

# *Cryptosporidium* spp. in drinking water

## Samples from rural sites in Switzerland

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### Summary

In most rural areas and small communities in Switzerland the drinking water is supplied to the consumers after a minimum or even no treatment at all. However, it is just in these areas where drinking water from sources of agricultural activities can be contaminated by liquid manure and faeces of pasturing animals. The Swiss drinking water regulations are limited to the monitoring of *E. coli*, *Enterococcus* spp. and total plate counts only. Hence, resistant pathogens, as for example *Cryptosporidium* spp., remain unnoticed. During a drinking water survey, which lasted from June 2003 to December 2004, water samples were collected from 3 selected rural sites in Switzerland. The drinking water was investigated for *Cryptosporidium* spp., *E. coli*, *Enterococcus* spp., *Clostridium perfringens* and other parameters. In all samples oocysts of *Cryptosporidium* spp. were detected at elevated concentrations of up to 0.18 oocysts/l. Between 28% and 75% of the oocysts were found to be vital by the excystation method. Sampled oocysts collected from the three sites were subjected to genotyping and in one case the isolate was found to belong to the genotype of *C. parvum*. No evidence for increased incidents of diarrhoea in the past years was noted by local authorities.

**Key words:** drinking water; *Cryptosporidium* spp.; *E. coli*; *Enterococcus* spp.; *Clostridium perfringens*; heterotrophic plate count

### Introduction

*Cryptosporidium* is a coccidian protozoan parasite that can infect the intestine of warm blooded animals and of human beings causing severe diarrhoea. Cryptosporidia develop enduring forms, so called oocysts, which are spread in the environment by faecal contamination where they can survive for several months [1]. The disease is normally transmitted faecal-orally by smear infection but also by contaminated food and water [2]. The low infection dose of less than 30 oocysts [3] and its persistence against chlorination [4] makes this pathogen, even today, a challenge for drinking water distributors. To date 13 species in the

genus *Cryptosporidium* are known that exhibit different human pathogenicity. The most virulent species for humans are *Cryptosporidium hominis* and *Cryptosporidium parvum* [5]. Whereas *Cryptosporidium hominis* is transmitted from humans to humans, *Cryptosporidium parvum* can be also transmitted from animals to humans and defines a so called zoonosis [6]. For a long time *Cryptosporidium* spp. received little attention. This changed with the numerous outbreaks of cryptosporidiosis due to contaminated drinking water were reported in industrialised countries such as the USA, GB, Japan and others [7]. Especially the outbreak in Milwaukee with estimated between 15,000–400,000 infected persons [8, 9] led to several investigations about this newly recognised pathogen and its risk to the health of drinking water consumers. Based on epidemiological studies of contaminated wells Haas and Rose defined an action level for *Cryptosporidium* in drinking water: “Whereas a *Cryptosporidium* concentration exceeding 0.1 oocysts/l can cause sporadically cryptosporidiosis cases in a population, concentrations over 0.3 oocysts/l will almost certainly cause a cryptosporidiosis outbreak [10].”

Nowadays, the regulations concerning *Cryptosporidium* in drinking water in the different countries are far from a common, generally accepted standard. In the USA the Environmental Protection Agency (US EPA) defines a tolerable yearly risk of 1 infected person in 10,000 from drinking water, which is often expressed as a risk of  $10^{-4}$  per person and year [11]. According to the published dose-infection model [12] this complies with a *Cryptosporidium* concentration of only 0.0000327 oocysts/l [13]. Furthermore, particularly for *Cryptosporidium* a Maximum Contaminant Level Goal (MCL) is set at zero in the USA. The drinking water treatments with supposedly safe catchment zones must reach at least a 99% removal for *Cryptosporidium* [14] whereas public water systems, which use either surface water or ground water directly influenced by surface water, have to perform additional treatment to reduce the cryptosporidiosis infection risk [14]. In Great Britain water companies must use a process for treating the water to ensure that the average number of oocysts is less than 1 per 10 litres of water [15]. This threshold is based on the already mentioned observation that outbreaks of crypto-

sporidiosis have only occurred, when the concentrations of oocysts were in excess of 1 in 10 litres [16]. The European Union Drinking Water Guideline declares the bacterium *Clostridium perfringens*, whose spores can survive for extended periods of time in soil, as a persistent faecal indicator and a surrogate for *Cryptosporidium* spp. Therefore, in 100 ml of drinking water sample no *C. perfringens* should be detectable [17]. In Switzerland no regulation at all exists concerning *Cryptosporidia* in drinking water. The Swiss regulation states generally that drinking water should not contain microorganisms at a concentration harmful to human health [18].

The detection of *Cryptosporidia* spp. is time-consuming and expensive. Therefore, drinking water is not regularly checked for this pathogen. The officially compulsory microbiological drinking water tests in Switzerland are limited to the detection of *E. coli* and *enterococci*, and the heterotrophic plate count [19]. While *E. coli* dies relatively quickly in the environment, the oocysts of *Cryptosporidium* remain infectious for weeks to months. In contrast to *E. coli*, *Cryptosporidium* also survives in chlorinated drinking water. Therefore, one has to take into account that water that fulfills the quality criteria for drinking water can still contain persistent pathogens such as *Cryptosporidium* [20]. In drinking water distribution systems that include an initial treatment of the raw water no *Cryptosporidium* in the drinking water are expected in Switzerland. However, in agricultural areas the water from the water catchment zones can come into contact with grazing animal's excrements and liquid manure. Animals that are infected with cryptosporidia excrete infectious microorganism that can infiltrate the groundwater. This water is usually not treated in any way and, therefore, the aim of this study was to estimate the risk for a *Cryptosporidium* infection through the consumption of drinking water in agricultural areas. A drinking water survey was performed from June 2003 to November 2004 analysing water samples from three rural sites in Switzerland. These three sites were selected out of 15 sites because of their elevated oocyst concentrations determined in a previous study [21]. The drinking water was investigated for *Cryptosporidium* spp. and bacterial faecal indicators such as *E. coli*, *Enterococcus* spp., *C. perfringens* and other parameters. Besides determining their concentration, the vitality of oocysts was assessed by the excystation method. Furthermore, the sampled oocysts were subjected to genotyping because different *Cryptosporidium* spp. exhibit different pathogenicity.

## Material and methods

### Description of the three sampling sites

In 2003 a drinking water survey was carried out which included 15 rural sites in Switzerland [21] where drinking water was investigated for *Cryptosporidium* spp., *E. coli*, *Enterococcus*, *Clostridium perfringens* and other parameters. Three of these sites exhibited increased oocyst concentrations and they were therefore investigated again. With the local authorities it was decided to publish the results anonymously; nevertheless, the general characteristics of the three sampling sites are described below.

### Site A: Alpine village

Samples were collected from the drinking water distribution system of a village of approximately 500 habitants situated in the Swiss Alps. The tap water originated from a well and was neither chlorinated nor treated in any other way. It was suspected that the drinking water was contaminated by grazing cattle kept in the drinking water catchment zone.

### Site B: Alpine camp

The second sampling site was the distribution network of a camp in the Swiss Alps. This camp has the capacity for several hundred people and has an independent well water supply. The drinking water was chlorinated after the first sampling campaign where faecal contamination was detected, probably caused by grazing cattle and sheep in the drinking water catchment zone.

### Site C: Mittelland town in Switzerland

From the drinking water distribution system of a small town (around 8,000 habitants) samples were taken from the public water distribution network. The water was neither chlorinated nor treated in any other way. *Cryptosporidium* oocysts may originate from agricultural activities in the catchment zone from either pasturing animals or organic fertilisers such as manure.

The local authorities were informed about the results of this study and the detection of *Cryptosporidium* sp. in their drinking water and if necessary measures were taken to improve its quality.

### Sampling procedure

Water samples for bacterial analysis were collected in sterile 500 ml PET-bottles (Huber & CO. AG, Basel, Switzerland), which contained sodium thiosulfate in order to neutralise residual chlorine. For *Cryptosporidium* analysis between 100 to 1000 litres tap water were filtered through a Filt-Max® filter (IDEXX, Westbrook, USA) with a pore size of one µm. Samples were transported to the laboratory within less than 8 hours, protected from light

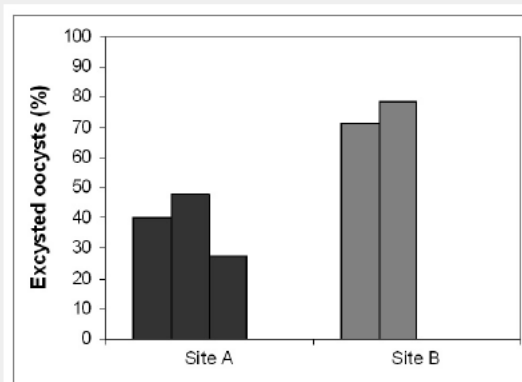


Figure 1

Viability of the oocysts in the drinking water samples determined by the excystation method. In the case of sites A 32, 42 and 125, in the case of sites B 11 and 12 oocysts, respectively, were analysed. Third sample of site B and samples from site C did not contain sufficient oocysts for a representative excystation testing.

and kept cool (12 °C). Samples were stored at 4 °C and analysed within 24 hours.

#### Detection of *Escherichia coli*, *Enterococcus* spp. and *Clostridium perfringens*

100 ml of sample were filtered through a 0.45 µm membrane filter (Millipore, Bedford, USA). *E. coli*, *Enterococcus* spp. and total plate counts were determined according to Swiss regulations [22], and *C. perfringens* according to the EU drinking water regulations [17]. Positive and negative controls were included.

#### Detection of *Cryptosporidium* spp.

Concentration of oocysts of *Cryptosporidium* spp. were determined according to method 1623, which is recommended by the Environmental Protection Agency [23]. The oocysts were detached from the Filt-Max<sup>®</sup> filter and further enriched and purified by Dynabeads<sup>®</sup> anti-*Cryptosporidium* (Invitrogen, Carlsbad, USA). The oocysts were stained with specific surface antibodies (Waterborne, New Orleans, USA) and finally enumerated by fluorescence microscopy. Loss of oocysts was not corrected for. Quality control of the method was performed with a commercial kit, Aqua-GloTM G/C Direct (Waterborne), obtaining a recovery of  $29.75 \pm 3.50\%$  for *Cryptosporidium* oocysts.

#### Determination of viability of oocysts

*In vitro* excystation was performed according to a published protocol [24], in short: an aliquot of suspended bead oocyst complex (50 µl) was mixed with 50 µl of acidified Hank's Buffered Salt Solution (HBSS); pH = 2.5, and incubated at 37 °C; after 30 minutes 50 µl of 3-times excystation medium (4.5% (wt/vol) tauroglycholic acid sodium salt, 1.5% bovine trypsin (2.2 U/µg), pH adjusted to 7.7 with NaHCO<sub>3</sub>) was added and the samples were further inoculated at 37 °C for at least 3 hours. The oocysts were washed with 0.22 µm-filtered PBS and later stained with fluorescent surface-specific antibodies (Waterborne, New Orleans, USA). The suspension was membrane-filtered and finally the ratio of the excysted oocysts to the total number of oocysts was determined under the fluorescence microscope.

#### Extraction of DNA

A part of the immunomagnetically enriched and purified oocysts were further processed in order to extract their DNA for genotyping as follows: three freeze-thaw cycles in liquid nitrogen and a hot water-bath (96 °C) for 1 minute, each, were followed by proteinase K digestion at 56 °C for 4 hours. DNA was isolated using spin columns (DNA mini kit, Qiagen, Basel, Switzerland) in 200 µl of elution buffer according to the manufacturer's instructions. DNA samples were frozen immediately at -20 °C.

#### Nested PCR for *cryptosporidium*

Nested PCR was done according to a published protocol [25]. In short: outer primers WR494F (TGA GTK AAG TAT AAA CCC CTT TAC) and AWA 1206R (CTC CAC CAA CTA AGA ACG GCC) were used amplifying a product of approximately 760 bp of the 18S rRNA – followed by the application of inner primers CBD-DiagF

(AAG CTC GTA GTT GGA TTT CTG) and PW99R (TAA GGA ACA ACC TCC AAT CTC), which is a slightly modified AWA995 reverse primer. The product of approximately 420 bps contains a variable region suitable for genotyping *Cryptosporidium* spp. PCR was done with 20 µl of the DNA extract (see above). The final concentration in the reaction mixture were 50 mM KCl, 20 mM Tris-HCl, pH 8.4, 2.5 mM MgCl<sub>2</sub>, 0.5% Tween 20, 400 ng/µl BSA, 200 µM dNTPs each, 1 µM of the primers, uracyl DNA glycosylase (UDG) and water to a final volume of 50 µl. Amplification was performed in a Genius Thermo Cycler (Techne, Cambridge, England) after incubating the reaction mixture for 10 min at 37 °C. Addition of Taq DNA polymerase (Sigma-Aldrich, St. Louis, USA) at 1.25 U per reaction was followed by an initial denaturation step at 94 °C for 10 min, 39 cycles at 94 °C for 30 sec, 58 °C for 40 sec, and 72 °C for 40 sec and a final extension step of 72 °C for 10 min. In the nested PCR, 1 µl of the first PCR array mixture was reamplified. Positive and negative controls were run in between every sample.

#### Detection and sequencing of PCR products

Reaction products were detected on agarose gels (1.5%) after staining with ethidium bromide (0.5 µg/ml) by visualisation with UV light, and their size was estimated by comparison with a sizemarker. A detection limit of 8 oocysts was experimentally defined in internal control experiments. Amplicons of the expected length of approximately 420 bp were purified by QIAamp DNA Mini Kit (Qiagen, Venlo, The Netherlands) and sent to a private company for sequencing in both directions (Microsynth, Balgach, Switzerland).

#### Sequence analysis

Only the part of the sequence that was confirmed by amplified fragments in both directions was used for further analysis. Sequence alignments for phylogenetic analysis were performed using MEGA version 3.1 [26]. After manual adjustment of the sequence with BLAST 2 SEQUENCES [27] and alignment by ClustalW [28] phylogenetic trees were constructed with neighbour joining methods using the Kimura 2-parameter model. Bootstrapping with 500 pseudoreplicates assessed the reliability of the tree. Additional *Cryptosporidium* 18S rDNA sequences were obtained from GenBank: *C. felis* (AF108862), *C. hominis* (DQ286403), *C. meleagridis* (AF180339), *C. muris* (AF093496.1), *C. muskrat* genotype (AY120904), *C. parvum* (AY030088), *C. parvum* (AF297513), *C. parvum* (AF030087), *C. parvum* (AF115377), *C. parvum* "pig genotype" (AF108861), *C. wrairi* (AF115378), *C. serpentis* (AF093499), *C. sp.* 5538 "C. muskrat genotype II" (AY545538). *Perkinsus marinus* (X75762) was used as an outgroup.

#### Nucleotide sequence accession numbers

The nucleotide sequence of the 18S rRNA gene of the *Cryptosporidium* sp. collected at drinking water sample site A has been deposited in GenBank under accession number EU263615.

## Results

### Oocysts concentrations at selected sites

Samples were taken from the three sampling sites at three different times (table 1). In the case of the alpine village (site A) all three samples had approximately the same oocyst concentration, whereas the oocyst concentrations in samples from site B varied strongly. The highest oocyst concentration was measured in the second sample after heavy rainfall. Due to chlorination no bacterial faecal indicators was found in this sample. Only the first sample of the town of the Swiss Mittelland had an elevated oocyst concentration whereas their concentrations in the second and third sample were close to the detection limit.

In addition to the *Cryptosporidium* oocysts concentration, the presence of the faecal indicators *E. coli*, *Enterococcus* spp. and *C. perfringens* was measured. Furthermore, the heterotrophic plate count and nitrate concentration were determined (table 1). Only sporadic faecal indicators were detected. Therefore, no correlation between faecal indicators and oocyst concentration was observed. Also, neither nitrate concentration nor the heterotrophic plate count seem to correlate positively with oocyst concentration.

### Viability

The viability of *Cryptosporidium* oocysts varied between 34% and 75% in the samples from site A and B (fig. 1). This is similar to values found in the literature, which assume that on average 40% of the oocysts in drinking water are viable [29]. The low number of oocysts in the sample from site C did not allow determination of their viability.

### Genotyping

Only in the case of second sample of site A the nested PCR assays resulted in an amplification product. We can only speculate why in all other samples no amplification product could be found. This could be due to problems with extraction of the nucleic acid, inhibitors or oocyst concentration below detection limit. Just the part of the sequence (263-bp), which was confirmed by amplified fragments in both directions, was used for further analysis. The PCR-product of site A was sequenced and exhibited a very high 99% (262/263) similarity with different isolates of *C. par-*

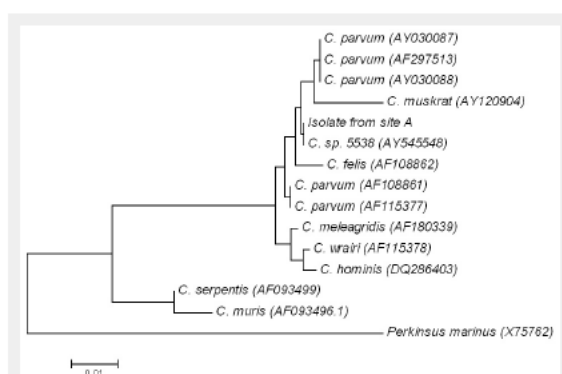
*vum* (see fig. 2) and a 100% agreement with *C. muskrat* genotype II AY545548 (263/263). A phylogenetic tree was constructed with the sequence obtained from the drinking water sample of the site A and Genbank sequences (fig. 2). The isolate is located in cluster of different *C. parvum* strains. During sampling grazing cattle were present in the catchment zone of the village well. As *C. muskrat* infects only muskrats [30], which are not part of the fauna in the sampled region. Therefore, it is very likely that the detected *Cryptosporidium* strain most probably belongs to the genotype of *C. parvum*.

### Risk assessment

The risk for the drinking water consumers of the site A was assessed based on the measured concentration, viability and genotype (table 2). The collected *Cryptosporidium* oocysts were viable and the amplified sequence is most probably belonging to the genotype of *C. parvum* suggesting a potential risk for the human health. The yearly risk of infection was calculated using the standard exponential model [12]. Assuming a daily consumption of 2 L of tap water with the average oocyst concentration of 0.154 oocysts/l resulted in a yearly infection risk of 40.8%. The assumed daily consumption of 2 L is very high. According to German study the average daily consumption of unboiled water is 0.17 L [31] and this assumption would lead to a yearly risk of 4.4%. We asked local authorities and the responsible physician of the district whether they observed increased numbers of diarrhoea cases. Both confirmed that no diarrhoeal outbreak was observed among the population in the alpine village. Located in the village is a home for elderly people, and also at this place, with around 70 inhabitants with a high proportion of potentially immunocompromised consumers, no unusually high rate of cases of diarrhoea was observed.

## Discussion

At three selected sites in Switzerland we detected *Cryptosporidium* oocysts in the concentration range between 0.04 and 0.18 oocysts/l in the drinking water. A number of reasons can cause the observed different oocysts concentrations of site A, B and C. This includes the number of oocysts in the faeces of the pasture livestock, the amount of faeces distributed by pasturing animals, or the amount of manure distributed. Furthermore, weather conditions influence the behaviour of oocysts; for example, during heavy rainfall fresh faecal contaminants can infiltrate the soil quickly, whereas during dry weather faecal contaminants remain on the surface. Also soil structure has an impact on the flow velocity of the infiltrating water and the retention capacity for oocysts. Long residence times favour the natural elimination of microorganisms and viability of oocysts will decrease with increasing exposure to environmental stress factors [32]. Oocysts isolated from samples of the site A were viable and most probably members of the *C. parvum* genospecies. This is in agreement with the fact that within the drinking water catchment zone solid manure or faeces of pasturing cattle was observed during the sampling campaign. Young cattle are often suffering from cryptosporidiosis and have a prevalence of up to 16.8% in Switzerland



**Figure 2**

A neighbour-joining tree, based upon partial 18S rRNA gene sequence of the positive sample. For comparison, different *Cryptosporidium* strains and *Perkinsus marinus* X75762 as out-group were used.

[33]. These animals excrete several millions of *C. parvum* oocysts per day in faeces [34] and can be a significant source of infection for humans. This could be an explanation, why in contrast to other countries, where *C. hominis* is the main reason for *Cryptosporidium* infection for humans, *C. parvum* seems to be the main pathogen in Switzerland [35]. For example, in an epidemiological study of diarrhoea patients in Switzerland carried out by Fretz and co-workers all of the nine isolates from stool samples belonged to the genotype II of *C. parvum* and the zoonotic cycle was believed to play a major role [35]. This is supported by an earlier study where seven out of 13 isolates from HIV-infected patients belonged to the bovine *C. parvum* genotype [36]. In another Swiss study on children suffering from diarrhoea mainly *C. hominis* was detected (11 of 14 children, nine with history of travel), whereas the other children had the zoonotic genotype II of *C. parvum* (three children, one with travel history). All this implies cattle as an important source of autochthonous infections [37].

In general, cryptosporidiosis is of minor importance in Switzerland. The prevalence of diarrhoea cases, i.e., the percentage of the *Cryptosporidium* infected diarrhoea patients for the total population in Switzerland at a specific time, is only 0.2% [38]. Diarrhoeic children are more often infected with *Cryptosporidium* at a proportion of 4.6%–5.5% [37, 39, 40], and for AIDS patients the prevalence rises even to 11.8% [41]. Based on extrapolated clinical data an estimate of only 340 annual cases of cryptosporidiosis in Switzerland was calculated [38]. It is believed that consumption of raw dairy products and oysters as well as travelling are the main risk factors whereas contaminated drinking water seems to be of minor importance [35, 38]. In several studies *Cryptosporidium* sp. oocysts were detected in Switzerland with highest reported concentration of 3.83 oocysts/l in surface water [25, 42–44], 1.6 oocysts/l in well water [42, 45], and 0.25 oocysts/l in tap water samples [42]. Despite this, no waterborne cryptosporidiosis have been reported in Switzerland up to now

[25]. The reasons for the fact that no outbreaks were observed although viable oocyst concentrations of *C. parvum* are above the action level can be only speculated about. It is possible that the specific *C. parvum* strain present in grazing cattle in Switzerland is less pathogenic than normally assumed. Furthermore, the determined viability may not correlate with infectivity, i. e., that the oocyst lose their infectivity but remain viable. Another reason may be that local tap water consumers get immunised already in their childhood after a first infection with *C. parvum* oocysts and are more or less protected from further infections. Studies with human volunteers indicate that prior infection with *Cryptosporidium* offers a certain degree of protection against infection with low doses of oocysts [46]. Such a situation can be expected by a regular consumption of contaminated drinking water. Furthermore, it was shown in a study from northern Italy that local people had an increased serological response against *Cryptosporidium* oocysts caused by previous exposure to *Cryptosporidium* and explaining the observed lower risk for cryptosporidiosis outbreaks in this region [47]. But also simple other reasons have to be considered, such that people consume very little unboiled tap water, probably even less than 0.17 L per day [31], or that infected persons suffering from mild diarrhoea would not consult a doctor. Therefore, one cannot exclude that sporadic cryptosporidiosis or even outbreaks were missed.

The European Union Drinking Water Guideline [17] declares the spore-forming bacterium *Clostridium perfringens* as a persistent faecal indicator and surrogate for *Cryptosporidium* spp. According to our results *C. perfringens* does not seem to be an appropriate indicator for *C. parvum*. Repeatedly, we detected *Cryptosporidium* oocyst in the absence of *C. perfringens* (table 1). This observation is also in agreement with other studies [21, 48]. No significant correlation with the *C. parvum* oocyst concentration was found for other chemical parameters such as nitrate or other microbial parameters. This suggests that there is

**Table 1:** Summary of microbial and chemical analyses of samples from the three sampling sites. For bacterial analysis only one sample was taken in the first sampling. Later triplicate samples were collected.

Place	Date	* <i>Cryptosporidium</i> spp. oocysts/l	<i>E. coli</i> CFU/100 ml	<i>Enterococcus</i> spp. CFU/100 ml	<i>Cl. perfringens</i> CFU/100 ml	Heterotrophic plate count CFU/1 ml	NO <sub>3</sub> -concentration mg/l
Site A	30.06.2003	0.146	0	5	0	1/6/1	15.46
	23.09.2004	0.133	0/0/0	0/0/0	0/0/2	3/0/2	1.24
	30.09.2004	0.183	0/0/0	0/0/0	0/1/2	3/3/1	1.23
Site B	26.08.2003	0.042	2	1	1	1/0/1	1.65
	20.10.2004	0.109	0/0/0	0/0/0	0/0/0	3/3/5	2.63
	16.11.2004	0.009	0/0/0	0/0/0	0/0/0	0/0/0	1.95
Site C	25.11.2003	0.038	0	0	0	6/11/7	26.55
	09.09.2004	0.010	0/0/1	0/0/0	0/0/0	16/16/18	25.45
	12.10.2004	0.010	0/0/0	0/0/0	0/0/0	2/2/4	25.27

\* Taking into account that in internal control experiments only 29.75 ± 3.50% of oocysts could be recovered, the reported oocysts concentrations have to be multiplied with the factor 3.3.

**Table 2:** Risk assessment by comparison between measurements of the site A with facts of concern.

	Results	Facts of concern	Possible consequences
Oocyst concentration	0.14–0.18/l	>0.1 Ooc./l [10]	Sporadically cryptosporidiosis cases or even outbreaks
Oocyst viability	28%–48%	>0	Viability is a preliminary for infectivity
Genotyping	According to our assessment the isolated strain is part of <i>C. parvum</i> genotype.	<i>C. parvum</i> is a pathogenic strain for human	Cryptosporidiosis among drinking water consumers possible

presently no valid surrogate concept, that could replace the time-consuming and expensive direct detection for *Cryptosporidium* spp. Even if the detection of *Cryptosporidium* oocysts in the alpine region in this study may be of minor risk for human health, their presence in tap water proves that this drinking water was faecally contaminated. This is an alarming signal, also because, other pathogenic microorganism from liquid manure or faeces of pasturing animals such as *Giardia* spp., *E. coli* O157:H7, *Salmonella* spp., rotaviruses and other enteric microorganisms might contaminate the drinking water [49]. Especially after heavy rainfalls there is a danger that pathogens can temporarily break through into drinking water wells. Our study has shown that especially in rural regions the drinking water can be temporarily microbiologically contaminated. Nevertheless, there is currently no indication of waterborne cryptosporidiosis in Switzerland as reported by public health authorities [38].

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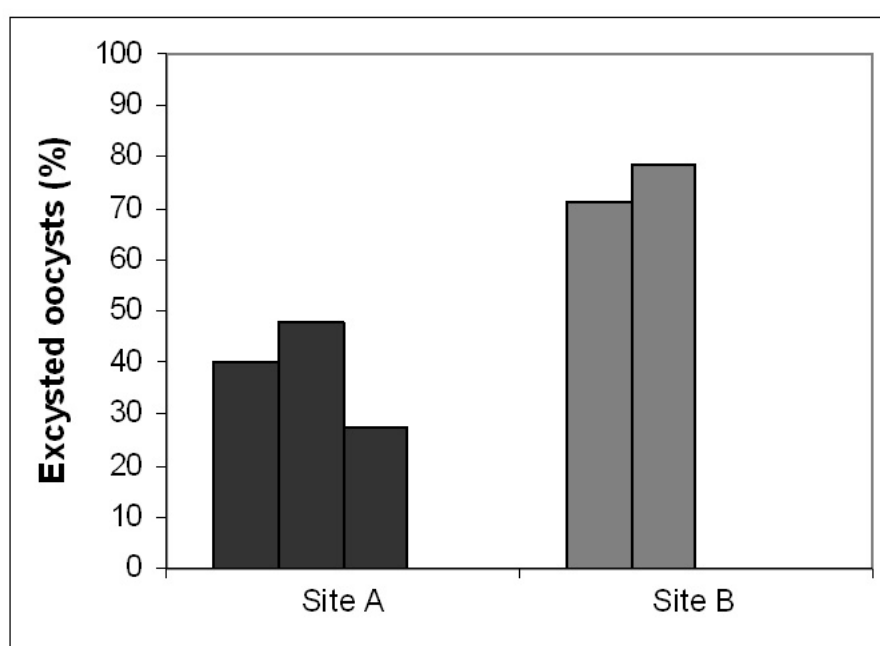
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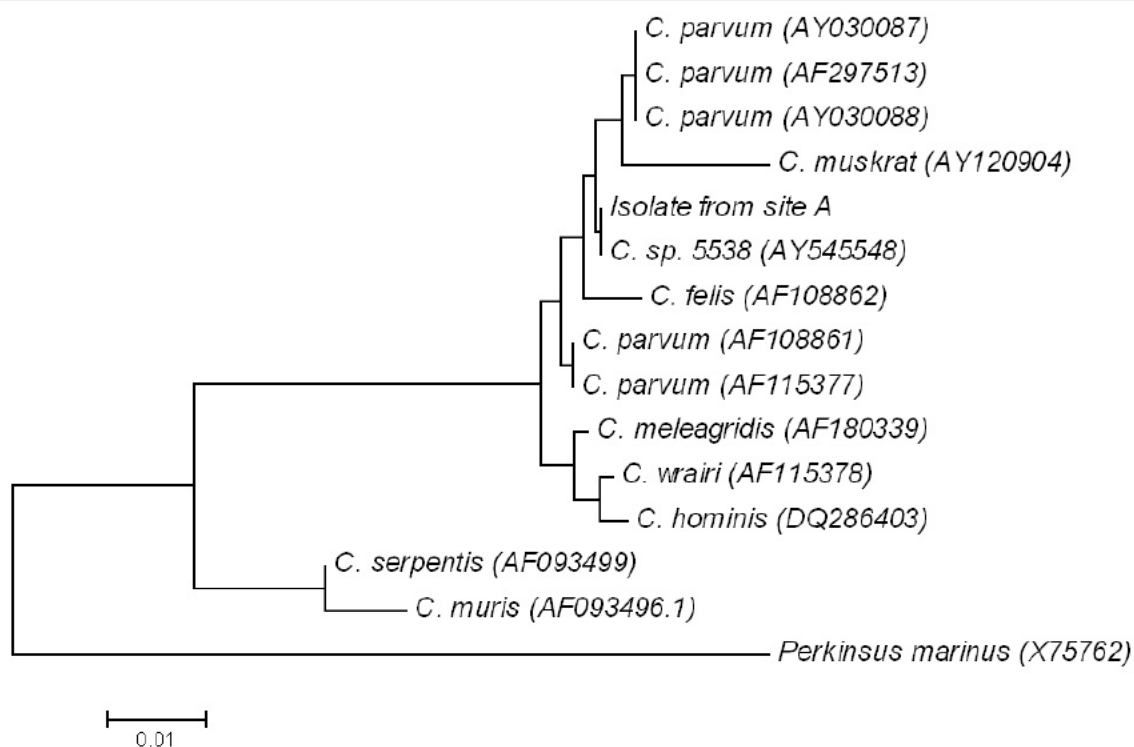
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## Figures (large format)

**Figure 1**

Viability of the oocysts in the drinking water samples determined by the excystation method. In the case of sites A 32, 42 and 125, in the case of sites B 11 and 12 oocysts, respectively, were analysed. Third sample of site B and samples from site C did not contain sufficient oocysts for a representative excystation testing.

**Figure 2**

A neighbour-joining tree, based upon partial 18S rRNA gene sequence of the positive sample. For comparison, different *Cryptosporidium* strains and *Perkinsus marinus* X75762 as out-group were used.