

Contaminated Drinking Water from Agricultural Areas?

Drinking water from rural catchments is normally either not at all or minimally treated. It is precisely this water which can be contaminated by liquid manure or excrement from grazing animals. The most worrying aspect are the environmentally persistent forms of cryptosporidia. In 9 out of 15 investigated drinking water catchments in rural areas, we could in fact detect cryptosporidia. It is still to be determined whether they present a hazard for humans.

The officially prescribed microbiological drinking water tests are limited to the detection of *E. coli* and enterococci, and the total plate count. Persistent pathogenic bacteria such as cryptosporidia (see box p. 10) therefore remain undetected. While *E. coli* die relatively quickly in the environment, the persistent form of *Cryptosporidium*, the so-called oocyst (Fig. 1), remain infectious for weeks to months. Cryptosporidia also survive in chlorinated drinking water, as opposed to *E. coli*. Therefore, water which otherwise fulfills the quality criteria for drinking water can still contain disease causing pathogens.

Particularly affected are drinking water catchments in agricultural areas, since this water can come into contact with grazing animal excrement and liquid manure. Animals which are infected with cryptosporidia excrete infectious oocysts which can reach drinking water. Since this water is usually not treated in any way, EAWAG wanted to estimate how great the risk was for a *Cryptosporidium* infection through the consumption of drinking water in agricultural areas.

Cryptosporidia are Relatively Widespread

We took water from 15 small rural area drinking water catchments in different parts of Switzerland. The drinking water was examined not only using the analysis prescribed in the food and drink regulations, but also for cryptosporidia (see box p. 10). 9 out of 15 of the water samples were contaminated with cryptosporidia (Fig. 2). To date, *Cryptosporidium* concentrations have been detected in Switzerland of up to 3.83 oocyst/l in surface waters [1], 1.6 oocyst/l

in karst spring water [2] and 0.25 oocyst/l in drinking water [3]. Our measurements are comparable to these findings.

In addition, 4 out of 9 *Cryptosporidium* containing drinking water catchments were

also contaminated with faecal bacteria *E. coli* (Fig. 2). The water from these catchments, therefore, did not meet the quality criteria for drinking water. For the remaining 5 catchments contaminated with crypt-

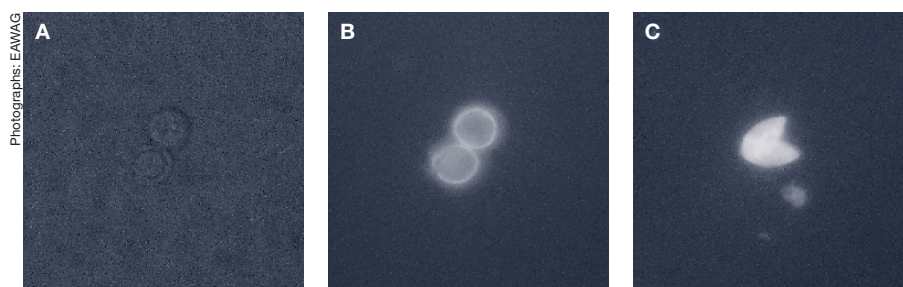


Fig. 1: Under a standard optical microscope, a *Cryptosporidium* oocyst is hardly visible (A). Specific fluorescent antibodies cause the oocyst surfaces to light up (B). In the small intestine of the host, the *Cryptosporidium* oocysts germinate (C) and release 4 sporocysts – a process known as excystation. The sporocysts enter the intestinal epithelium and create new oocysts there.

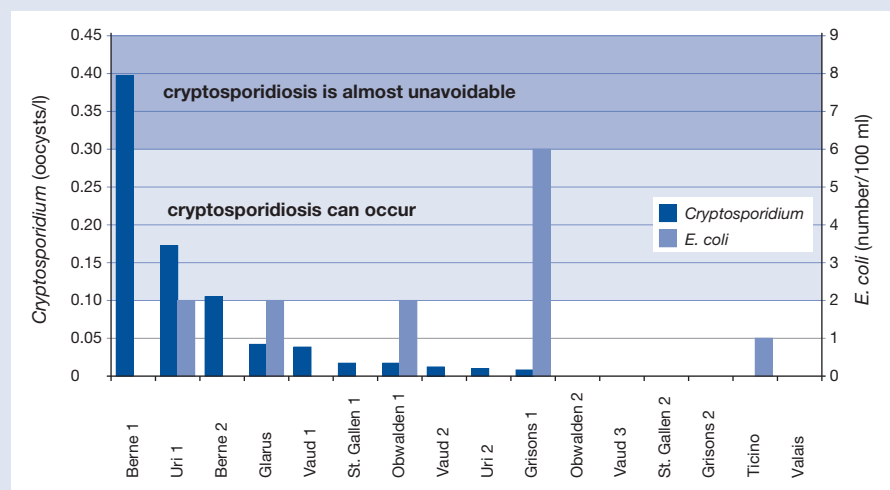


Fig. 2: Measured *Cryptosporidium* and *E. coli* concentrations in 15 drinking water catchments of rural areas displayed in descending order of oocyst concentrations. Berne 1 is a public fountain. In the range of 0.1–0.3 oocysts/l disease outbreaks in the population can be expected, above 0.3 oocysts/l epidemics are possible.

tosporidia, the *E. coli* indicator had not detected the faecal contamination. In another catchment we detected *E. coli* but no cryptosporidia.

Clostridium is not an Indicator for Cryptosporidia

The Drinking Water Guideline from the European Union [4] declares the bacterium *Clostridium perfringens*, which can survive for extended periods in the soil with help of its spores, as persistent faecal indicator on the basis of the following assumption: if in 100 ml drinking water there are no clostridia detectable, the water should also be free of any other persistent parasite forms such as *Cryptosporidium* oocysts.

If this association is correct, then water samples which are contaminated with *Cryptosporidium* should also contain *Clostridium*. We wanted to test this hypothesis and analyzed water samples of the 15 drinking water catchments also for *Clostridium*. However, we found no correlation between the occurrence of *Cryptosporidium* and the persistent faecal indicator *C. perfringens*. Only two drinking water catchments were contaminated with clostridia: in Glarus cryptosporidia as well as clostridia were present (1 spore in 100 ml water), while the investigated drinking water catchment in Ticino contained no cryptosporidia but 96 *Clostridium* sporidia in 100 ml water. It is therefore doubtful that *C. perfringens* can be used as an indicator for *Cryptosporidium*. Most likely, clostridia and cryptosporidia are not similar enough in their environmental behavior.

The Situation in Switzerland

Legal regulations for defining threshold limits for cryptosporidia in drinking water exist neither in Switzerland nor abroad. There is therefore uncertainty as to how to assess the hazard presented by the presence of cryptosporidia in drinking water.

Even a *Cryptosporidium* concentration of 0.1 oocyst/l can lead to disease outbreaks in the population, and concentrations of more than 0.3 oocyst/l will almost certainly

lead to cryptosporidiosis cases [5]. In 20% of the drinking water samples we investigated, the *Cryptosporidium* concentration was above 0.1 oocyst/l and exceeded in one case even the value of 0.3 oocyst/l (Fig. 2). In 9 of the 15 drinking water catchments the risk level assessed in the USA as a 10^{-4} residual risk for cryptosporidiosis was exceeded (1 infected person per 10,000 persons per year, at an oocyst concentration of more than 0.0000327 oocyst/l). Since our measurements are just momentary sample extractions, it must be assumed that after large precipitation events, the *Cryptosporidium* concentration will rise considerably.

So Far No Cryptosporidium Epidemics in Switzerland

Despite this noteworthy count of cryptosporidia in the tested drinking water,



Fig 3: Manure spreading and ...

Switzerland has so far been spared an epidemic. The prevalence of diarrhea cases – i.e. the percentage of the *Cryptosporidium* contaminated diarrhea patients at a given time – is in Germany and Switzerland for the general population at 0.4–1.9%. Children are harder hit, at a rate of 1.1–4.8%, while

Cryptosporidia

What are cryptosporidia?

Cryptosporidia are protozoal intestinal parasites of considerable diameter (5 µm diameter), which create oocysts in a persistent form (Fig. 1). They belong to the most important pathogenic protozoa in drinking water. The *Cryptosporidium* genus contains 13 species. *Cryptosporidium parvum* is the most widespread and pathogenic also for humans. *C. parvum* is thought to be able to infect any mammal [6].

What are the symptoms of a *Cryptosporidium* infection?

The disease contracted through cryptosporidia, cryptosporidiosis, is a zoonosis – i.e. an animal disease which can be transferred to humans. Human infections were first documented in 1976 and water-borne cryptosporidiosis has been known since 1984. Since then a number of epidemics occurred in the USA, Great Britain and Japan: the largest estimate of cases being 400 000 in 1993 in Milwaukee (Wisconsin, USA) [7]. The oocysts excreted with faeces stay alive in cold water for several months. Cryptosporidiosis begins with the intake of oocysts (Fig. 1A + B). After an incubation period of 2 to 12 days, in which the oocysts germinate in the intestine (Fig. 1C) and multiply, the infection leads to watery diarrhea and stomach cramps, usually without temperature rise, nausea, faintness, or vomiting. The sickness onset is variable, but normally the disease cycle is over within 30 days. However, for people with weakened immune systems, especially HIV positive cases, the infection takes a chronic or fulminant course, and can even lead to death in some exceptional cases. To date, there is no medication available for treating cryptosporidia.

How are Cryptosporidia detected?

The American environmental authorities and the British Drinking Water Inspectorate recommend Detection Method 1623 for *Cryptosporidium* in drinking water. In the field a large volume of water, 100 to 1000 l, is passed through a filter with 1 µm pores. In the laboratory, the particles are dissolved from the filters and the cryptosporidia separated from the other particles with help of immunomagnetic methods. The cryptosporidia are colored by surface antibodies and counted under a fluorescent microscope (Fig. 1B).

Active oocysts from environmental samples can be brought to germination in suitable media and temperatures of 37 °C in laboratory cultures. In this way, the percentual proportion of active oocysts in drinking water can be determined.



... pasture grazing can lead to animal faecal contamination of drinking water catchments.

the value rises for AIDS patients to 11.8%. It is calculated that in Switzerland annually there are 340 cryptosporidiosis cases [8]. In reality, only a few are clinically identified. This could be due to the following reasons:

- Cryptosporidia originating from cattle are less infectious than first thought. With the detection methods we use, *Cryptosporidium* species which are either not at all or little pathogenic are also detected.
- Cryptosporidia in drinking water are no longer necessarily vital or infectious. Depending on the environmental conditions, oocysts can survive for several months. They die in time, but remain detectable.
- People who are sick with cryptosporidiosis seldom consult a doctor. Consequently, clinical tests are not usually made for cryptosporidia.
- The public consumes very little unboiled water.

Practical Consequences

The regulations in the current legislation for drinking water in specified groundwater protection zones must be better respected. Close to drinking water catchments, in the so-called groundwater protection zone I, all grazing and fertilizer usage is forbidden [9]. These regulations are not always complied with, as Fig. 3 shows. Periodic inspections are therefore necessary. Above all, bad weather can raise the risk of faecal matter being washed into drinking water catchments. Therefore, for certain locations with poor soil filtration, it may be necessary to

treat the water by means of, for example, UV disinfection.

Implications for Further Research

There is a general uncertainty whether periodically higher *Cryptosporidium* concentrations, which are to be expected after heavy precipitation, represent a hazard for consumers of drinking water. Too little is known about the exact species allocation of cryptosporidia which occur in Switzerland, and the vitality of oocysts in drinking water, to provide a clear answer to this question.

In a further stage of the project, we would like to close some of these gaps in our knowledge. For this purpose, at three locations with high oocyst concentrations, the vitality should be determined by sporulation tests (see box p. 10), and the exact species of *Cryptosporidium* should be characterized through genotyping. In addition, a risk assessment should be made on the basis of results obtained. We would also like to survey the local authorities and physicians concerning whether in the past years there has been any increase in the number of cryptosporidiosis cases in the population. Cryptosporidia, even if they are classed as weak pathogens in further investigations, are environment persistent faecal indicators, which should be excluded in any case from drinking water.

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