First investigations into the prevalence of *Cryptosporidium* and *Giardia* spp. in Hungarian drinking water

J. Plutzer, M. H. Takó, K. Márialigeti, A. Törökné and P. Karanis

ABSTRACT

Safe drinking water is a top priority in preventing disease outbreaks and is of general concern to everyone. This study examines the occurrence of *Cryptosporidium* and *Giardia* in Hungarian drinking water supplies for the first time. A total of 76 raw and drinking water samples were examined using the U.S. EPA Method 1623. From these 15 of 34 (48.4%) raw water samples tested positive for *Giardia* and 7 (26.6%) for *Cryptosporidium*. Twelve of 45 (26.7%) drinking water samples were positive for *Giardia* and 6 (13.3%) for *Cryptosporidium*. Overall, *Giardia* cysts and/or *Cryptosporidium* oocysts were detected in 48% of the raw water samples and 35% of the drinking water samples. The highest levels in drinking water were found to be 3 oocysts/100 litres of *Cryptosporidium* and 63.6 cysts/100 litres for *Giardia*, enough to cause giardiasis. The highest levels in raw water were 1,030 cysts/100 litres for *Giardia* and 50 oocysts/100 litres for *Cryptosporidium* and higher oocyst densities were associated with source water receiving effluents from sewage treatment plants or originating from a forest environment. In addition to this monitoring, riverbank filtrated water and raw water from the River Danube in Budapest were monitored in order to ascertain protozoan removal efficiency of riverbank filtration (RBF). A total of 157 samples, including 87 samples from the River Danube and 70 samples post RBF, were examined. *Cryptosporidium* and *Giardia* were detected regularly in the river water but never in riverbank filtered water suggesting the effectiveness of RBF as a purification method.

The occurrence of *Cryptosporidium* oocysts and *Giardia* cysts in the investigated water supplies may require the water utilities and water authorities in Hungary to apply additional monitoring and treatment and/or watershed controls.

Key words | *Cryptosporidium*, drinking water, *Giardia*, Hungary, water sources

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INTRODUCTION

The presence of *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts in water is an increasing problem throughout the world. *Giardia* causes an intestinal illness called giardiasis, leading to explosive, foul smelling watery diarrhoea while *Cryptosporidium* is responsible for a similar diarrhoeal disease called cryptosporidiosis. Several species of *Giardia* and *Cryptosporidium* exist including *G. lamblia*, *C. parvum* (genotype II) and *C. hominis* (formerly known as

anthroponotic genotype or genotype I) which comprise the most prevalent group of species causing disease in humans (Hunter & Thompson 2005) although *C. felis*, *C. meleagridis*, *C. canis*, and *C. muris*, *C. andersoni*, *C. suis* have also been reported to cause human disease (Jiang & Xiao 2003; Read *et al.* 2004; Leoni *et al.* 2006). Drinking water sources can become contaminated through the introduction of infected faeces from wild and/or farm animals or from sewage flushed

into surface waters (Erlandsen *et al.* 1990; Karanis *et al.* 1996*a*, *b*; Bednarska *et al.* 1998; Hunter & Thompson 2005). A number of outbreaks have been reported worldwide owing to a combination of chlorine resistance of the dormant parasite and inadequate water treatment leading to sufficient numbers of protozoa to cause illness (Karanis *et al.* 2007). However, it is unusual to find these parasites in boreholes or deep well water unless fissures are present, thereby allowing surface water into the source. In Hungary 95% of water supplies originate from groundwater (confined aquifers and karst water) with, riverbank filtration (RBF) and surface water treatment plants making up the remaining 5%. In our study, we investigated the public drinking water systems in order to gain information on the distribution of *Giardia* and *Cryptosporidium* oocysts and estimate the efficacy of applied water treatment techniques in Hungary.

MATERIALS AND METHODS

Sampling sites and sampling designs

The geographic location of Hungary and sampling sites are shown in Figure 1. From the year 2000 until 2005 suspected contaminated drinking water resources were examined on an irregular basis taking into consideration particular events such as heavy rains or/and dry seasons.

Springs

Three springs and three karsts wells were examined.

Karsts are a special type of landscape which is formed by dissolution of soluble rocks, including lime rocks and dolomite. Karsts regions contain aquifers that are capable of providing large supplies of water. These aquifers are very productive, but they are more susceptible to contamination, than those in other geologic media.

In all cases, the water is used without treatment or treatment by chlorination or in the case of one spring, the water is stored in an open pool. There is extensive animal husbandry in the vicinity of the karst wells and 2 springs. The sample code is 1, 17 in Table 1 and Figure 1.

Wells

Two groundwater wells were sampled several times, one of which showed bacteriological problems and was the site of
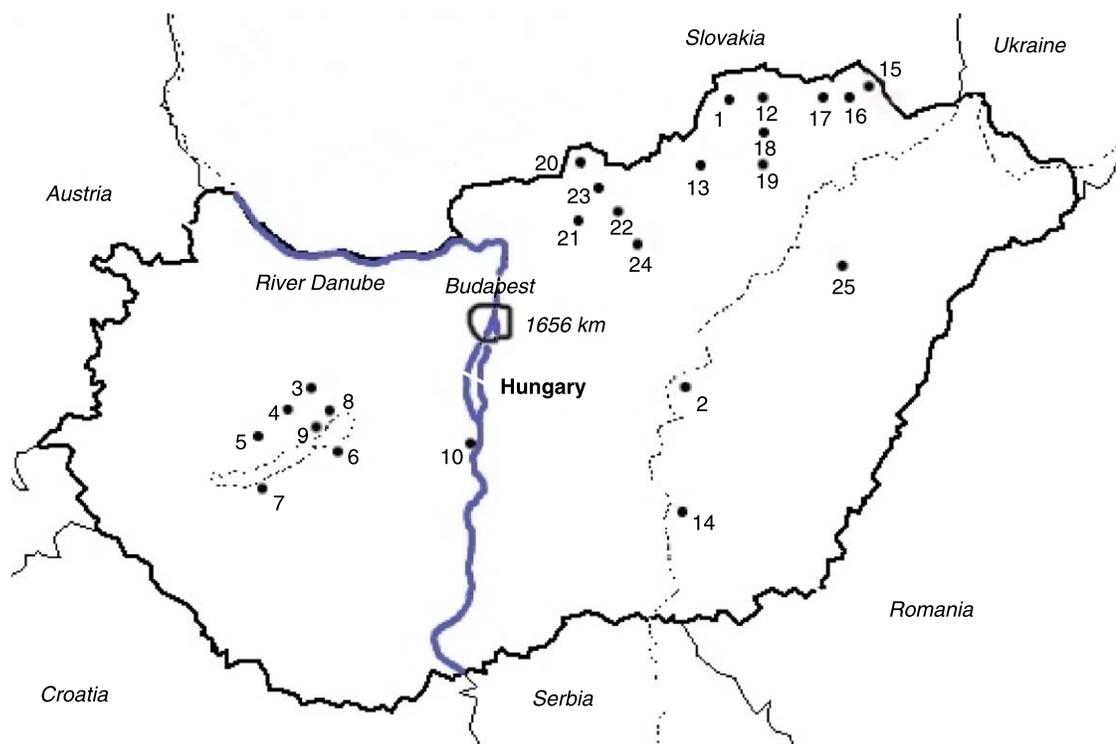

Figure 1 | The map of Hungary, the location and the codes of sampling sites.

Table 1 | *Giardia* and *Cryptosporidium* detected in Hungarian drinking water resources and in drinking water. Sample code, date of sampling, positive samples of the investigated drinking waters and the characteristics of the investigated raw water samples collected from water sources in Hungary during the investigation period 2000–2005

Sample code	r = raw water, d = drinking water	Date of sampling	*Giardia* cysts/100 litres	*Cryptosporidium* oocysts/100 litres	Water source/Water treatment characteristics
1	d	09/10/2000	2	0	Karst spring/chlorination
	d	13/11/2000	0	0	
2	r	22/03/2001	0.3	0	Surface water, 157, 000 km² watershed/flocculation,
	d	22/03/2001	0	0	filtration, ozonation, activated carbone, chlorination
	r	10/09/2001	6	0	
	d	10/09/2001	1.2	0	
	r	25/03/2002	60	5	
	d	25/03/2002	0	2.6	
	r	12/08/2002	0	0	
	d	12/08/2000	0.2	0	
8	r	04/11/2002	5	0	Surface water, 5,800 km² watershed/flocculation,
	d	04/11/2002	0	0.1	filtration, activated carbon and chlorination or chlorine dioxide
9	r	04/11/2002	0	0	Surface water, 5,800 km² watershed/flocculation,
	d	04/11/2002	0	0.1	filtration, activated carbon and chlorination or chlorine dioxide
10	r	10/06/2002	135	5	Surface water, 372,000 km² watershed/flocculation,
	d	10/06/2002	0.4	0	filtration, activated carbon and chlorination
	r	13/09/2005	300	40	
	d	13/09/2005	0	0	
13	r	21/06/2005	90	20	Surface water, 218 km² watershed/flocculation, filtration,
	d	21/06/2005	0	0	chlorination
16	d	28/06/2005	0	3	Surface water, water treatment is 112 m from high up of
	r	16/08/2005	40	0	brook/flocculation, filtration, activated carbon and
	d	16/08/2005	12.8	0	chlorination
17	d	28/06/2005	4	0	Spring/chlorination
	d	16/08/2005	3.5	0	
18	r	06/09/2005	20	0	Surface water, 1,730 km² watershed/flocculation,
	d	06/09/2005	0	0	filtration, activated carbon and chlorination
19	r	05/07/2005	1030	30	Surface water, 1,730 km² watershed/flocculation,
	d	05/07/2005	0	0	filtration, chlorination
	r	06/09/2005	60	20	
	d	06/09/2005	0	0	
20	r	12/07/2005	0	0	Surface water, 5.4 km² watershed/flocculation, filtration,
	d	12/07/2005	0.7	1.4	activated carbon and chlorine dioxide
22	r	26/07/2005	10	0	Surface water, 8.38 km² watershed/flocculation, filtration,
	d	26/07/2005	1	1	chlorination
	r	23/08/2005	10	0	
	d	23/08/2005	0	0	
24	r	09/08/2005	840	0	Surface water, 4 km² watershed, this brook collects the
	d	09/08/2005	27.1	0	water of springs, the water treatment is ~1 km from
	r	14/09/2005	850	0	high up of brook/Sedimentation, chlorination
	d	14/ 9/2005	63.6	0	
25	r	10/09/2001	8	0	Canal from River Tisza/flocculation, filtration,
	d	10/09/2001	0	0	ozonation, activated carbone, chlorination
	r	13/09/2005	0	50	
	d	13/09/2005	0	0	

a second giardiasis outbreak in Hungary. During this sampling period, numerous giardiasis cases were identified among local people, mainly among children. This outbreak has been highlighted in the public media but was never reported in a scientific journal. Since we could not detect any protozoa in these samples, these are not included in Table 1.

Tap and raw water from treatment plants

Hungary has a total of 16 treatment plants abstracting surface water from lakes and rivers for drinking water consumption. Source water near all 16 plants and finished water were tested a minimum of two times during the investigative period of 2000–2005, in different seasons or after rainfalls, when the water level of reservoirs and rivers were raised. Four surface water treatment plants are located around Lake Balaton, one is located on the River Danube, one on the River Tisza, one on the Eastern Main Canal, two on the River Bódva and one at Brook Nagy. There are six water reservoirs in Hungary. In general the main steps of the surface water treatment are flocculation by alum and iron salts and/or synthetic organic polymers (alone, or in combination), settling or sedimentation, before sand (or gravel) filtration and chlorination (with chlorine or chlorine dioxide). The maximum level of combined chlorine, chlorite and chlorine (Cl_2) never exceeded 3 mg/L, 0.2 mg/L and 0.5 mg/L in the final water. Chlorine dioxide can be added to a maximum of 0.4 mg/L final concentration.

Lake Balaton.   The ecological state of Lake Balaton was at its lowest in 1995, but due to environmental investments, sewage treatment, waste management reforms it is now in a good state. Its catchment area is 5,800 km² with the River Zala providing the largest source of water and the canalized Sio being the only outflow. The possibility of *Giardia* and *Cryptosporidium* contamination comes from small inflows, ducts, treated sewage, and from birds. The drinking water treatment contains additional activated carbon filtration. Sample code is 8, 9 in Table 1 and Figure 1.

River Danube.   This is the second largest river in Europe and the only major European River to flow from west to east.

It receives water from the Black Forest Mountains of Germany and empties after 2,850 km into the Black Sea on the Romanian cost. Along its way the Danube flows through 9 countries and drains an area slightly larger, than 817,000 km². It has 300 tributaries, the principal one being the River Tisza in Hungary. A small water treatment plant services the River Danube to produce water for only 8000 people. Authorized sewage outflows are located at the 1,576, 1,577, 1,577.2, 1,580.2 and 1,584 km points and water treatment is located at the 1,576 km point from the issue. The drinking water treatment contains additional activated carbon filtration. Sample code is 10 in Table 1 and Figure 1.

River Tisza.   This river is the second largest river in Hungary and the longest Danube tributary. It is approximately 966 km long with approximately 160 km laying in the Ukraine and Romania and about 800 km in the Great Hungarian Plain. It has a catchment area of 157,000 km² with 29.4% being in Hungary. Like the River Danube, it receives many authorized and sometimes illegal sewage outflows, although none are located near the water treatment plant. The drinking water treatment contains additional activated carbon filtration, ozonization. Sample code is 2 in Table 1 and Figure 1.

Eastern Main Canal.   The main function of this canal is agricultural, and it supplies water to fish ponds, year round irrigation-water to the plain and receives the inland water. It does not receive sewage, although the possibility of *Giardia* and *Cryptosporidium* contamination exists from animal husbandry. The drinking water treatment contains additional activated carbon filtration, ozonization. Sample code is 25 in Table 1 and Figure 1.

River Bódva.   Its catchment area is 1,730 km². The tributaries are polluted with sewage effluents and at 10.7 km from the issue there is an authorized sewage outflow. Water treatments are located at the 0.1 and 5.7 km points. If the raw water turbidity is high, the water treatment contains additional activated carbon filtration. If the raw water turbidity is low the water is pumped through sandpools into the groundwater prior to chlorination and delivery into the distribution system. Sample code is 18, 19 in Table 1 and Figure 1.

Brook Nagy. This brook flows through forests, where contamination may occur from wild animals. There is also a small water treatment plant at the 0.1 km point of the brook that produces water for only 1000 people. The drinking water treatment contains additional activated carbon filtration. The sample code is 16 in Table 1 and Figure 1.

Water Reservoirs. Reservoir Hasznos is established on the Kövicses Brook, Komravölgy on the Brook Ipoly (catchment area is $5.4 \, km^2$), Köszörűvölgy on the Brook Köszörű, Csórrét on the Brook Nagy (catchment area is $8.38 \, km^2$), Lázbérc on the Brook Bán and Csernely (catchment area is $218 \, km^2$) and Mátrafüred on the Brook Csatorna (catchment area is $4 \, km^2$, this brook receives water from 9 springs, which are $50-500 \, m$ from the brook). Only the Brooks Bán, Kövicses, Ipoly, and Nagy receive authorized sewage. In the case of Brook Bán, water treatment is at km 10.3, and sewage outflow is at km 26.3. This reservoir is surrounded by forests where the possibility of contamination exists by wild animals. Hasznos and Komravölgy have additional activated carbon filtration. Mátrafüred does not include flocculation or a filtration step in water treatment. Sample codes are indicated as 13, 20, 22, 24 in Table 1 and Figure 1.

Riverbank filtered water

The water supply of the city of Budapest and its suburbs originates from river bank filtration, and approximately 2 million people consume RBF water. In total 700 riverbank filtration wells are located $30-100 \, m$ from the River Danube, mainly on the Island Szentendrei and on the Island Csepel. On the Island Szentendrei, water flows thorough sand and gravel layers and is directed to the drinking water system after chlorination. On the Island Csepel water is filtered through muddy layers and after treatment (ozonization, sand and activated carbon filtration, removal of iron and manganase, and chlorination) is directed to the water distribution system. During 2004–2005, the River Danube in Budapest was investigated once per week at the 1,656 km point along with its riverbank filtered water (Figures 2 & 3). For sampling post riverbank filtration water, we selected four sampling points for the drinking water at the distribution system.

Sample collection

For primary concentration of 50–1,000 litres of drinking water, Filta-Max (IDEXX, Genera) foam filters were used (USEPA 2001). Water and pressure meters were connected to the sampling tap. During the filtration, the pressure was adjusted to 1–2 Bar, and the flow rate to 1–2 litres/minute. Raw water samples with high turbidity were collected into 4-20-L cans from sampling taps inside of the water treatment plants and the samples were transferred immediately to the laboratory in order to perform chemical flocculation for recovery of *Cryptosporidium* oocysts and *Giardia* cysts (Vesey *et al.* 1993). During flocculation, the formation of a calcium carbonate precipitate adsorbs and pulls water particulates and protozoa to the bottom of the vessel, giving a dense, stable form within 4 hours. After discarding the supernatant, the precipitate was dissolved in 10% sulphamic acid, giving a concentrate containing the oocysts.

In the case of riverbank filtration monitoring, all samples were concentrated using membrane filtration according to U. S. EPA 1623 method (USEPA 2001). We used 5–10 litres when samples originated from the River Danube and 400 litres of drinking water for post riverbank filtration samples. The pressure filtration equipment was connected to the tap of system pipes and filtration was achieved through 142 mm diameter, $3 \, \mu m$ pore size cellulose nitrate membrane filters (Millipore). For raw water sampling with higher turbidity multiple membrane filters were used for each sample before final filtered sediments were combined.

Cryptosporidium and *Giardia* concentration/separation

The purification of the parasites was carried out according to the standard method U.S. EPA 1623. Briefly, after Filta-Max filtration, the filter was decompressed and washed with phosphate buffered saline and sediment from the surface of membrane filters was washed with Envirocheck buffer solution (USEPA 2001). Oocysts were further concentrated by centrifugation, and separated from debris by Immuno magnetic separation (IMS) (Dynabeads GC-Combo kit, Dynal Biotech), according to the manufacturer's description. This method replaces flotation techniques
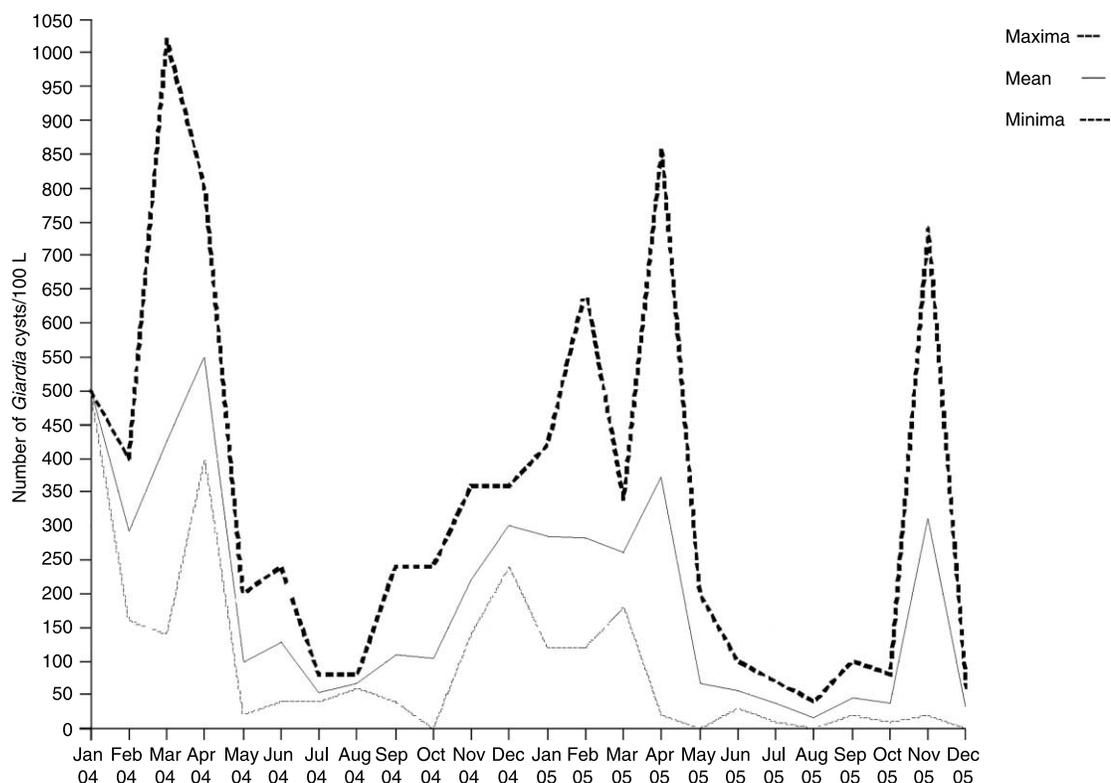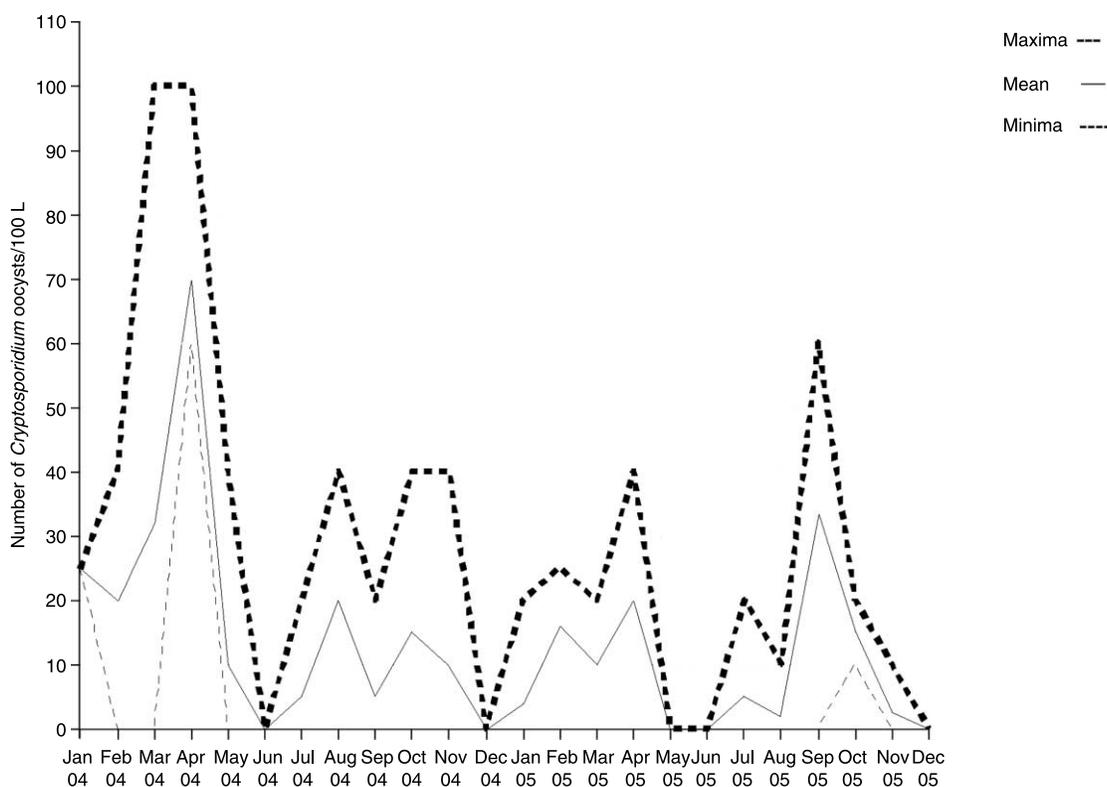
Figure 2 | *Giardia* oocysts count in River Danube at 1,656 km during the years 2004–2005.

used for separating oocysts from other debris. Briefly, anti-*Cryptosporidium* and anti-*Giardia* Dynabeads were incubated with the water sample concentrate along with the supplied buffer. The antibodies coated on the bead selectively bind oocysts. The bead-oocyst complexes are separated using a Dynal magnetic particle concentrator and subsequently the oocysts are dissociated from the beads. The resultant suspension for screening was clean and it had the small volume of 50 μl.

Microscopic examination

After IMS, separated oocysts were mounted onto slides, fixed with methanol and stained with fluorescently labelled monoclonal antibodies (FITC) (Waterborne, Inc, New Orleans, LA) and a nucleic acid stain [DAPI, 2-(4-amidi-nophenyl)-6-indolecarbamidine dihydrochloride, Fluka] as described (USEPA 2001). Slides were examined using epifluorescent microscopy (LEICA DM IRB) and FITC

positive structures were checked for the appropriate size and shape. Such presumptive organisms were further examined to detect the DAPI stained nuclei, and with DIC (Differential Interference Contrast) microscopy for the confirmation of characteristic surface and internal structures (USEPA 2001). DIC examination was carried out with the same microscope using Nomarski polarisation optics. All samples found positive by IFT were examined with DAPI and by DIC under 1,000 × magnification in order to confirm the presence of internal structures of oocysts. Identification was confirmed when those same oocysts exhibited the oocyst wall, and two or more internal morphological features, characteristic of *Giardia* cysts and *Cryptosporidium* oocysts. *Giardia* cysts were confirmed by observing the cyst wall, internal cytoplasm, peritrophic space and nuclei. *Cryptosporidium* oocysts were confirmed by the oocyst wall and the observed sporozoites, nuclei or densely packed cytoplasm. The structure of the oocyst surface was visible many times. Only samples in which

Figure 3 | *Cryptosporidium* oocysts counts in River Danube at 1,656 km during the years 2004–2005.

oocysts fulfilling defined and published morphological criteria, according to the USEPA protocol, were enumerated and samples deemed positive.

RESULTS

236 water samples within Hungary (31 raw water, 45 drinking water, 87 river water, and 70 post RBF samples) were collected and investigated for the presence of *Cryptosporidium* oocysts and *Giardia* cysts.

Spring water

In one spring, 2 *Giardia* cysts/100 L were found (sample code 1, Table 1 & Figure 1) while in another, 4 *Cryptosporidium* oocysts and 3.5 *Giardia* cysts/100 L were detected once. The drinking water in the last case was stored for a long time in an opened pool. The sample code is depicted as 17 in Table 1 and Figure 1.

Ground water

No protozoa were detected in any sampled groundwater and therefore are not included in Table 1, and the sample code is 14 in Figure 1.

Raw water

The raw water of 10 treatment plants was contaminated with both protozoa, ranging from 5 to 50 *Cryptosporidium* oocysts per 100 L and 0.3–1,030 *Giardia* cysts/100 L (samples 2, 8–10, 13, 16, 18–20, 22, 24–25, Table 1 & Figure 1).

Final (drinking) water

The final water of 8 water treatment plants was contaminated with *Cryptosporidium* and *Giardia* varying between 0.1–3 *Cryptosporidium* oocysts and 0.2–63.6 *Giardia* cysts/100 L (samples 2, 8–10, 13, 16, 18–20, 22, 24–25, Table 1 and & Figure 1).

Only the data with positive results is shown in Table 1.

Danube surface river water and River bank filtrate

No *Cryptosporidium* oocysts (0/100 L) were detected in approximately 60% of surface river water samples. In those samples found to be contaminated oocysts values varied between 0–50/100 L. *Giardia* was detected more frequently with cyst numbers ranging within 0–500/100 L in ~90% of river water samples, with 1% of the samples containing more than 1,000 cysts/100 L. The highest *Giardia* levels were in January to April in both 2004 and 2005 with a second peak in November 2005. During these time a record high of 1,020 *Giardia* cysts/100 L was found with mean numbers ranging between 260–550 cysts/100 L. The lowest *Giardia* levels were found to be in July and August each year with mean numbers ranging between 16–67 cysts/100 L. Similar to *Giardia*, *Cryptosporidium* peaked in March and April 2004, with the highest recorded number being 100 oocysts/100 L; the mean varied between 32–70 oocysts/100 L. The minimum and maximum counts for the detection of *Cryptosporidium* and *Giardia* in River Danube samples are shown in Figures 2 and 3 and are presented for each month in the investigated period of 2004 and 2005. Interestingly, no *Cryptosporidium* oocysts or *Giardia* cysts could be detected in the distribution system of drinking water from riverbank-filtered sources.

DISCUSSION

In Eastern European countries, investigations into contamination of water supplies with *Giardia* and *Cryptosporidium* are very rare. In 1987–1988, small Hungarian waterworks (water originating from springs and streams) were examined for the occurrence of *Giardia* cysts by membrane filtering 70–380 litres of drinking water before examination of the pellet by direct microscopy. *Giardia* was detected regularly in one of the springs with the authors emphasizing that the hazard from *Giardia* contaminations in Hungary exists, especially where spring water originates from a forest environment (Andrik & Komuves 1989). Raw water sources in the Czech Republic were found to contain 0 to 7,400 *Cryptosporidium* oocysts per 100 litres and 0 to 485 *Giardia* cysts per 100 litres (Dolejs *et al.* 2000) and recently, high levels of contamination have been reported

for both protozoa in water supplies in Russia and Bulgaria (Karanis *et al.* 2006).

In our investigation, samples were taken from water treatment plants posing a high risk of protozoan contamination; 6 surface water treatment plants were in contact with effluents from sewage treatment plants located between 1 to 40 km away, 3 surface water treatment plants took water from a forested area containing abundant wild animals, and 2 surface water treatment plants' source water was near agricultural activities (livestock rearing). In all cases the raw water was contaminated, however, except for two water treatment plants parasite removal seemed effective during the investigative period.

Raw water from the River Danube was found to be highly contaminated with both *Giardia* cysts and *Cryptosporidium* oocysts. Changes in water level and the introduction of differently treated sewage led to high variability in the numbers of these protozoa with counts varying over two or three orders of magnitude with peaks found in winter/spring months.

One interesting finding pertained to the lack of protozoa after RBF demonstrating the high potential of an RBF system for the reduction or elimination of protozoan. Another group (Weiss *et al.* 2005) previously suggested the potential of RBF for a substantial reduction in microorganism such as *Giardia*, *Cryptosporidium*, viruses, and potential surrogate parameters. A Hungarian study investigated serological responses to the 15/17-kDa and 27-kDa cryptosporidial antigens in women using groundwater and or surface water for drinking (Frost *et al.* 2005). Serological responses were significantly lower in women who drank water from a confined aquifer or surface water following RBF compared to those, drinking water from Karst wells or non-RBF treated surface water. Strikingly, among women using bank-filtered water, the intensity of response was less than one-third of that observed for women using conventionally filtered and disinfected surface water.

The current protozoan detection methods likely underestimate the number of organisms and, therefore, when protozoa are detected they should be treated seriously. Infectivity of these oocysts/cysts depends on several factors including the species present and whether they are capable of producing human infection, clumping of the oocysts/cysts, water temperature, and age (Li *et al.* 2004). We have

taken into consideration useful information such as internal structure and DAPI staining for the accurate identification of *Cryptosporidium* and *Giardia* oocysts using microscopy (Ho *et al.* 1995; Thiriat *et al.* 1998; USEPA 2001; Smith *et al.* 2002). While PCR identification of oocysts may be more sensitive than microscopic examination, morphological characteristics cannot be distinguished (Jiang *et al.* 2005).

Genotyping isolates found in either source or treated water can give further information on the likely sources of contamination and whether strains may be infectious to humans (Ryan *et al.* 2005). Classification of *Giardia* and *Cryptosporidium* taxonomy with the aid of molecular typing techniques is still being developed but is promising to distinguish isolates in the environment able to infect humans as well as their transmission patterns (Appelbee *et al.* 2005; Alves *et al.* 2006; Xiao *et al.* 2006). Different genotypes have been linked to different symptomatology in sporadic human giardiasis and cryptosporidiosis but more information is required with regards to the association between different risk factors and different genotypes, particularly for human adapted *Giardia* and *Cryptosporidium* genotypes and for zoonotic genotypes. Genetic analysis using isolates from cattle samples confirmed the presence of *C. parvum* on animal farms in Hungary (Plutzer & Karanis 2007) suggesting that cattle may act as infection reservoirs for water supplies in the country.

Our results confirm the need to examine water treatment technologies concerning *Giardia* and *Cryptosporidium* inactivation/removal efficacy in Hungary. Currently drinking water plants are not prepared for unexpected events or worst-case scenarios. Additional treatments are necessary, particularly, at the 14 surface water treatment plants where the water treatment is not effective against the protozoa (Table 1) as well as the introduction of watershed control. Whereas the combination of filtration and chlorine disinfection is considered fundamental for treatment of surface water originated drinking water, unusual raw water conditions or inattentive operation often led to an infection of the purified water and distribution system (see e.g. number 16, Table 1 & Figure 1). Moreover, it has been demonstrated that disinfectant levels adequate for *Giardia* treatment are only marginally effective against *Cryptosporidium*. At typical levels of drinking water chlorination over prolonged periods *Giardia* is killed whereas *Cryptosporidium* can only be rendered harmless via long exposure to concentrated ultra violet light or special chemical treatment (Medema *et al.* 2006). Ozonation is an effective measure in killing both these pathogens; however the most successful method of removing oocysts/cysts from a water supply is through filtration (membrane filtration, diatomaceous earth filtration) (Medema *et al.* 2006). Indeed, our study demonstrated the ineffectiveness of disinfection against protozoa at 14 surface water treatment plants while also hinting at the efficacy of riverbed filtration.

Giardia can cause disease at a level as low as 3–5 cysts/100 litres in treated drinking water (Wallis *et al.* 1996) while *Cryptosporidium* requires as few as 10–30 oocysts/100 L to pose a risk of outbreak (Haas & Rose 1995). The highest protozoan levels found in Hungarian drinking waters was 63.6 *Giardia* cysts/100 litres and *Cryptosporidium* 3 oocysts/100 litres. During the investigation period, two water treatment plants in Hungary were found to harbour levels of protozoans above this threshold for disease outbreak suggesting the need for re-evaluation of the purification systems.

In the United Kingdom, direct monitoring of drinking water is embodied in drinking water regulation. Water supply systems with a risk of *Cryptosporidium* infection are obligated to sample their treated water at least daily, in order to demonstrate average concentrations of *Cryptosporidium* below 10 oocyst/100 L of treated water (Medema *et al.* 2006). U.S. EPA Surface Water Treatment Rule require water systems using surface water or ground water in direct contact with surface water to disinfect and/or filter their water in order to render at least 99.9% of *Giardia* cysts harmless or physically removed (USEPA 1989). This level of removal/inactivation is believed to reduce the risk of waterborne giardiasis to less than one person of 10,000 people per year. In addition, it has been determined that raw water should not contain more than seven cysts/100 litres (USEPA 1989). Raw water from nine surface water treatment plants in Hungary was found to contain *Giardia* counts above this level during our study (Table 1). One component of the Interim Enhanced Surface Water Treatment Rule regulates *Cryptosporidium* in drinking water by requiring filtered surface water systems serving at least 10,000 people to physically remove at least 99% of *Cryptosporidium*; for systems without filtration a watershed control program must be adapted in order to protect the source water from *Cryptosporidium*

contamination (USEPA 1998). In our investigations, sampling site number 24 has no filtration, only sedimentation; therefore, a watershed control would be necessary.

In Hungary the statutory orders 201/200 (X.25.) and 47/2005 (III.11.) regulate the handling and testing of drinking water and state that *Cryptosporidium* must be determined for water intended for human consumption if *Clostridium perfringens* is detected. *Clostridium* detection is required at water systems that use surface water or ground water in direct contact with surface water. The bacteriological quality and parameters have been examined in parallel for samples in our study by either the waterworks or the Department of Bacteriology at our Institute according to the Hungarian standard MSZ EN 26461-2:1994. *Clostridium* could not be detected in any drinking water samples.

It is known that the major reservoir for *C. parvum* is domestic livestock, predominantly cattle, and direct contact with infected cattle is a major transmission pathway of human infection along with indirect transmission through drinking water (Hunter & Thompson 2005). It should be emphasised here, that in Hungary in 2003, the cow and calf (dairy cow, beef cow and heifers in calves) stock totalled 714,000, and the lamb (ewes and shear lings, lambs) stock was estimated to be 1.3 million (Anonymous 2004). In England, the cow and calf and lamb stocks are eight times higher per square kilometre, than in Hungary (Defra Statistics 2004). Based on Hungarian investigations focused on enteric diseases, *Cryptosporidium* is the third most frequent pathogen of calves (detected in 70% of the herds) while 22.6% of lambs and 37.5% of goat kids with diarrhoea were found to carry *Cryptosporidium* (Nagy 1995). In stool samples of patients with gastro-enteritis in Hungary 562 tested positive for *Giardia* and only six for *Cryptosporidium* in 2003 (Anonymous 2003). Accordingly, *Giardia* infections are more frequent than *Cryptosporidium* but a more detailed study needs to be undertaken. Currently there is no data monitoring the presence of these protozoa in wild animals in Hungary. According to data from the Czech Republic deer can be a potential reservoir of *C. parvum* (Hajdusek *et al.* 2004). A Polish study emphasized that small rodents should be considered as an important reservoir for different *Cryptosporidium* genotypes (Bajer *et al.* 2003). Review of world-wide reports showed *C. parvum*

to be found in 11 wild mammals, mainly rodents, but also insectivores (e. g. common shrew), lagomorphs (e. g. brown hare) and ungulates (e. g. deer) while *C. muris* has been reported specifically in wild rodents (Sturdee *et al.* 1999). *G. lamblia* genotypes A and B are widespread, found in dogs, cats, farm and wild animals (mainly rodents); zoonotic origin for waterborne outbreaks of *Giardia* infection appears to be uncommon or unclear. Therefore, the impact of the wildlife cycle cannot be underestimated (Appelbee *et al.* 2005) nor can the risk appear posed by cats, dogs, and other animals (Karanis *et al.* 1996a, b; Karanis & Ey 1998; v. Keulen *et al.* 2002; Thompson 2004). Future genotyping of parasites will be helpful in the identification of contamination sources (Caccio *et al.* 2005). Water pollution is one of the most urgent health problems currently facing several European countries and emerging pathogens leading to waterborne disease is a pan-European problem. Safe drinking water is a general concern to all European countries yet it has been recognised that sophisticated surveillance against many diseases and especially those associated with water are not available. There is a need for wider dissemination of information on waterborne and emerging diseases amongst member states of the EU and Hungary.

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