

A Review of Current Knowledge

CRYPTOSPORIDIUM
in
WATER SUPPLIES

FR/R0005

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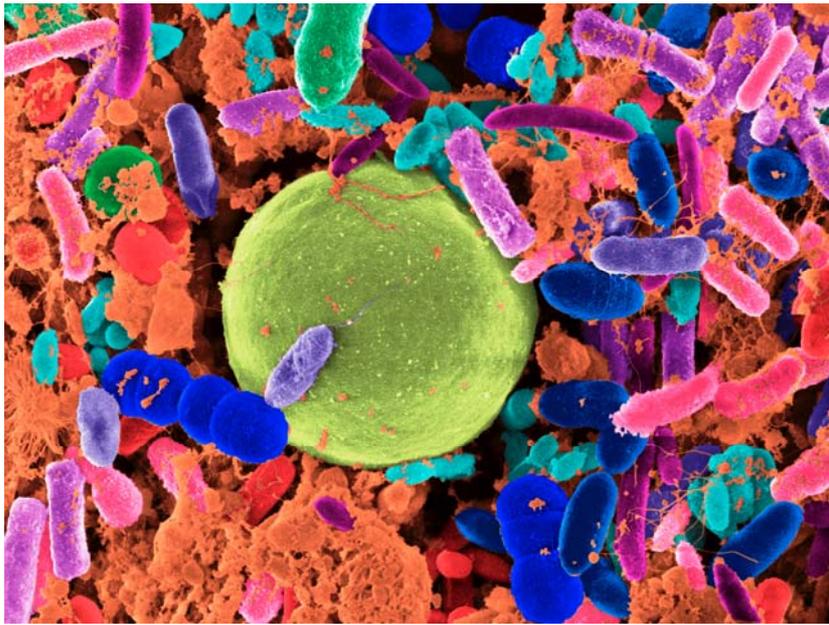
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CRYPTOSPORIDIUM in WATER SUPPLIES



Cryptosporidium protozoan
Cover page image © Science Photo Library

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Third Edition revised by Laura Keay

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1 Introduction

Most developed countries have regulations which specify a minimum quality for public and private drinking water supplies. This is to ensure that the water is both pleasant and safe to drink.

In the European Union (EU) the quality required in member states for public supplies of drinking water was first specified in 1980 by the 'Drinking Water' Directive. The Directive defined limits for the chemical and microbiological content of the drinking water. A revised EU Directive was approved in 1998 (EU Council Directive 98/83/EC) which has been implemented in the UK by means of Statutory Instruments (SI) which define drinking water regulations. The overall requirement for drinking water is that it shall be "Wholesome". This is defined in the regulations as "water which does not contain any micro-organism or parasite or any substance at a concentration or value which would constitute a danger to human health". Further information is available from the website of the Drinking Water Inspectorate (for England & Wales) at www.dwi.gov.uk.



Small water reservoir below Malvern Hills. © R G Ainsworth

In the UK water companies are required to carry out risk assessments on all their water supply sites to ascertain the level of risk *Cryptosporidium* poses to the final treated water quality. Those at high risk need additional treatment (in the form of properly controlled coagulation/flocculation filtration systems or membrane or UV treatment systems (see section 9)). The UK regulations also require water

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companies to design and continuously operate adequate treatment and disinfection. A proven failure to comply with this is now an offence.

The current Water Supply (Water Quality) Regulations 2000 were amended in 2007 (SI 2000 No. 3184) to remove Section 29, the requirements previously in place to monitor all treated water supplies for *Cryptosporidium*. Whereas previously the regulations had been very specific (continuous monitoring of high risk sites post treatment to achieve <1 oocyst per 10 litres) they now only require water companies to produce water which is “Wholesome” with regard to *Cryptosporidium* (Regulation 4). An oocyst is the infective form of the parasite *Cryptosporidium* and is later described in this review. Other potential parasites are found in surface waters, notably *Giardia*.

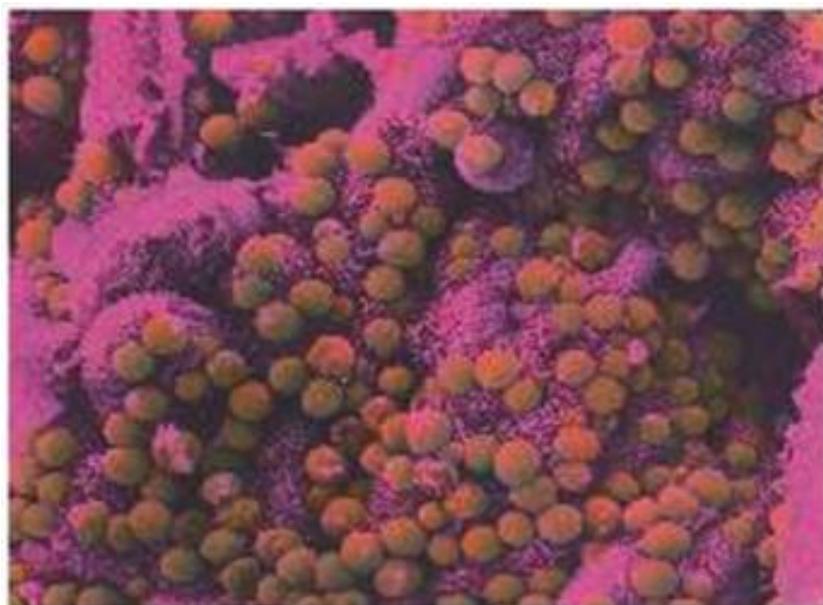
A wide range of parasites can cause disease (for example malaria is caused by the parasite *Plasmodium* and sleeping sickness is caused by the parasite *Trypanosoma*). A number of parasites (or their infective form) are found in lakes, rivers, and groundwaters and if they are ingested when the water is drunk illness can follow. *Entamoeba histolytica*, which is principally a tropical parasite causing amoebic dysentery, is the third largest cause of parasitic death in human beings after malaria and schistosomiasis. The American Water Works Association Manual on Waterborne Pathogens (AWWA, 2006) lists at least 18 different waterborne parasites most of which are found principally in tropical climates.

The waterborne parasites of greatest concern in countries with temperate climates are *Cryptosporidium* and *Giardia*. This review provides specific information on *Cryptosporidium*, a parasite which causes an unpleasant illness and which is well known for its resistance to drug therapy in infected animals and human beings and its resistance to the normal methods used to disinfect drinking water. Another review in this series addresses *Giardia* (FWR, 2006).

2 What is *Cryptosporidium* and Cryptosporidiosis?

Cryptosporidium is a waterborne parasite found widely distributed around the world including Europe. When ingested it can cause an unpleasant illness called **cryptosporidiosis**. Infection is transmitted by tiny spore- or egg-like cells called oocysts. These oocysts are small, roughly spherical in shape and about 4 to 6 µm in diameter; a µm is a micrometre, one millionth of a metre.

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Surface of the small intestine infected with *Cryptosporidium parvum* parasites. © Moredun Scientific Ltd / Science Photo Library

The symptoms of cryptosporidiosis are diarrhoea (92% of patients), mild abdominal pain (45% of patients), nausea and vomiting (51% of patients), mild fever (63% of patients) and fatigue. The incubation period of the disease is between 4 and 28 days with an average of 7 days. Specific antibiotics can be used to treat the illness if diagnosed correctly however recovery from the illness is usually dependent on the body's immune system alone. As a consequence cryptosporidiosis may be very serious in people whose immune system is weakened or less effective such as the very young, the elderly, AIDS sufferers and those on immuno-suppressant drugs. Although the disease is not usually fatal it can be life-threatening for individuals with weakened immune systems.

3 *Cryptosporidium* in the environment

Cryptosporidium is found in man and many other mammals and also in birds, reptiles and fish. It is principally an infection of new-born and young animals except in man in which it can readily infect all ages. Although known as a cause of sickness in farm animals it was not recognised as a causative agent of illness in man until 1976 (Casemore *et al*, 1985). Oocysts are passed in the faeces of infected animals, including humans. Typically, an infected calf can excrete ten thousand million oocysts daily for up to 10 days. Infection occurs either by zoonosis (animal-person contact), by ingestion of contaminated food and drink, or by inhalation of droplets contaminated with oocysts (e.g. from farm animals).

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A UK survey (Carrington & Smith, 1995) found that between 37% and 74% of samples of treated sewage from 14 plants in the UK contained oocysts. Rivers into which treated sewage is discharged are thus likely to contain oocysts. Groundwater can also become contaminated with oocysts. In 1993 an outbreak of cryptosporidiosis in the UK was associated with a borehole supply (Morgan *et al*, 1995) and other studies have also shown that groundwaters can be contaminated by both *Cryptosporidium* and *Giardia* (Hancock *et al*, 1997). Swimming pools are also potential sources of oocysts and infection (DWI, 2000; Puech *et al*, 2001).

There are many different species of *Cryptosporidium* found in animals (DWI, 2004). Some species are adapted to infecting several hosts - for example *Cryptosporidium parvum* infects both human beings and ruminant animals (Chalmers *et al*, 1995). Other species are more host specific, for example *Cryptosporidium hominus* is largely confined to human beings.

The oocysts of *Cryptosporidium* are passed in huge quantities in the faeces of infected people and animals (which is why oocysts are found in sewage effluent and sewage sludge). When animal slurry is spread on farmland oocysts may well be present, and as a consequence runoff from rain can carry oocysts into streams, rivers, lakes and reservoirs. It has been reported that many waterborne outbreaks of cryptosporidiosis occur during and after heavy rainfall (Atherholt *et al*, 1998; Curriero *et al*, 2001).



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4 The life-cycle of *Cryptosporidium*

The life cycle of *Cryptosporidium* is very complex and consists of a number of stages which may take 1-8 days to complete. The cycle is continually repeated so that the host re-infects itself. The oocyst is ingested; in the gut it excystates (or hatches out) releasing four sporozoites which attach themselves to the lining of the small intestine. The sporozoite then enters a surface cell and develops into a spherical trophozoite which subdivides forming a meront which eventually releases merozoites. These then form either macrogametes or microgametes; the microgametes fertilise the macrogametes which become zygotes which then form oocysts. The oocyst is the infective stage which then either passes out with the faeces, or excysts and starts another cycle.

Oocysts have thick protective walls which ensure their survival in the environment and which are also highly resistant to the disinfectants used to treat drinking water (disinfection and removal are discussed later).

5 Cryptosporidiosis in the community

In England and Wales, the Centre for Infections (CfI) which is part of the Health Protection Agency (HPA) produces routine reports on the levels of "notifiable diseases". Regulations (SI 1988 No. 1546) require that the CfI is informed when certain diseases are identified. Although cryptosporidiosis is not itself a notifiable disease its symptoms are very similar to those of food poisoning which is notifiable, and as a consequence data on *Cryptosporidium* are collected and published by the CfI. In England and Wales, in the region of 4000 cases are identified annually. In Scotland there are about 600 cases identified annually. These figures underestimate the actual cases to a large extent because those infected may not visit a doctor and because those that do visit may not provide a sample for analysis. However, the collected data indicate that *Cryptosporidium* is the third leading cause of non-viral gastro-intestinal illness in the UK.

Studies have shown that between 21% and 49% of the UK population might have been infected at some time by *Cryptosporidium* (Wheeler *et al*, 1999; Hunter, 1999). Prior infection with *Cryptosporidium* can confer immunity against re-infection (Chappell *et al*, 1999).

6 Outbreaks of Cryptosporidiosis

An 'outbreak' of cryptosporidiosis or intestinal disease is defined as a level of disease above the normal background level (Badenoch, 1990). The first recorded

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outbreak of cryptosporidiosis associated with a public drinking water supply was in Bexar County, Texas, in 1984 which affected 79 people. The first identified outbreak in the UK was in Ayrshire in 1988, affecting 27 people. In 1989 there was a major outbreak in the Swindon area which affected over 500 people. It was this outbreak which led to the establishment of the Group of Experts under the chairmanship of Sir John Badenoch which produced two reports (Badenoch, 1990; Badenoch, 1995). After the death of Sir John Badenoch in 1996 Professor Ian Bouchier was appointed chairman of the Group of Experts which produced its third report in 1998 (Bouchier, 1998). The third report identified 25 outbreaks of cryptosporidiosis in the UK between 1988 and 1998 that had been associated with the consumption of public drinking water supplies.

The largest recorded outbreak of cryptosporidiosis was in Milwaukee in the USA in 1993 (MacKenzie *et al*, 1994) when approximately 54 people (85% of whom were immuno-deficient) died as a consequence (Hoxie *et al*, 1997).

Most outbreaks of cryptosporidiosis in the UK appear to be associated with swimming pools. In the period January 1999 to December 2000 there was a total of 18 outbreaks of cryptosporidiosis of which 2 were attributed to public water supplies, 1 to a private water supply, 1 uncertain and 14 were attributed to swimming pools. The total number of people infected in these outbreaks was 667, of whom 405 were infected by the public water supply, whereas the total number of individual cases notified to the Cfl for the same period in England & Wales was 10,037. This indicates that the great majority of infections of cryptosporidiosis (i.e. about 96%) are not from the drinking water supply but from other sources such as contact with infected pets and farm animals, contaminated food and person to person contact in families and nurseries.

7 Can all oocysts cause an infection?

There is usually a so-called "infective dose" with many infective agents. An infective dose depends on the physical condition of the person who is infected and the state of their immune system so the size of an "infective dose" will vary. In the case of *Cryptosporidium* oocysts the infective dose may be between 30 and 1 million viable oocysts. However, not all oocysts are viable (viable means that they are able to "hatch out" and start the reproductive cycle of the parasite).

Available analytical methods for detecting viable oocysts in the environment are not very reliable (although they are improving all the time). An infective dose of 30 oocysts is quoted by the American Water Works Association Manual on Waterborne Pathogens (AWWA, 2006). A study on healthy volunteers showed that

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the infective dose can vary between 30 and 1 million oocysts with a mean dose of 132 oocysts (DuPont *et al.*, 1995). In this study only 20% of the volunteers became infected after ingesting 30 oocysts, 88% became infected after ingesting 300 oocysts whereas all those ingesting 1 million oocysts developed the disease. However, no data was available on how many of these oocysts were viable, so there is still considerable uncertainty on the levels of infective dose.

8 How are oocysts detected in water supplies?

The analysis of a water sample for the presence of oocysts consists of three stages: - concentration, separation and detection. *Cryptosporidium* sample filters come in various forms which range from the older spiral wound filters to the more current cellulose acetate membrane or compressed foam filters with a nominal pore size of 1µm. Due to the complex nature of the sampling and analysis of a water sample there is ample opportunity for oocysts to be missed and there is a wide variation in the results of the analyses (Nahrstedt and Gimbel, 1996). Recovery of oocysts can vary by as much as 30% to 60%. The Drinking Water Inspectorate in the UK has produced detailed protocols for the approved methods of analysis for *Cryptosporidium* oocysts (Standard Operating Protocols, 1999).

Although the detection of *Cryptosporidium* oocysts (or *Cryptosporidium* like bodies) in treated water is common place, detecting whether oocysts are non-viable (i.e. the DNA has been denatured) and therefore harmless is more difficult. There are methods to detect denatured oocysts but these are mostly theoretical and not practiced in any UK labs on a routine basis (Al-Adhain *et al.*, 2007, Clancy Environmental Consultants, 1999, Chalmers, 2011).

With the change in regulations oocysts can now be detected in treated water and not be in breach of the regulations as long as a suitable deactivation treatment method has been used i.e. UV. If oocysts are detected post treatment the water can be considered as “Wholesome” as long as the main treatment and disinfection processes can be proven to be operating correctly (see Chapter 9). However the DWI is still requesting water companies to advise them whenever any oocysts are detected.

9 How do we prevent *Cryptosporidium* entering water supplies?

Preventing pathogenic micro-organisms entering a drinking water supply depends firstly on catchment control measures to minimise pollution risks, secondly physical removal in a well-operated treatment plant, and thirdly by disinfection or inactivation of the organisms.

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Increased *Cryptosporidium* detection in treated waters has been associated with increased turbidity levels in raw waters. This is mainly due to runoff in the catchment area which will increase the number of oocysts found as they are collected up with solids washed into the raw water source. Hence high risk *Cryptosporidium* sites are mainly those using surface waters and/or around farmland or have inputs from sewage effluent. However investigations into *Cryptosporidium* outbreaks have often found that inadequate provision of a suitable process and/or inadequate operation of the water treatment works have been the main causes of outbreaks. Water treatment plants at high risk from *Cryptosporidium* tend to have systems in place to ensure shut down on detection of high turbidity in the raw water as they cannot operate in highly turbid conditions (unless pre-filters are in place). For example membrane plants will get clogged by increased solid loads and UV plants will be less effective (as oocysts can potentially be shielded by particulates in the water being treated).

A well-operated water treatment plant using conventional coagulation and filtration can reduce oocysts by as much as 99% or even 99.8% (Hall *et al.*, 1994). Contrary to received opinion, oocysts, although small (4 - 6 μm), can be removed by well-operated sand filters. However, sudden changes in flow rates dislodge some of the solids retained within the filters, including oocysts (Badenoch, 1995; Glasgow and Wheatley 1998). Water companies operate slow start up and flow to waste regimes on Rapid Gravity Filters being operated at high risk *Cryptosporidium* sites to minimise this.

Polymeric microfiltration membranes provide an effective barrier against *Cryptosporidium* oocysts. These plants have to be run under strict controls and parameters and if a membrane module has less than 90% integrity (as an operational rule) then it is considered not to be an effective barrier and is shut down. The integrity of a membrane module relates to how many membrane strands are whole and unbroken. Membranes are seen as a total barrier method and are often run without any other treatment units except for chlorine disinfection.

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Membrane Plant
© Laura Keay

The traditional techniques available for disinfecting drinking water consist of chemical dosing with chlorine, chloramines, chlorine dioxide and/or ozone. An alternative technology is irradiation with ultra-violet (UV) light.

Oocysts are protected by a thick wall which is highly resistant to chlorine. Free chlorine is effective against bacteria and viruses but ineffective against *Cryptosporidium* oocysts because it requires impracticably large concentrations that would create undesirable chemical by-products. Unfortunately, the evaluation of the performance of all disinfectants is strongly dependent on the method used for determination of the degree of oocyst inactivation and the different methods give inconsistent results. Most of the work carried out in the UK has used viability measurements which suggested that the disinfectants used in water treatment, at practicable dose levels, are ineffective as a means of reducing risk from *Cryptosporidium*.

Irradiation with UV light is an effective form of disinfection - or inactivation - for *Cryptosporidium*. The UV damages DNA in cells, disrupting their replication thereby preventing new cells being created. Although early work on UV light suggested that it was not very effective against *Cryptosporidium* a number of later studies using different techniques to measure inactivation claimed reductions in oocyst viability of 99% to 99.99% (Dyksen, 1998; EPA, 1999; Leech, 2000). A

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detailed description of the use of UV light for disinfection can be found in the EPA Manual on Alternative Disinfectants (EPA, 1999). The use of UV light for disinfection of water and inactivation of oocysts is becoming increasingly widely used, especially on waters taken from sources which are known (or suspected) to be susceptible to contamination by *Cryptosporidium* and *Giardia*.



UV disinfection equipment.
Photograph courtesy of Hanovia Ltd.

Since the de-regulation of the UK *Cryptosporidium* standard, UV plants have found greater favour as doses $> 10 \text{ mJ/cm}^2$ have been shown to effectively deactivate oocysts (Al-Adhain *et al*, 2007, Clancy Environmental Consultants, 1999). If oocysts are detected post treatment then deactivation is assumed as long as the UV system is properly validated and operating correctly, i.e. the UV transmittance (percentage of UV light at 254nm not absorbed after passing through 1cm of water sample) is optimal. This is affected by the levels of suspended solids in the raw water, levels of Total Dissolved Solids, Total Hardness, UV sleeve cleanliness, and flowrate through the UV system. An automated UV system will observe all of these parameters and raise an alarm/shut down the system if the UV validation is not sufficient to ensure effective *Cryptosporidium* inactivation.

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Sites which use UV as an effective *Cryptosporidium* deactivation method must have a low turbidity water as oocysts can be protected from UV by particulates in the water. Again this is either achieved by additional upstream filtration or monitoring of the raw waters (i.e. the plant will shut down if the turbidity rises above a certain preset level). This is in parallel with the new regulations which require water to be <1.0 NTU prior to disinfection.

10 Summary

Cryptosporidium is a parasite which can produce an unpleasant gastric illness known as cryptosporidiosis. The parasite is transmitted in an encysted form known as an oocyst. At the present time there is nothing to treat cryptosporidiosis other than the body's defence systems. Cryptosporidiosis can, therefore, be a serious problem in people with weak immune systems such as young children, the elderly, people on cytotoxic drugs and those suffering from AIDS. *Cryptosporidium* is the third leading cause of non-viral gastro-intestinal illness in the UK

There are a number of different species and strains of the parasite, but those which can infect human beings are also capable of infecting some animals, and vice versa.

Cryptosporidium is frequently waterborne in natural waters and infections have occurred from drinking contaminated water supplies. However, there are many other possible sources of infection such as zoonosis (animal-person contact), contaminated food and contaminated swimming pools and other recreational waters (rivers and lakes), or foreign travel.

A well-operated drinking water treatment plant can physically remove over 99.99% of oocysts from an affected raw water. Traditional processes such as coagulation, clarification and filtration remain the best defence against this parasite entering supplies. However, high risk sites are required to remove or render harmless any oocysts present based on raw water analysis and site specific risk assessments.

Chemical disinfectants are not generally effective against *Cryptosporidium* however a barrier method (membranes) and/or UV treatment are the only truly effective treatments at high risk sites.

Since 2007 in the UK the regulations are no longer specific for the control of *Cryptosporidium*, however water companies are now required to design and continuously operate adequate treatment and disinfection. Any oocysts found in the final treated water need to be reported to the DWI so the inspectorate can make checks to confirm if the regulations were contravened or any offences committed.

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