Occurrence of *Cryptosporidium* oocysts and *Giardia* cysts in Norwegian groundwater wells in bedrock

Sylvi Gaut, Lucy Robertson, Bjørn Gjerde, Atle Dagestad and Bjørge Brattli

ABSTRACT

The occurrence of *Cryptosporidium* oocysts and *Giardia* cysts in Norwegian groundwater wells in bedrock has been investigated for the first time. Wells close to risk areas such as farming and septic tanks were chosen. In all, 20 water samples from 20 different waterworks were collected. The samples were analysed for *Cryptosporidium* and *Giardia*, using US EPA Method 1623. Turbidity was also measured. Water samples from 10 of the waterworks were also analysed for *Clostridium perfringens* by membrane filtration. *Cryptosporidium* was detected in the groundwater samples from 3 of the waterworks. *Giardia* and *Clostridium perfringens* were not detected. Too few samples were analysed to verify whether *Giardia* is indeed absent from bedrock wells, and further studies are recommended to give more reliable data.

Key words | *Clostridium perfringens*, *Cryptosporidium*, drinking water, *Giardia*, groundwater in bedrock

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INTRODUCTION

Reports from USA and UK show an increasing problem with outbreaks of waterborne diseases caused particularly by *Cryptosporidium parvum* and *Giardia duodenalis* (= *G. intestinalis* = *G. lamblia*) in groundwater (Bridgman *et al.* 1995; Ball 1997; Craun *et al.* 1998; Hancock *et al.* 1998). Some outbreaks have been associated with heavy rainfall (Bridgman *et al.* 1995; Willocks *et al.* 1998; Rose *et al.* 2000; Curriero *et al.* 2001), and studies have revealed that *Cryptosporidium* oocysts can travel far and fast in the subsoil especially when preferential flow paths exist (Darnault *et al.* 2003).

About 85% of the Norwegian population use surface water as their drinking water source, whereas groundwater, often from wells in crystalline bedrock, is mainly supplied by small and medium sized waterworks (<1000 people), or used by private households or holiday cottages. Little is known about the occurrence of *Cryptosporidium* and *Giardia* in Norwegian drinking water, and groundwater is regarded as being well protected against these parasites. Robertson & Gjerde (2001) analysed 408 water samples of raw-water from 147 drinking water sources in Norway, and oocysts/cysts of one or both of these protozoans were detected in water samples from 47 of the localities. Only two of the 408 samples were taken from a groundwater well, however, *Cryptosporidium* was detected in one of these samples.

In Norway, the intestinal diseases giardiasis and cryptosporidiosis are seldom diagnosed in humans, and infections have usually been assumed to be associated with foreign travel. However, studies by Nygård *et al.* (2003) and Robertson *et al.* (2006*a*; 2006*b*) indicate that both cryptosporidiosis and giardiasis acquired in Norway are underdiagnosed. None of the waterborne outbreaks caused by these parasites have been related to groundwater, but a large outbreak of giardiasis due to contamination of a main surface drinking water source occurred in Bergen in the autumn/winter of 2004/2005 (Søbstad 2004; Eikebrokk *et al.* 2006; Robertson *et al.* 2006*c*).

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During the 1990s, investigations through the national Program for Improved Water Supply (PROVA) revealed clear evidence of water quality problems in bedrock wells. Therefore a study was initiated in 1998 by the Geological Survey of Norway (NGU) to investigate the vulnerability of groundwater wells in bedrock to microbiological contamination (Gaut 2005). Part of this study was dedicated to investigate the possible presence of *Cryptosporidium* and *Giardia* in water from bedrock wells. The results of these studies are presented in this paper.

METHODS

Selection of sampling sites

Wells close to specified risk areas (e.g. arable land, pasture and septic tanks) were chosen for analysis, due to the fact that both animal and human fecal matter can be sources of these parasites (Craun *et al.* 1998; Olson *et al.* 2004). In all, 15 sites were found among 49 water works (135 wells) inspected while examining the vulnerability of the wells to microbiological contamination (Gaut 2005). The remaining 5 waterworks were selected i) in dialog with local offices of the Norwegian Food Safety Authority, and ii) from waterworks registered in the National Waterworks Register which had detected coliforms. In each of the 5 latter cases the waterworks' owners were contacted to verify a suitable location. For three of the waterworks the water sample represented more than one well, thus the total number of wells examined by the 20 water samples was 26.

Water sampling

In all, 20 samples of raw-water were collected from 20 waterworks (Figure 1(a)) and analysed at the Norwegian School of Veterinary Science. Water samples were collected from 28 April to 27 May 2004. At 10 of the waterworks, an additional water sample was collected for analyses of *Clostridium perfringens* (*C. perfringens*) at the nearest laboratory (Figure 1(b)).

Personnel from NGU or from the different waterworks collected the water samples for *Cryptosporidium* and *Giardia* analyses. Each sample consisted of about 10 L of untreated groundwater collected in an ordinary plastic carboy from the sampling tap at the well or inside a house supplied by the well. One of the samples was taken from the water reservoir by submerging the uncapped carboy into the water. The samples were transported to the laboratory at the Norwegian School of Veterinary Science as soon as possible after sampling, either by post, courier service or direct delivery to the laboratory by personnel from NGU. Due to the wide geographic distribution of the waterworks, it was not possible to standardise the collection and delivery of water samples.

Water samples analysed for *C. perfringens* were collected by personnel from the different waterworks or the Norwegian Food Control Authority (NFCA). The water was sampled in sterile plastic bottles specially designed for water to be analysed for microbiological parameters. Sampling volume was normally 0.5 L and the sampling occurred simultaneously with the collection of the water samples analysed for *Cryptosporidium* and *Giardia*. The bottles were kept cool and brought to the laboratory normally used by each waterwork for analysis within 24 hours.

Laboratory analyses

Before analysing for *Cryptosporidium* and *Giardia* the carboy was shaken well and a small sub-sample taken for turbidity measurements (Hach turbidimeter 2100A).

Cryptosporidium and *Giardia* were analysed by US EPA Method 1623. The method makes it possible to isolate both *Cryptosporidium* oocysts and *Giardia* cysts simultaneously from water samples. In brief, the analytical technique can be divided into 5 stages as follows: a) Filtration of the water sample, b) elution of the material from the filter, c) concentration of the eluted material by centrifugation, d) isolation of the parasites from the concentrated eluted material by immunomagnetic separation (IMS), and e) detection and identification of the parasites by immunofluorescence assay (IFA), using light microscopy with Normaski (DIC; differential interference contrast) optics for confirmation of identity. More detailed descriptions of the five stages used can be found in Robertson & Gjerde (2001). Lowest detection limit is 1 oocyst/cyst per test volume.

C. perfringens was analysed by the membrane filtration methods described in the European Council Directive 98/83/EC or NS-ISO 6461-2 with verification. Both methods

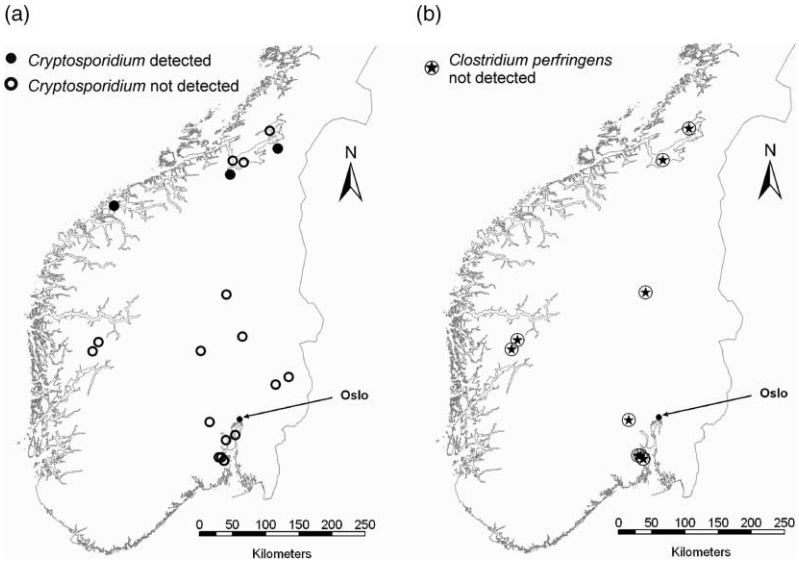

Figure 1 | a) Geographical distribution of the 20 waterworks from which water samples were analysed for *Cryptosporidium* and *Giardia*. Filled circles represent waterworks where *Cryptosporidium* oocysts were detected. *Giardia* cysts were not detected. b) Geographical distribution of 10 of the waterworks in a) from which water samples were also examined for *Clostridium perfringens*. This bacteria was not detected at any of the waterworks. All waterworks are based on groundwater from bedrock (Gaut 2005).

use membrane filtration of a 100 ml water sample and have a lower detection limit of 1 *C. perfringens* per test volume.

Video inspections of wells

A downhole video camera (Tiny CS 3002 S, Rico EAB) was used to inspect the internal appearance of the wells at 13 of the 20 waterworks. The camera was lowered into the wells and well data like casing length, existence or lack of sealing between bedrock and casing and water inflows were registered. If possible, the well was logged down to 15–20 m depth. Data was recorded on an Archos video AV380.

RESULTS

Cryptosporidium oocysts were found in 3 of the 20 water samples (Figure 1(a) & Table 1). Only one *Cryptosporidium* oocyst was found in each of the three positive samples, which were all 10 L volumes. *Giardia* cysts (Figure 1(b) & Table 1) and *C. perfringens* (Figure 1(b) & Table 1) were not found. Unfortunately water samples analysed for *C. perfringens* were not collected from any of the waterworks where *Cryptosporidium* oocysts were found.

Turbidity measurements for all 20 water samples ranged between 0.3 and 1.4 NTU (Table 1). Median and average turbidity values were slightly higher in the samples in which *Cryptosporidium* oocysts were detected.

Water leakages between bedrock and well casing and water inflow through fractures <10 m below ground level were observed in most of the wells investigated by a downhole video camera.

DISCUSSION

The occurrence of *Cryptosporidium* and *Giardia* in groundwater from bedrock wells in Norway has been investigated for the first time. It is shown that *Cryptosporidium* can be found in groundwater derived from bedrock wells, at least when the well is close to a possible contamination source. The viability of the oocysts was not examined. Therefore it is possible that only dead shells were detected, which are not infectious, and therefore not of any public health significance. Nevertheless the oocysts have reached the groundwater, demonstrating that the groundwater is vulnerable to this kind of contamination.

Several species/genotypes of *Cryptosporidium* exist, of which some are known to infect humans (Fayer 2004; Hamnes *et al.* 2006*a*). Due to the small number of oocysts in the 3 positive samples in the present study, the genotype(s)

Table 1 | Occurrence of *Cryptosporidium* oocysts, *Giardia* cysts and *Clostridium perfringens* and turbidity measurements in water samples from waterworks based on groundwater from bedrock. In all, 20 samples were analysed for (oo)cysts, and 10 samples were analysed for *Clostridium perfringens* as shown in Figure 1. NTU = nephlometric turbidity units

		Turbidity measured (NTU)		
	Number of water samples	**Range**	**Median**	**Average**
Parasites not detected	17	0.3 – 1.4	0.4	0.5
Only *Cryptosporidium* oocysts detected	3	0.4 – 0.9	0.7	0.7
All water samples examined	20	0.3 – 1.4	0.4	0.5
Clostridium perfringens detected				
(10 samples analysed)	0	0.3 – 0.65*	0.4*	0.5*

*Turbidity was measured on water samples collected simultaneously with those analysed for *C. perfringens*.

could not be determined, and they might be of a species not infective to humans.

Giardia cysts were not found in the present study, although studies of both Norwegian dairy calves (Hamnes *et al.* 2006*a*) and wild cervids (Hamnes *et al.* 2006*b*) show a higher frequency of *Giardia* cysts than of *Cryptosporidium* oocysts in the faecal samples. The lack of *Giardia* cysts in the water samples can be due to a more rapid decrease in viability for the cysts than the oocysts as found by Robertson & Gjerde (2006). Transport of both *Cryptosporidium* and *Giardia* is influenced by straining and/or mechanical filtration in porous media (Bradford & Bettahar 2005; Bradford *et al.* 2006). A possible explanation is that the larger size of the *Giardia* cysts (10–15 μm) compared to the *Cryptosporidium* oocysts (about 5μ) causes the cysts to be more easily removed by these processes.

It is possible that sampling in August or September, rather than April/May, would have revealed a larger occurrence of the parasites. Studies by Robertson & Gjerde (2004) indicated that *Cryptosporidium* and *Giardia* will not persist in the Norwegian terrestrial environment over winter because freeze-thaw cycles probably induce shear forces which cause the parasites to disintegrate. Secondly, *Cryptosporidium* and *Giardia* occurrence is related to fecal contamination, and studies carried out by Gaut (2005) showed a higher frequency of fecal coliforms in groundwater from bedrock wells in the autumn.

For surface water Robertson & Gjerde (2001) found a significantly higher probability that samples with turbidity ≥ 2 NTU contained one or both of the parasites *Cryptosporidium* and *Giardia*. A similar correlation was also found by Atherholt *et al.* (1998) and LeChevallier *et al.* (1991), whereas Thurman *et al.* (1998) did not find any significant relationship between the presence of parasites and turbidity. In the current study the turbidity of all samples was <2 NTU (Table 1) but, as only three samples contained oocysts, there were too few positive samples for meaningful statistical comparison between turbidity and presence of *Cryptosporidium*.

The spore-forming bacteria *C. perfringens* is used as an indicator for *Cryptosporidium* and *Giardia* in Norwegian drinking water (Helse- og omsorgsdepartementet 2001), although the postulated correlation between these microorganisms is not clear (Østensvik 2002). In this study, samples from 10 of the waterworks were analysed for *C. perfringens*, but none were found to be positive. Of these 10 waterworks, only 5 carried out this analysis regularly. None of the 3 waterworks from which the water samples tested positive for *Cryptosporidium*, had ever analysed for *C. perfringens*. Therefore, it is not possible by this study to verify whether *C. perfringens* and *Cryptosporidium* occurred simultaneously. Neither is it possible to evaluate if *C. perfringens* is a good indicator for *Cryptosporidium*. However, if *C. perfringens* is continually used as an indicator, all groundwater from

bedrock wells should be analysed, and not only water from wells known to be influenced by surface water. This is because shallow water leakages/inflows which are likely to be influenced by surface water or groundwater with short residence time in the subsoil were observed in wells from which water was not analysed for *C. perfringens*.

Further work

Based on the results from this limited investigation, and the fact that waterborne diseases caused by both *Giardia* and *Cryptosporidium* in groundwater are an increasing problem (Ball 1997; Craun *et al.* 1998; Hancock *et al.* 1998), a more comprehensive study of the occurrence of both parasites in a representative selection of Norwegian groundwater wells in superficial deposits and bedrock should be carried out. The study by Robertson & Gjerde (2001) showed that the probability of detecting the parasites in surface water increased with number of samples collected at each site. Therefore further studies should include sampling at each groundwater well or waterwork throughout the year to increase the probability of detecting cysts/oocysts and to investigate seasonal changes in the occurrence of the parasites. Both single, private wells and waterworks should be sampled to study possible differences in water quality. The water samples should also be analysed for *C. perfringens* to verify whether this is a suitable indicator organism for *Giardia* and *Cryptosporidium*.

CONCLUSION

Cryptosporidium, but not *Giardia*, has been detected in the groundwater from Norwegian bedrock wells. The assumed contamination sources are manure from grazing animals or manure spreading, and overflow from a septic tank. Too few samples were analysed to verify whether *Giardia* is indeed absent from bedrock wells and further studies are recommended to provide more reliable data.

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