Review

A review of an emerging waterborne medical important parasitic protozoan

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SUMMARY

Water borne parasites are ubiquitous protozoan pathogens that affect humans, domestic animals and wildlife throughout the world. From a water perspective, several protozoan parasites are important but till now mostly *Giardia* and *Cryptosporidium* have been highlighted as significant waterborne parasitic pathogens. For many years WHO took under consideration the intestinal protozoa and *Giardiasis* and *Cryptosporidiosis* are already included into the "Neglected Diseases Initiative". At least 325 water associated outbreaks of parasitic protozoan diseases have been reported worldwide. *Giardia lamblia* and *Cryptosporidium parvum* account for the majority of the outbreaks, since *Entamoeba histolytica, Cyclospora cayetanensis, Toxoplasma gondii, Isospora belli, Blastocystis hominis, Balantidium coli*, Microsporidia, *Acanthamoeba* and *Naegleria fowleri* were respon-

sible for only a small part of the reported outbreaks. This review is intended to provide an overview of the current state of knowledge regarding these medically important parasites, to present results on their prevalence in water supplies and to stimulate research questions on different aspects of their developmental biology and water transmission.

INTRODUCTION

Waterborne diseases occur worldwide, and outbreaks caused by the contamination of community water systems have the potential to cause disease in large numbers of consumers. Waterborne outbreaks have economic consequences beyond the cost of health care for affected patients, their families and contacts, and the economic costs of illness and disease, as they also create a lack of confidence in potable water quality and in the water industry in general. A number of outbreaks have been associated with drinking and recreational water worldwide but most of the information is available from USA and GB (Barwick, et al.,

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Table 1: Waterborne parasitic protozoa and the water route of transmission

Organism	Disease/symptoms	Geographic distribution	Transmissive stage (size range) and route of infection
Entamoeba histo-lytica	dysentery, liver abscess	cosmopolitan	cyst (9 - 14.5 µm) ingestion
Giardia intestinalis	diarrhoea, malabsorption	cosmopolitan	cyst (8 - 12 µm) ingestion
Cryptosporidium spp.	diarrhoea	cosmopolitan	oocyst (4 - 6 µm) ingestion
Balantidium coli	diarrhoea, dysentery	cosmopolitan	cyst (50 - 60 µm) ingestion
Sarcocystis sp.	diarrhoea, muscle weakness	cosmopolitan	oocyst (7.5 - 17 µm) ingestion
Toxoplasma gondii	lymphadenopathy, fever, congenital infections	cosmopolitan	oocyst (10 - 12 µm) ingestion
Neospora caninum	Abortion, meningoencephalitis, myositis	cosmopolitan	oocyst (10 - 12 µm) ingestion
Cyclospora cayeta-nensis	protracted diarrhoea	cosmopolitan	oocyst (8 - 10 µm) ingestion
Microsporidia	enteritis, hepatitis, peritonitis, kerato-conjunctivitis	cosmopolitan	spore (1.8 - 5.0 µm) ingestion/contact with eye
Acanthamoeba Naegleria Balamuthia	Granulomatous amoebic encephalitis, amoebic keratitis, primary amoebic meningoencephalitis, headache, fever, abnormal behaviour, intense pain, photophobia, vomiting, loss of consciousness	cosmopolitan	trophic amoeba (5-85 µm), cyst (10-37 µm)

(adapted and modified from: Dowd et al., 2003; Karanis et al., in press; Mota et al., 2000, Schuster and Visvesvara, 2004a, b; Shields & Olson 2003; Smith et al., 1995; Smith, 1999, Soave, 1996).

2000, Kramer et al., 2001, Lee et al., 2002; Karanis et al., in press). Interest in the contamination of drinking water by enteric pathogenic protozoa has increased considerably during the past three decades and a number of protozoan parasitic infections of humans are transmitted by the waterborne route (Table 1). In industrialized countries, *Giardia lamblia* and *Cryptosporidium* spp. are of major concern as waterborne pathogens. Infective (oo)cysts are environmentally robust, sufficiently small to penetrate the physical barriers of water treatment (Table 1) and insensitive to disinfectants used in the water industry (Smith and Grimason; 2003).

A variety of properties of these protozoan pathogens, particularly the size of the transmissive stage, their environmental abundance and robustness, augment their potential for waterborne transmission. *Cyclospora cayetanensis* is a recently recognized waterborne parasite, with small oocysts that are environmentally robust, but no validated methods, currently used to assure the safety of drinking water supplies, are available for its detection (Soave, 1996). Information on the waterborne

transmission of *E. histolytica*, *B. hominis*, *B. coli*, *I. belli*, *T. gondii*, microsporidia, *Naegleria*, *Acanthamoeba*, *Balamuthia* is even scarcer. Protozoan infections are common in humans, worldwide. In September 2004, *Giardia* and *Cryptosporidium* were included in the 'Neglected Diseases Initiative' (Savioli, in press) .

GENERAL ASPECTS

The topic of emerging pathogens and related waterborne diseases is a worldwide problem. Human activities are expanding and travel between different countries is increasing, for both tourism and business. Occurrence of an infectious disease or emerging infection in one area, therefore, is of concern, should it be able to quickly spread to other areas. Data on the distribution of parasites in water supplies of different countries will be useful and may contribute to the protection of public health and to effective surveillance strategies. Parasitic illness is one significant health aspect associated with the planning and management of the drinking water resources and waterborne parasites are the most prevalent waterborne agents worldwide. They are important causes of diarrhoea, nutritional and other disorders in developing and developed countries. Emerging pathogens that may be of concern to drinking water supplies needs to be introduced as emerging pathogens in relation to drinking water in order to focus respectively the problem and to define any related research needs. We need to keep in mind that some outbreaks or infections have recently appeared in a population even they have been existed but are rapidly increasing in incidence or geographical range. Furthermore there is an apparent emergence of pathogens due to increased medical knowledge, recognition of a previously undetected infections or realisation that a disease is caused due to a special pathogen.

There are many reasons why a pathogen may become emergent and these reasons have been reported from the WHO and can be summarised into 4 categories: a) new environments; b) changes in human behaviour and vulnerability; c) new technologies; d) scientific advances; (WHO 2003). The phenomenon of emergence is a continuous process which involves ongoing recognition of organisms that can be pathogenic to humans. Considering the distribution of emerging pathogens according to the main group of micro-organisms to which they belong, Taylor et al. (2001) estimated that 11% of the emerging pathogens were protozoa, 6% were helminths in comparison to viruses or prions (44%), bacteria or rickettsia (30%), and fungi (9%). These percentages illustrate the significant contribution of parasites as emerging pathogens of concern to human health, and this may be also true for some unknown or potentially waterborne emerging protozoan parasites.

Detection of emerging or re-emerging waterborne parasites relies upon appropriate investigations, reporting and assessment of information from the medical community, coupled with dissemination of the information to increase awareness of newly characterised potential parasites or re-emerging parasites to appropriate people in the medical, public health and water and food communities. Further understanding of such parasites would then be dependant upon their developmental biology, the application of suitable methods, conventional and molecular tools for their detection in clinical and environmental samples, genotyping and infectivity studies. Taking into consideration that there are a lot of discussions, recommendations, strategies and concepts and the reasons for emergence, it is necessary to be focused on a larger group of pathogens including viruses, bacteria, and parasites, which are currently of interest for suppliers. Knowledge of emerging waterborne parasites (biology and transmission routes, pathogenicity, dose-response, zoonotic transmission) is

needed in combination with a worldwide information exchange system evaluating the significance of waterborne parasites for the water industries.

HOW CAN WE IDENTIFY RELEVANT PARASITES IN DRINKING WATER?

Epidemiological investigations are a key activity that can lead to the identification of waterborne parasites, and establish relationship between outbreaks and waterborne parasitic diseases. The main purposes of such investigations are: a) documenting disease characteristics and distribution, for which a good system of surveillance is necessary; b) understanding life cycle and disease transmission; c) epidemiological approaches can be used in order to investigate associations between parasites and contaminated drinking water.

Well established disease surveillance systems have been very effective in detection and control of outbreaks of waterborne parasitic disease from both treated and untreated drinking water supplies as well as recreational waters. Several examples from the UK, USA, Germany, Japan, and few other countries have been already reported (Craun et al., 2005, Jakubowski and Craun, 2002, Karanis et al., 1996, 1998, Olson et al., 2002; Nichols et al., 2003; Ono et al., 2001, Yamamoto et al., 1996). These studies are valuable that they resulted in an improved understanding of the protection of the water sources and protection of water supplies from contamination, improvements of water treatment technologies, and initiation to develop strategies to prevent further outbreaks.

HOW TO ADDRESS THE PATHOGEN PROBLEM?

The WHO Water Safety Plans (WSPs) will be the most appropriate way forward in managing drinking water treatment and distribution and understanding of the environmental occurrence, survival in the nature and removal by the water treatment process of emerging protozoan (WHO, 1991, 2003). This, however, is strongly associated and depends upon techniques that allow the detection of these protozoans in source water. Analytical methods for assaying protozoan parasites in water are being developed and molecular diagnostic tools became a part of the detection techniques (Appelbee et al., 2005; Caccio et al., 2005, Monis et al., 2005). This creates new opportunities: more specific and rapid detection of protozoan parasites, direct detection and identification of contamination sources. Emerging technologies for the detection and genetic characterization of waterborne protozoan parasites provides a large research horizon for analytical tools, which will be very useful for clinical, environmental and biosecurity settings.

Such research work has been developed in the USA, UK, in part in some European countries, and in Australia. Although water treatment technologies are effective to remove microorganisms in water during the treatment process, current data on the prevalence of *Giardia* and *Cryptosporidium* in water clearly show that these parasites evade the filter barriers in the absence of treatment deficiencies and contaminate drinking water (Karanis et al. 1996, 1998). It remains to estimate the same risk for the other waterborne or potentially waterborne parasites. In general, protozoan parasites are resistant to chlorine and chlorine dioxide at the dosage levels that can be used for disinfection of drinking water. Disinfection by UV-light became more reliable for parasites and water utilities, worldwide, because UV could be used as an additional disinfection step as has been shown for *Giardia* and *Cryptosporidium* (Karanis et al., 1992, Wallis and Campbell 2002). UV-irradiation (7 – 25 mJ/cm^2) inactivates *C. parvum* (Rochelle et al., 2004; Rochelle et al., 2005) and *G. duodenalis* [100 – 400 mJ/cm^2 (Linden et al., 2002)], but also this alterna-

tive needs more detailed investigations in order to define the conditions in the practical water treatment process of large scale disinfection systems. Protection of the raw water from contamination including identification of the sources; protection of the watersheds as much as possible from human and animal wastes and avoiding re-introduction of cysts and oocysts e.g. by recycling of backwash water will be the best protection measures for the protection of water supplies. Using always the multiple barrier concept (filtration, disinfection) for production of drinking water, production of filtered water with low turbitidy of 0.1 Ntu and improving drinking water technologies comprise the only effective approach to treatment strategies.

MORE SPECIFIC INFORMATION ON THE PARASITES

Cryptosporidium/Giardia

Giardia lamblia and *Cryptosporidium parvum* account for the majority of outbreaks (Karanis et al., in press). *Cryptosporidium* and *Giardia* are waterborne pathogens that have caused many waterborne outbreaks, especially in systems without filtration or where the filtration was compromised. They are very resistant to chemical disinfection. The first priority for water utilities is to provide information on the occurrence or absence of the pathogens, as well as on practices to protect the water resources, and on treatment processes for effective (oo)cyst removal. United states EPA validated a method (Method 1623) for simultaneous detection of *Cryptosporidium* and *Giardia* and developed quality control acceptance criteria (Anonymous, 2001). The taxonomy of both protozoa is still developing with a large extent of the molecular typing methods. For the water industry, it is important to be able to genotype isolates that are found either in source or treated waters, for source tracking, for setting of treatment goals and for use in the investigation of outbreaks that are considered waterborne. Studies on the taxonomy of *Giardia* and *Cryptosporidium* will shed new light to the identification of isolates in nature that are able to infect humans and their potential for zoonotic transmission. Different genotypes have been linked to different symptomatology in sporadic human giardiasis and cryptosporidiosis cases, but more information is required regarding association between different risk factors and different genotypes, particularly for human adapted *Giardia* and *Cryptosporidium* genotypes and for zoonotic genotypes.

Toxoplasma and *Isospora*

Infection by *Toxoplasma gondii* is widely prevalent in humans and virtually any species of warm-blooded animals throughout the world. *T. gondii* is the causing agent of toxoplasmosis, one of the most common parasitic zoonoses, which is especially dangerous in congenital form and in people with immunologic deficiency. It is estimated that up to 30% of the human population, i.e. every third person in the world, has been exposed to *T. gondii* (Jackson and Hutchison, 1989; Wong and Remington, 1993). However, the frequency of infection varies considerably among different countries, different geographical areas within one country and different ethnic groups living in the same area (Tenter et al., 2000). Postnatal transmission may be more important in the epidemiology of human toxoplasmosis and is believed to occur via two major routes: a) ingestion of tissue cysts by consumption of raw or undercooked infected meat, and b) ingestion of sporulated oocysts by consumption of contaminated food or water, or after handling contaminated soil and infected cat feces.

T. gondii oocysts exhibit remarkable resistance to environmental influences and sporulated oocysts could keep infectivity for long time period. *T. gondii* oocysts may be introduced into the envi-

ronment via infected domestic cats or wild felines. Further spread may occur by wind, rain, water, manure, or even by invertebrate hosts such as earthworms and arthropods. They may subsequently contaminate surface water, soil, harvested feeds, fruits and vegetables. Although *Toxoplasma* oocysts have been recovered from contaminated soil, they have never been recovered from water due to the lack of detection techniques. In the last years few papers have been published on the development of strategy for the detection of oocysts in water (Dumetre and Darde, 2003; Kourenti et al., 2003; Kourenti and Karanis, 2004; Villena et al., 2004). Six acute toxoplasmosis outbreaks in humans have been correlated so far with ingestion of oocysts via contaminated soil or water (Karanis et al., in press). The best-documented outbreak occurred in British Columbia (Canada) in 1994 and affected approximately 8.000 people. It was the first toxoplasmosis outbreak to be linked to municipal drinking water. Fecal contamination of the unfiltered water supplies by infected animals (cougars, domestic and feral cats) was suspected. Field investigations for the detection of the parasite in the implicated reservoir 12 weeks after the last human infection were unsuccessful (Isaac-Renton et al., 1998). Two more waterborne toxoplasmosis outbreaks in humans were associated with untreated stream water and unfiltered ground water, respectively. The outbreak in a dairy farm in Brazil in 1982 was the only case so far where successful recovery of infectious oocysts from the implicated matrix (soil) was documented (Coutinho et al., 1982). However, because only cats shed oocysts that survive in water, concentrations of oocysts in surface water could be probably generally low. It is expected that coagulation and filtration processes will remove *Toxoplasma* in a similar manner as *Cryptosporidium* and *Giardia* but nothing is known about the removal of *Toxoplasma* oocysts by the water treatment process.

Neospora

   Neospora caninum was first recognized as a cause of neonatal paralysis in dogs and has been found to be the major cause of bovine abortions. It is similar in the structure to *T. gondii* and according to Lindsay et al. (1999) and McAllister et al. (1998) dogs are the definitive hosts of *N. caninum*, but they are one of the most important causes of abortion in cows (Haddad et al., 2005). Nothing is known about the water transmission of *Neospora* and there is a need for data on its occurrence in water.

Cyclospora

   Cases of cyclosporiasis have been recognised in the late 1970s after Ashford (1979) described the parasite in an infected man in Papua New Guinea. Ortega et al. (1993; 1994) classified the parasite and several foodborne outbreaks have been described in America (Curry and Smith, 1998; Mansfield and Gajadhar, 2004, Mota et al., 2000, Shields and Olson, 2003) and also one in Europe. The outbreak in Germany was associated with mixed lettuce imported from Italy (Doeller et al., 2002). Except this outbreak very little information is available on the occurrence of *Cyclospora* in water in Europe. Although this is a newly recognised pathogen, the number of cases is limited and information from surveillance does not suggest a strong increase of prevalence in European countries. This is partly due to the lack of appropriate detection methods. Most food (fresh produce) outbreaks of *Cyclospora* have been observed in the US and water has been identified as a source in particular in South America and Nepal (Soave 1996, Shields and Olson, 2003). In countries like Haiti, Peru, Nepal, *C. cayetanensis* is endemic. A high positive rate has been reported in children in Nepal (Kimura et al., in press, Mota et al. 2000). Our knowledge about *Cyclospora* widely increased in the last years but the epidemiology of this parasite especially the role of waterborne transmission

and the role water of plays in the contamination of fresh products. Only one study confirmed identification of *C. cayetanesis* in source water used for consumption (Dowd et al., 2003). Detection methods are based on those for other parasitic protozoans like *Giardia* and *Cryptosporidium* but information on occurrence in the aquatic environment and removal/inactivation by water treatment processes doesn't exist.

Entamoeba, Acanthamoeba, Balamuthia, Naegleria

Entamoeba histolytica has been the aetiological agent in six (1.8%) outbreaks. *Acanthamoeba* and *Naegleria fowleri* were responsible for one outbreak, each (0.3%) (Karanis et al., in press). *Entamoeba histolytica* and *dispar* is a typical parasite on tropical countries and often associated with drinking water but less likely to be an issue for Europe. Normal water treatment practice is able to remove the large cysts. Only in the case of gross contamination with sewage transmission through water may occur.

Acanthamoeba culbertsonii and *Balamuthia mandrillaris* can cause rare but fatal granulomatous amoebic encephalitis, but this is not considered as water–related. Keratitis by *Acanthamoeba polyphaga* and *A. castellanii* amongst contact lens wearers is water-related. The risk factors are poor hygienic practices with contact lenses and control should be aimed at improved information and hygiene. There is increasing interest in the potential role of free-living amoebae as reservoirs and vectors of pathogenic bacteria. The best example of such pathogenic bacteria is *Legionella*, which cause pneumonia. An indirect health issue of *Acanthamoeba* and other free-living protozoa is that they live in distribution networks and they may act as protective coat against disinfection and transmission vehicle and passage may even increase the virulence of the endosymbiontic pathogens. *Acanthamoeba* keratitis is an emerging com-

plication of orthokeratology in young myopes. There have been an increasing number of reports of microbial keratitis in association with overnight wear of orthokeratology lenses. Most cases of microbial keratitis were reported from East Asia (80%) and most affected patients were Asian (88%) and the peak age ranged was from 9 to 15 years (61%) (Watt and Swarbrick, 2005).

Naegleria fowleri causes rare but fatal primary amoebic meningoencephalitis. Most cases have occurred through exposure to warm surface water, although in long overground pipes in hot climate, *N. fowleri* may occur in drinking water. The rare but fatal occurrence of the infections of the nervous system, it is important to ascertain the availability of expertise on these amoebas. This should include clinical aspects and the water-related aspects (detection methods, treatment efficiency); (Schuster and Visvesvara, 2004a, b; Watt and Swarbrick, 2005).

Microsporidia, Isospora, Balantidium, Blastocystis.

Microsporidia have been responsible for one outbreak (0.3%), *Isospora belli* has been responsible for three outbreaks (0.9%), *Balantidium coli* for one outbreak *(0.3%), Blastocystis hominis* for two outbreaks (0.6%) (Karanis et al., in press). Of these, only Microsporidia have been included in the Contaminant Candidate List of the US EPA. Only one study confirmed identification of *Encephalitozoon intestinalis* in source water used for consumption (Dowd et al., 2003). Of the others, transmission through water is considered not impossible but unlikely (Mota et al., 2000). Microsporidia are opportunistic pathogens that cause infections in severely immunocompromised persons (Sheikh et al., 2000). There are suggestions but not proof that these organisms may be transmitted through water. The number of microsporidia infections in humans has increased rapidly in the last years due to the accurate diagnosis and

microsporidiosis has been considered as one of the most common opportunistic infections in AIDS patients (Mota et al., 2000, Weiss and Keohane, 1997; Vavra et al., 1998). They are transmitted through the fecal-oral route and produce life stages that are environmentally robust and small, hence more difficult to remove by conventional water treatment. These parasites are not high on the priority list of emerging pathogens and of course the question is how much the water route is involved in the transmission of these parasites and needs to be investigated.

DIAGNOSIS AND BIOLOGICAL/MOLECULAR ASPECTS

Microscopy is the gold standard for detecting protozoan parasites. Conventional diagnosis will be always important for protozoan parasites and it needs to remain a strong part in parasitology teaching and research. In the last 15 years, progress has been made in developing and validating non-morphologically based diagnostic tests for some referred parasite's antigen (immunofluorescence microscopy, enzyme linked immunosorbent assay, and parasite DNA) which can offer increased sensitivity. Sensitivity of detection is influenced by the method(s) used. To overcome any problems for an accurate diagnosis a strong protocol for each one organism has to be developed, modified and adapted to the sampling conditions. Molecular tools for specific diagnosis of the parasites remain too costly for diagnostic laboratory use, based on the requirement to screen samples for a variety of pathogens.

Human-derived *Giardia* are often assigned to a separate species (*G. lamblia*) but there is no definitive evidence that they differ genetically from organisms of the "duodenalis" type isolated from various animals. Isolates from humans are in fact very heterogeneous genetically and they can be divided into two major genetic assemblages on the basis of enzyme electrophoresis data and analysis of DNA amplified from structural genes.

The extreme genetic diversity represented by the human-derived isolates composing genetic assemblages A and B raises two important questions. Firstly, what is the relationship between other *Giardia* of the "duodenalis" type (in particular, those from animals that share the human environment) and those from the two genetic assemblages discerned among isolates from humans? Secondly, are any "duodenalis" type organisms capable of zoonotic transmission between animals of different species or between animals and humans? Some animal-derived *Giardia* are similar to some isolates from humans but others, whilst clearly of the "duodenalis" type, possess genotypes not yet observed in humans. Infectivity studies utilising *Giardia* isolated from both humans and animals have shown that zoonotic transmission is possible (Thompson and Monis, 2004; Karanis and Ey 1998, Traub et al., 2004, Thompson et al., 2005). In many situations, giardiasis is clearly transmitted between humans by the faecal-oral route, either directly or by contamination of water by human sewage. However, some epidemics in North America have been linked circumstantially to contamination of water with cysts excreted by animals such as beavers and muskrats, which have reported carriage rates of 7-16% and > 95% respectively. Other animal species, including agricultural livestock, are potential contaminators of surface waters. Mean *Giardia* spp. carriage rates of 30% have been reported for 5 livestock species from diverse geographic localities (Thompson et al., 1994, 2005, Olson et al., 2002).

The occurrence of numerous foodborne and waterborne outbreaks of cryptosporidiosis worldwide has led to a need for the detection of *Cryptosporidium* isolates from various hosts and contaminating sources. Current research is now emphasizing on the detection of various *Cryptosporidium*

isolates, as well as on the identification of genetical and phenotypic differences in *C. parvum* and other species in this genus (Gobet et al., 2001; Hunter and Thompson 2005; Sulaiman et al., 2000; Peng et al., 1997; Patel et al., 1999; Thompson et al., 2005, Widmer et al., 1998; Xiao et al., 1999). Although current knowledge has certainly increased our understanding about *Cryptosporidium* heterogeneity patterns, however, this knowledge is far from the reality of the systematics of this genus. The determination of the biological properties of different strains worldwide in association of phenotypic characterization to genomic identification of these strains level constitutes a big challenge for the future. Potentially contaminated material has to be sampled and examined for (oo)cyst presence and evaluate viability and infectivity of *Cryptosporidium* oocysts from animal or environmental sources. It is important to identify potential pathogen pathways from humans and domestic animals to wildlife populations. To achieve this goal, a great variety of sampling will include faecal material from: a) animals living near large surface water supplies, b) livestock, and c) domestic animals. In addition, sampling should include water from: a) surface supplies (lakes, rivers, springs), b) underground supplies (wells), c) distribution systems (drinking water), and d) recreational areas (swimming pools). Examination of the collected material will be processed using molecular (PCR) methods. Especially to target small numbers of (oo)cysts molecular based methods are the only way for assessing occurrence of the parasites. Methods for evaluating viability, infectivity and genotyping of *Cyclospora*, *Toxoplasma*, *Microsporidia* from environmental sources are not available. Additional bioassay experiments should be set up, in order to assess the transmissibility, virulence or dose-effect relationships of *Cryptosporidium* isolates in animal hosts with diverse genetic background (Karanis and Schoenen 2001, Matsubayashi et al., 2005). *Cryptosporidium* oocysts of

different origin, strain and dose will be orally transmitted to groups of immunocompetent and immunosupressed animals. An *in vivo* SCID mouse infectivity assay has been developed to determine the capacity to detect the infectivity of low concentrations of *C. parvum* oocysts in water. This biological test can be applied to demonstrate oocysts infectivity in water samples derived from drinking water supply and/or other environmental sources. The SCID mouse model allows detecting the presence in an inoculum of a single infectious *Cryptosporidium* oocyst (Hikosaka et al., 2005). The result confirms the assumption that a single viable oocyst is sufficient to induce an infection in a suitable host. The sensitivity of this model in determining the oocyst viability and infectivity is comparable to that of the *in vivo* neonatal mouse infectivity model so that both animal models are possible to apply. The SCID mouse model is considerably more suitable for determining the infectivity, pathogenicity and virulence of *C. parvum*, because this model is sensitive enough for monitoring the presence of the lowest levels of infectious *Cryptosporidium* oocysts in the environmental samples. As compared to the immunocompetent mice, the dosages required to induce infection in the SCID mice by oocysts from a variety of sources are considerably lower. Cell culture system has been proposed as an alternative to the animal models. Cell culture systems have been developed for various purposes on *Cryptosporidium* research in particular for pharmaceutical research. Sporozoites of *C. parvum* have been developed into mature meronts after inoculation at human rectal tumor cells, and sporozoites have been developed to oocysts in human embryonic, porcine kidney and primary kidney cells. Advances in *C. parvum* cultivation systems were sparse and they did little to enhance parasite development. Although development of the parasite is possible in monolayers of some epithelial cells, different cells lines showed different results. In some cell lines

only the asexual part of the life cycle of *Crypto-sporidium* has been developed. Full development of the parasite and the production of oocysts were missing. The *in vitro* cultivation of *Cryptosporid-ium* in host cell free system may allow the production of pure parasite material for future vaccine development. For many years the cell free medium culture has been avoided to search, most probably because of the extraordinary and unique position (intracellular but extracytoplasmic position) of *Cryptosporidium* in the host cells particular in the intestine invading the microvillus membrane of the intestinal cells. Unlike other intestinal parasite like *G. lamblia*, *Cryptosporidium* has no specific organelles to attached his hosts epithelial cells and cause diseases. On the other site *Cryptosporidium* does not penetrate to cells like other parasites (i.e. *Leishmania* or *Toxoplasma*) which are able to penetrate and multiply inside the cells using host-specific molecules involved in excystation, motility and host cell invasion (Smith et al., 2005). A recent study (Hijjawi et al., 2004) described the complete in vitro development of *Cryptosporidium* (cattle genotype) in cell free medium. Principally it has been shown that *Cryptosporidium* has the ability to develop in cell free medium devoid of host cells and this method is further developmental (Karanis, unpublished observations). The axenic culturing system will be of great value for drug evaluation studies and routine water and environmental water monitoring for the contamination with *Cryptosporidium*. *In vitro* culture system in combination with *in vivo* studies on histopathology will contribute to explain several questions on developmental stages of *Cryptosporidium* life cycle that remains unclear in several aspects with an exceptional extra - cytoplasmatic development of the parasite in the enterocytes, and it will enhances diagnostic possibilities in particular for clinical samples and molecular diagnostic tools on *Cryptosporidium*. It has been pointed out that the animal mouse model should be the golden standard for the evaluation of the infectivity of *Cryptosporidium* oocysts and the SCID mice model can probably deal also with the high degree of parasite biodiversity. Despite *in vitro* axenic cultivation for *Giardia* is possible since 1979 and contributed to the better diagnosis of the parasite, research and data of *Cryptosporidium* axenic culture system is very limited.

CONCLUSIONS

a) *Giardia* and *Cryptosporidium* are well recognized waterborne parasites, caused a number of outbreaks worldwide; a variety of studies have shown that both *Giardia* and *Cryptosporidium* are widely dispersed in environmental waters which are used for drinking sources; a number of studies have shown that both *Giardia* and *Cryptosporidium* are also dispersed in tap water but also in bottled water.

b) *Cyclospora cayetanensis* has been also recognized as a waterborne parasite, not readily detected by methods currently used to assure the safety of drinking water supplies.

c) Water treatment research is being conducted to obtain information on other potential waterborne parasites as well, such as *Acanthamoeba*, *Balamuthia*, *Naegleria*, B*alantidium coli*, *Blastocystis hominis*, *Entamoeba histolytica*, *Isospora belli*, *Microsporidium*, and *Toxoplasma gondii*.

d) There are no firm data regarding the importance of *Neospora caninun*, a parasite closed to *T. gondii*, and the possible association of this parasite to water supplies. It is hypothesized, however, that water contaminated with fecal material containing *Neospora* oocysts might be a possible way of infection.

e) Appropriate monitoring data on environmental occurrence of waterborne parasites of concern

are needed, for which suitable methods of detection are required. Research on the use of molecular methods to detect waterborne parasites in the environment should encompass research into the efficacy of concentration and purification methods and into the relation with the infectivity.

f) There is a need for the compilation of all the available information in a suitable world wide data base.Training courses on medical important protozoa and the usefulness of molecular methods for detecting them should be encouraged for primary health and day care workers and diagnostic laboratory staff.

g) More effective diagnoses carried out in clinics and laboratories globally will enhance the targeting of treatment and lead to a reduction in morbidity. There is an urgent need to standardize the concentration and enumeration methods for these parasites used throughout laboratories in different countries, to give some perspectives to water industries for the significance of the parasites and real reduction of protozoan parasites in practical water treatment process.

h) In addition, providing accurate statistics are collected, the view of the importance of intestinal protozoal diseases held by governments, national and international organisations and advocacy groups will be more realistic, and lead to correct targeting of aid and research funds. It is evident that the prevention and control of intestinal protozoan infections is now more feasible than ever before.

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REFERENCES

Anonymous (2001) Method 1623, *Cryptosporidium* in water by filtration / IMS / FA. United States Environmental Protection Agency, Office of Water. *Fed. Register* 63, 160

Appelbee, A.J., Thompson, R.C. and Olson, M.E. (2005) *Giardia* and *Cryptosporidium* in mammalian wildlife--current status and future needs. Trends Parasitol., 21, 370-376.

Ashford, R.W. (1979) Occurrence of an undescribed coccidian in man in Papua New Guinea. Ann. Trop. Med. Parasitol. 73, 4997-4500.

Barwick, R.S., Levy, D.A., Craun, G.F., Beach, M.J. and Calderon, R.L. (2000) Surveillance for waterborne-disease outbreaks-United States, 1997-1998. MMWR 49, 1-21.

Caccio, S.M., Thompson, R.C., McLauchlin, J. and Smith, HV. (2005) Unravelling *Cryptosporidium* and *Giardia* epidemiology. Trends Parasitol. 21, 430-437.

Coutinho, S.G., Lobo, R. and Dutra, G. (1982) Isolation of *Toxoplasma* from the soil during an outbreak of toxoplasmosis in a rural area in Brazil. J. Parasitol. 68, 866-868.

Craun, G.F., Calderon, R.L. and Craun, M.F.

(2005) Outbreaks associated with recreational water in the United States. Int. J. Environ. Health Res. 15, 243-262.

Curry, A, and Smith, H.V. (1998) Emerging pathogens: *Isospora*, *Cyclospora* and microsporidia. Parasitol. 117, 143-159.

Döller, P.C., Dietrich, K., Filipp, N., Brockmann, S., Dreweck, C., Vonthein, R., Wagner-Wiening, C. and Wiedenmann, A. (2002) Cyclosporiasis Outbreak in Germany Associated with the Consumption of Salad. Emerg. Infect. Dis. 8, 992-994.

Dowd, S.E., John, D., Eliopolus, J., Gerba, C.P., Naranjo, J., Klein, R., Lopez, B., de Mejia, M., Mendoza, C.E. and Pepper, I.L. (2003) Confirmed detection of *Cyclospora cayetanesis*, *Encephalitozoon intestinalis* and *Cryptosporidium parvum* in water used for drinking. J. Water Health 1, 117-23.

Dumetre, A. and Darde, M.L. (2003) How to detect *Toxoplasma gondii* oocysts in environmental samples? FEMS Microbiol. Rev. 27, 651-661.

Gobet, P. and Toze, S. (2001) Sensitive genotyping of *Cryptosporidium* parvum by PCR-RFLP analysis of the 70-kilodalton heat shock protein (HSP70) gene. FEMS Microbiol. Lett. 200, 37-41.

Haddad, J.P., Dohoo, I.R. and VanLeewen, J.A. (2005) A review of *Neospora caninum* in dairy and beef cattle--a Canadian perspective. Can. Vet. J. 46, 230-243.

Hijjawi, N.S. Meloni, B.P., Ng'anzo, M.., Ryan, U.M., Olson, M.E., Cox, P.T., Monis, P.T. and Thompson, R.C. (2004) Complete development of *Cryptosporidium parvum* in host cell free culture. Int. J. Parasitol. 34, 769-777.

Hikosaka, K, Satoh, M., Koyama, Y. and Nakai, Y. (2005) Quantification of the infectivity of *Cryptosporidium parvum* by monitoring the oocyst discharge from SCID mice. Vet. Parasitol. 134, 173-176.

Hunter, P.R. and Thompson, R.C. (2005) The zoonotic transmission of *Giardia* and *Cryptosporidium*. Int. J. Parasitol. 35, 1181-1190.

Isaac-Renton, J., Bowie, W.R., King, A., Irwin, G.S., Ong, C.S., Fung, C.P., Shokeir, M.O. and Dubey, J.P. (1998) Detection of *Toxoplasma gondii* oocysts in drinking water. Appl. Environ. Microbiol. 64, 2278-2280.

Jackson, M.H. and Hutchison, WM. (1989) The prevalence and source of *Toxoplasma* infection in the environment. Adv. Parasitol. 28, 55-105.

Jakubowski, W. and Craun, F.C. (2002) Update on the Control of *Giardia* and water Supplies. *In: Giardia. The Cosmopolitan Parasite.* Olson, B., Olson, M., Walllis P. (ed.). CABI Publishing, CAB International Wallingford, Oxon OX108 DE UK, pp. 239-247.

Karanis, P., Maier, W.A., Seitz, H. M. and Schoenen, D. (1992) UV sensitivity of protozoan parasites. J. Water SRT-Aqua 41, 95-100.

Karanis, P., Schoenen, D. and Seitz, H.M. (1996) *Giardia* and *Cryptosporidium* in backwash water from rapid sand filters used for drinking water production. Zentbl. Bakteriol. 284, 107–114.

Karanis, P., and Ey, P. (1998) Characterization of axenic isolates of *Giardia intestinalis* established from humans and animals in Germany. Parasitol. Res. 84, 442-449.

Karanis, P. and Schoenen D. (2001) Biological test for the detection of low concentrations of infectious *Cryptosporidium parvum* oocysts in water. Acta hydrochim. hydrobiol. 29, 242-245.

Karanis, P., Schoenen, D. and Seitz, H.M. (1998) Distribution and removal of *Giardia* and *Cryptosporidium* in water supplies in Germany. Wat. Sci. Tech. 37, 9-18.

Karanis, P., Kourenti, C. and Smith, H.V. Waterborne transmission of protozoan parasites: A worldwide review of outbreaks and lessons

learnt. Water and Health, (in press).

Kimura, K., Rai, K.S., Rai, G., Insisiengmay, S., Kawabata, M., Karanis, P. and Uga, S. (2006) Study on *Cyclospora cayetanensis* associated with diarrhoeal diseases Nepal and Lao PDR. Southeast Asian J. Trop. Med. Public Health, (in press).

Kourenti, C. and Karanis, P. (2004) Development of a sensitive polymerase chain reaction method for the detection of *Toxoplasma gondii* in water. Water Sci. Technol., 50, 287-291.

Kourenti, C., Heckeroth, A., Tenter, A. and Karanis, P. (2003) Development and application of different methods for the detection of *Toxoplasma gondii* in water. Appl. Environ. Microbiol. 9, 102-106.

Kramer, M.H., Quade, G., Hartemann, P. and Exner, M. (2001) Waterborne diseases in Europe-1986-96. J. Am. Water Works Assoc. 93, 48-53.

Lee, S.H., Levy, D.A., Craun, G.F., Beach, M.J. and Calderon, R.L. (2002) Surveillance for waterborne-disease outbreaks-United States, 1999-2000. MMWR Surveill. Summ. 51,1-47.

Linden, K., Shin, G.A., Faubert, G., Cairns, W. and Sobsey, M.D. (2002) UV disinfection of *Giardia lamblia* cysts in water. Environ. Sci. Technol. 36, 2519-2522.

Lindsay, D.S., Dubey, J.P. and Duncan, R.B. (1999). Confirmation that the dog is a definitive host for *Neospora caninum*. Vet. Parasitol. 82, 327-333.

Mansfield, L.S. and Gajadhar, A.A. (2004) *Cyclospora cayetanensis*, a food- and waterborne coccidian parasite. Vet. Parasitol. 9, 73-90.

McAllister, M.M., Dubey, J.P., Lindsay, D.S., Jolley, W.R., Willis, R.A. and McGuire, A.M. (1998) Dogs are the definitive hosts of *Neospora caninum*. Intern. J. Parasitol. 28, 1473-1478.

Matsubayashi, M., Kimata, I., Iseki, M., Hajiri, T., Tani, H., Sasai, K. and Baba, E. (2005) Infec-

tivity of a novel type of *Cryptosporidium andersoni* to laboratory mice. Vet Parasitol. 129, 165-168.

Monis, P.T., Giglio, S., Keegan, A.R. and Andrew, Thompson, R.C. (2005) Emerging technologies for the detection and genetic characterization of protozoan parasites. Trends Parasitol. 21, 340-346.

Mota, P., Rauch, C.A. and Edberg, S.C. (2000) Microsporidia and *Cyclospora*: epidemiology and assessment of risk from the environment. Crit. Rev. Microbiol. 26, 69-90.

Nichols, R.A.B., Campbell, B.M. and Smith, H.V. (2003) Identification of *Cryptosporidium* spp. oocysts in United Kingdom noncarbonated natural mineral waters and drinking waters by using a modified nested PCR-Restriction Fragment Length Polymorphism Assay. Appl. Environ. Microbiol. 69, 4183-4189.

Ono, K., Tsuji, H. Rai, H.K., Yamamoto, A., Masuda, K., Endo, T., Hotta, H., Kawamura, T. and Uga, S. (2001) Contamination of river water by *Cryptosporidium parvum* oocysts in western Japan. Appl. Environ. Microbiol. 67, 3832-3836.

Ortega, Y.R., Sterling, C.R., Gilman, R.H., Cama, V.A. and Diaz, F. (1993) *Cyclospora* species-a new protozoan pathogen of humans. N. Engl. J. Med. 328, 1308-12.

Ortega, Y.R., Gilman, R.H. and Sterling, C.R. (1994) A new coccidian parasite (Apicomplexa: Eimeriidae) from humans. J. Parasitol. 80, 625-629.

Olson, B.E., Olson M.E. and Wallis, P.M. (2002) *Giardia* the cosmopolitan parasite. CABI Publishing CAB International Wallington Oxon OX 10 8DE UK.

Patel, S., Pedraza-Diaz, S. and McLauchlin, J. (1999) The identification of *Cryptosporidium* species and *Cryptosporidium* parvum directly from whole faeces by analysis of a multiplex PCR of the 18S rRNA gene and by PCR/RFLP

of the *Cryptosporidium* outer wall protein (COWP) gene. Int. J. Parasitol. 29, 1241-1247.

Peng, M.M., Xiao, L., Freeman, A.R., Arrowood, M.J., Escalante, A.A., Weltman, A.C., Ong, C.S., Mac Kenzie, W.R., Lal, A.A. and Beard, C.B. (1997) Genetic polymorphism among *Cryptosporidium parvum* isolates: Evidence of two distinct human transmission cycles. Emerg. Infect. Dis. 3, 567-573.

Rochelle, P.A., Fallar, D., Marshall, M.M., Montelone, B.A., Upton, S.J. and Woods, K. (2004) Irreversible UV inactivation of *Cryptosporidium* spp. despite the presence of UV repair genes. J. Eukaryot. Microbiol., 51, 553-562.

Rochelle, P.A., Upton, S.J., Montelone, B.A. and Woods, K. (2005) The response of *Cryptosporidium* to UV light. Trends Parasitol., 21, 81-87.

Rose, J.B., Lisle, J.T. and LeChevallier, M. (1997) Waterborne cryptosporidiosis : incidence, outbreaks, and treatment strategies; *In: Cryptosporidium* and Cryptosporidiosis. Fayer, R. (ed.). CRC Press, Boca Raton New York London Tokyo, pp. 65-92.

Savioli, L. *Giardia* and *Cryptosporidium* join the "Neglected Diseases Initiative". Trends Parasitol., (in press).

Satoh, M., Kimata, I., Iseki, M. and Nakai, Y. (2005) Gene analysis of *Cryptosporidium parvum* HNJ-1 strain isolated in Japan. Parasitol. Res., 97, 452-457.

Schuster, F. and Visvesvara, G. S. (2004a) Free living amoebae as opportunistic and non-opportunistic pathogens of humans and animals. Int. J. Parasitology, 34, 1001-1027.

Schuster, F. and Visvesvara, G. S. (2004b) Opportunistic amoebae: challenges in prophylaxis and treatment. Drug Resistance Updates 7, 41-51.

Sheikh, R.A., Prindiville, T.P., Yenamandra, S., Munn, R.J. and Ruebner, B.H. (2000) Microsporidial AIDS cholangiopathy due to *Encephalitozoon intestinalis*: case report and review. Am. J. Gastroenterol. 95, 2364-2371.

Shields, J.M. and Olson, B.H. (2003) *Cyclospora cayetanensis*: a review of an emerging parasitic coccidian. Int. J. Parasitol. 33, 371-391.

Smith, H.V., Robertson, L.J. and Ongerth, J.E. (1995) Cryptosporidiosis and giardiasis: the impact of waterborne transmission. J. Water Supp. Res.Technol. - *Aqua* 44, 258-274.

Smith, H.V. (1999) Detection of parasites in the environment. In: Infectious diseases diagnosis: current status and future trends. Smith, H.V. and Stimson, W.H., co-ordinating, Chappel, L.H. (ed.) Parasitology 117, pp.113-141.

Smith, H.V. and Grimason, A.M. (2003) *Giardia* and *Cryptosporidium* in water and wastewater. In: The Handbook of Water and Wastewater Microbiology (Mara, D. and Horan, N., (eds.), Elsevier Science, pp. 619-781.

Smith H.V., Nichols, R.A. and Grimason A.M. (2005) *Cryptosporidium* excystation and invasion: getting to the guts of the matter. Trends Parasitol. 21, 33-42.

Soave, R. (1996) *Cyclospora*: an overview. Clin. Infect. Dis. 23, 429-435.

Sulaiman, I.M., Morgan, U.M., Thompson, A.R.C., Lal, A.A. and Xiao L. (2000) Phylogenetic relationships of *Cryptosporidium* parasites based on the 70-Kilodalton Heat Shock Protein (HSP70) Gene. Appl. Environ. Microbiol. 66, 2385-2391.

Taylor, L.H., Latham, SM. and Woolhouse, M.E. (2001) Risk factors for human disease emergence. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 356, 983-989.

Tenter, A.M., Heckeroth, A.R. and Weiss, L.M. (2000) *Toxoplasma gondii*: from animals to humans. Int. J. Parasitol., 30, 1217-1258. Erratum in: Int. J. Parasitol. 31, 217-20.

Thompson, R.C.A., Reynoldson, J.A. and Lymbery, A.J. (1994) *Giardia:* from molecules to diseases. CAB International Wallington Oxon

OX10 8DE UK.

Thompson, R.C. and Monis, P.T. (2004) Variation in *Giardia*: implications for taxonomy and epidemiology. Adv. Parasitol., 58, 69-137.

Thompson, R.C., Olson, M.E., Zhu, G., Enomoto, S., Abrahamsen, M.S. and Hijjawi, N.S. (2005) *Cryptosporidium* and cryptosporidiosis. Adv. Parasitol. 59, 77-158.

Vavra, J., Yacnis, A.T., Shaddruck, J.A. and Orenstein, J.M. (1998) Microsporidia of the genus Trachipleistophora-causative agents of human microsporidiosis: description of *Trachipleistophora anthropophthera* n.sp. (Protozoa: Microsporidia). J. Eukaryot. Microbiol. 45, 273-283.

Traub, R.J., Monis, P.T., Robertson, I., Irwin, P., Mencke, N. and Thompson, R.C. (2004) Epidemiological and molecular evidence supports the zoonotic transmission of *Giardia* among humans and dogs living in the same community. Parasitology 128, 253-262.

Villena, I., Aubert, D., Gomis, P., Ferte, H., Inglard, J.C., Denis-Bisiaux, H., Dondon, J.M., Pisano, E., Ortis, N. and Pinon, J.M. (2004) Evaluation of a strategy for *Toxoplasma gondii* oocyst detection in water. Appl. Environ. Microbiol. 70, 4035-4039.

Wallis, P.M. and Cambell, A.T. (2002) Rethinking disinfection of *Giardia* cysts with Ultraviolet Light: old light through a new window. *In*: *Giardia.* The Cosmopolitan Parasite. Olson, B., Olson, M., Wallis, P. (ed.). CABI Publishing, CAB International Wallingford, Oxon OX108 DE UK, pp. 239-247.

Watt, K. and Swarbrick, H.A. (2005) Microbial keratitis in overnight orthokeratology: review of the first 50 cases. Eye Contact Lens. 31, 201-208.

Weiss, L.M. and Keohane, E.M. (1977) The uncommon gastrointestinal Protozoa: Microsporidia, *Blastocystis*, *Isospora*, *Dientamoeba*, and *Balantidium*. Curr. Clin. Top. Infect. Dis., 17, 1471-87.

WHO (1991) WHO/PAHO informal consultation on intestinal protozoal infections.

WHO (2003) Emerging issues in water and infectious disease. ISBN 9241590823.

Widmer, G., Tchack, L., Chappell, C.L. and Tzipori S. (1998) Sequence polymorphism in the beta-tubulin gene reveals heterogeneous and variable population structures in *Cryptosporidium parvum*. Appl. Environ. Microbiol. 64, 4477-4481.

Wong, S.Y. and Remington, J.S. (1993) Biology of *Toxoplasma gondii*. AIDS 7, 299-316.

Xiao, L., Escalante, L., Yang, C., Sulaiman, I., Escalante, A.A., Montali, R.J., Fayer, R. and Lal, A.A. (1999) Phylogenetic analysis of *Cryptosporidium* parasites based on the Small-Subunit rRNA gene locus. Appl. Environ. Microbiol. 65, 1578-1583.

Yamamoto, N., Urabe, K., Takaoka, M., Nakazawa, K., Gotoh, A., Haga, M., Fuchigami, H., Kimata, I. and Iseki, M. (2000) Outbreak of cryptosporidiosis after contamination of the public water supply in Saitama Prefecture, Japan, in 1996. Kansenshogaku Zasshi 74, 518-526.