10^{10} cysts
G.duodenalis cysts entering medium-sized STW daily	A x B	10.8×10^{10} cysts/day
Approximate number of infected persons in community served by STW	$\frac{A \times B}{C}$	10.8

Potential contamination sources during waterborne outbreaks of giardiasis

As the *G.duodenalis* isolates which are infective to humans have the potential to be zoonotic, regardless of whether they are in Assemblage A or B, when considering potential sources of contamination during a waterborne outbreak, both human and animal sources must be included. A selection, but not exhaustive, of possible sources are listed in Table 3.

It may also be useful to consider previous outbreaks of waterborne giardiasis, and the sources, or potential sources, of the pollution of the water supply in those events. For the majority of previous outbreaks the initial origins of the *Giardia* cysts in the water supply is unknown. However, when the origin is documented, it is mostly from sewage, either raw sewage or sewage effluent, with contamination of the water supply either following water treatment, due to mechanical or human error, or in the water source prior to water treatment, where the water treatment in place is insufficient to remove or inactivate the cysts. In a couple of waterborne outbreaks of giardiasis in US, infected beavers have been implicated as the origin of the contamination. However, there has never been conclusive proof that they are the original source, and have not been infected themselves due to the same, or a separate, contamination event. Nevertheless, obviously aquatic mammals such as beavers, if infected, have the potential to amplify the numbers of cysts present in that water source.

Table 3: Some potential sources of *G.duodenalist* cyst contamination of water supply causing waterborne outbreaks of giardiasis

Potential animal sources	Potential human sources
Grazing/pasturing livestock	Sewage effluent (e.g. discharge from municipal works, discharge from private treatment, septic tanks, leaking pipelines etc.)
Agricultural practices (e.g. slurry spraying, manuring etc.)	Raw (untreated) sewage (e.g. leaking pipelines, discharge from septic tanks etc.)
Effluent from abattoirs	Sewage sludge (e.g. disposed of in landfill, or being used for agriculture, building projects etc.)
Wild animals (e.g. beavers)	Deliberate contamination (bio-terrorism)
Other animal-related activities (e.g. zoos, fur-farms, dog-walking, kennels, stabling etc.)	

Assessment of the most likely sources of contamination of a water source must use the widest number of tools available. The most useful tools are probably the most obvious; namely catchment knowledge and practical observation. If the relevant authorities are aware of the most likely sources of faecal pollution within a catchment then they can be monitored and/or controlled before an outbreak event occurs. However, unusual circumstances may arise, perhaps due to weather conditions (heavy precipitation or snow melt) that may convert a usually 'safe' activity such as slurry spraying or pasturing of animals into one that may result in a contamination event. Sewage pipelines, which are normally intact, may rupture under particular circumstances such as heavy building work, land-shift or earth tremors, or may corrode over time. In an ideal situation such possibilities would be noted and rectified as they occur, but in 'real life' this is often not the case. Consideration, recognition and testing for such possibilities after the contamination event may be the next best approach.

Although suspected sources of contamination can be analysed for *Giardia* cysts, two points must be taken into consideration: a) during an outbreak, there are likely to be more *Giardia* cysts in the larger environment directly as a result of the outbreak; thus it may not be possible to distinguish between cysts resulting from the outbreak and cysts which caused the outbreak to occur (this is particularly the case for sewage), and b) if a considerable time elapses between the contamination event and the identification of a possible source, all the cysts may be eliminated or damaged beyond recognition before the analysis is performed.

Comparison of the genotype of cysts from a suspected pollution source and comparison with the genotype of *G.duodenalis* causing the outbreak is unlikely to 'prove' a source, but may be a valuable tool for providing evidence either for or against an implicated source. This was, indeed, the case in the Bergen outbreak.

The Bergen outbreak of giardiasis – possible sources of contamination

Retrospective analysis of turbidity and faecal coliform data from the contaminated water supply suggested that the pollution event, which caused the waterborne giardiasis outbreak in Bergen, occurred towards the end of August, when precipitation in Bergen (never considered the most arid of places) was particularly and notably heavy. The relatively long period between the presumed contamination event and the first documentation of a rise in cases of giardiasis (a period of some 7-8 weeks) can probably be explained by the fact that doctors in Norway do not normally ask for patient samples to be tested for giardiasis unless there is a history of travel, in combination with symptoms. The process of collecting patient samples, having them analysed for bacterial and other infections, further samples being

taken and finally being analysed for parasitic infection could take a period of two to three weeks. Additionally, it is unclear how much time would elapse before the polluted raw water would reach the consumers in Bergen; this is dependent upon the contamination site of the raw water source. The incubation time for giardiasis (the time between cyst ingestion and appearance of symptoms) is generally considered to be between approximately 1-2 weeks.

Although pasturing of animals had occurred on the hillsides around the water supply up until the end of August, local knowledge suggested that this was unlikely to be the source of contamination. No other agricultural activities, such as slurry spraying or manuring, was reported to have been undertaken in the catchment area. The catchment area is very popular with dog walkers, and dog faecal samples were collected and analysed at NVH. *Giardia* cysts were not detected in any of them. Various sewage installations were also suggested as possible sources of the pollution. These were both from residential areas and also from a tourist installation that had been closed from the end of August.

Many of the suggested sewage sources were difficult to test for being the origin of the contamination, due to the occurrence of post-outbreak *Giardia* cysts occurring in them. However, one source which was given particular media attention at the time of the outbreak, was considered to have been unlikely to have received faecal material post-outbreak. Analysis of this potential contamination source by NVH revealed large numbers of *Giardia* cysts occurring in it. This information spurred the media to increased speculation. However subsequent genotyping at NVH of the cysts in this source, revealed that they did not belong to the same Assemblage as the *G.duodenalis* Assemblage associated with the outbreak. This was authoritative evidence that this particular sewage was probably not the source of the pollution event.

Although it is highly unlikely that the contamination source associated with this outbreak will ever be definitively identified, examination of the available evidence suggests that sewage leakage from a residential area into the water source is the most probable explanation. If this is the case, then a resident or visitor using that sewage system in August must have been already infected, probably quite heavily, with *G.duodenalis*. It is unlikely that retrospective tracking will be able to determine whether this is the case.

What have we learned from this outbreak and how should we proceed to minimise the chances of a similar situation occurring in Norway again?

Probably the most important general lesson from this outbreak is that such outbreaks of waterborne parasitic infection CAN happen in Norway. Norwegians are particularly proud of their water supply, and often rightly so. However, this outbreak has punctured this perhaps somewhat

naïve pride, and hopefully heightened the awareness of both the relevant authorities and the wider population that contamination of water sources can occur. If this occurs, and the water treatments in place are insufficient to remove or inactivate the pathogens, then community-wide outbreaks can occur and large numbers of the population might be affected.

Although the water supply associated with this outbreak was routinely monitored for parasitic pathogens, this monitoring was insufficient to detect the contamination. Perhaps this demonstrates that monitoring should not be conducted on an *ad hoc* basis or following a calendar routine, but by following trigger events, both within the water supply (rises in turbidity or faecal coliform counts above a predetermined level) or when particular events, which may impact on the water supply, occur. (e.g. heavy precipitation).

Within Norway there are very few laboratories that have the necessary competence or experience for water analysis for parasites. There are currently two that undertake such analysis; namely the parasitology laboratory at NVH in Oslo and M-Lab in Stavanger. The former of these 2 currently has very limited capacity for this type of analysis (one part-time, short-term contract research scientist). Neither of these laboratories has participated in a recognised accreditation scheme. During the outbreak in Bergen both these laboratories were utilised, but the majority of the analysis of water for *Giardia* cysts was performed by SMI in Stockholm, Sweden. It seems obvious that if another outbreak had occurred simultaneously, either in Norway or Sweden, analytical capacity would have been much stretched. Clearly there is a need for building up competence and experience in this analysis within Norway.

The method of water analysis for parasites should also be considered. Although the techniques have improved hugely within the last 10-15 years, particularly with the development of immunomagnetic separation, they are still of limited efficiency. The setting of an NMKL standard method is presently in progress, but this should not limit the incorporation of new, improved techniques and methodologies as they become available.

The use of genotyping was demonstrated to be particularly important during this outbreak, by providing strong evidence for eliminating a suspected contamination source. The utility of genotyping has perhaps been previously underestimated by the Norwegian water industry; in this instance, the power and value of this technique has been clearly demonstrated.

Other countries, notably USA and UK, have experienced waterborne outbreaks of parasitic infection that have resulted in the implementation of particular legislation designed to minimise the recurrence of such outbreaks. In USA, the Information Collection Rule (a period of monitoring lasting about 2 years), was followed by the implementation of the Surface Water Treatment Rule. In UK,

following an outbreak of waterborne cryptosporidiosis, a standard operating protocol specific for the monitoring of treated drinking water for *Cryptosporidium* oocysts, was implemented.

The relevant Norwegian authorities would be wise to consider the decisions of these countries, and compare and contrast the situations there, with those here in Norway. Particularities of geography, population structure and size for each water supply, ownership of water supply (private or municipal) and other factors may make decisions that are sensible elsewhere, less suitable for the Norwegian situation. It is obviously sensible to try to learn from these experiences, and avoid repeating any mistakes that were made.

One approach that would appear to be of value would be for risk assessments to be conducted for water sources and supplies throughout Norway. This would ensure that raw water supplies at particular risk of contamination, or communities particularly at risk if contamination does occur (due to minimal treatment barriers being in place), could be identified, and where necessary appropriate action, improvements or monitoring schedules implemented. It is understood that preliminary risk assessments of Bergen's water supplies are currently in progress.

Cryptosporidiosis?

Following the outbreak of giardiasis in Bergen and the accompanying widespread media attention, many people who previously had no knowledge that such a parasite even existed, have become aware of it. The relevant authorities have become particularly alerted to the potential for future outbreaks of giardiasis, and there has been a marked increase in water suppliers wishing for analysis of their water for this parasite. However, there has been minimal corresponding increase in awareness or interest in the parasite *Cryptosporidium.*

The parasites *Cryptosporidium parvum* and *Cryptosporidium hominis*, whilst unrelated to *G.duodenalis*, have a similar epidemiology. Both cause diarrhoeal infection, and *C.parvum* is zoonotic, occurring in livestock and domestic animals as well as wild animal populations. However, unlike with giardiasis, there is currently no effective chemotherapy for cryptosporidiosis; particularly in the immunocompromised population, the infection can fail to resolve and can be fatal. Furthermore, *Cryptosporidium* oocysts are both smaller and more robust than *Giardia* cysts, and thus less likely to be removed by filtration or inactivated by traditional disinfection regimes. Thus, where an outbreak of waterborne giardiasis has the potential to occur, there is also the potential for an outbreak of waterborne cryptosporidiosis to occur, provided an initial contamination event occurs. In the most widely quoted waterborne outbreak of cryptosporidiosis, which occurred in Milwaukee, USA in 1993, over 400,000 individuals were estimated to

have been infected, and approximately 50 deaths were associated with the outbreak.

In the light of this information, perhaps the authorities and population of Bergen might be grateful that their 'wake-up call' was a waterborne outbreak of giardiasis, and not cryptosporidiosis. And perhaps it should be remembered that by focussing upon giardiasis, and neglecting cryptosporidiosis, if another 'wake-up call' occurs, it may be equally, or even more, unpleasant than that which occurred in Bergen.