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Effectiveness of water treatment for the removal of *Cryptosporidium* and *Giardia* spp.

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SUMMARY

Cryptosporidium and *Giardia* are intestinal parasites of humans and of many other species of animals. Water constitutes an important route of transmission for human infections in both developed and developing countries. In Poland, contamination of water sources with oocysts/cysts is not routinely monitored and scientific research in this field is scarce. Our aim was to compare the contamination of surface and treated water and thus the success of water treatment processes. Water samples ($n=94$) of between 30 l (surface water) to over 1000 l for tap water, were taken in the period of 2008–2009 using specially constructed equipment with cartridge filtration (Filta-Max; IDEXX, USA). Immunofluorescent assay, and nested polymerase chain reaction were used for the detection of parasites. *Cryptosporidium* oocysts were found in 85% of surface water and in 59% of raw (intake) water samples. Oocysts were also detected in treated water (16%) but were absent in samples of swimming pool water. The highest mean number of *Cryptosporidium* oocysts [geometric mean (GM) = 61/10 l] was found in samples of rinsing water. *Giardia* cysts were observed in 61% of surface water samples, in 6% of raw water and in 19% of treated water, with the highest number of cysts noted in rinsing water samples (GM = 70 cysts/10 l). Our study highlights the frequent occurrence of parasites in surface waters in Poland and the effectiveness of water treatment for the removal of parasites from drinking water.

Key words: *Cryptosporidium*, genotyping, *Giardia*, water contamination, water treatment.

INTRODUCTION

Cryptosporidium and *Giardia* spp. are intestinal parasites that are prevalent and widespread pathogens of humans and many other species of animals [1, 2]. Infective stages of parasites, oocysts and cysts, are excreted in high quantities in the faeces of infected hosts and this process often leads to contamination of surface water. Contaminated water is believed to constitute an important route of transmission for human infections [3]. In Poland, surface water covers 5572 km² (1·8%) of the country and often constitutes the principal source of drinking water for cities. The Vistula River supplies water to the capital city of Warsaw and its 1·7 million inhabitants, and many other cities situated along the river also exploit water from this source. Poland's many lake districts are attractive areas for thousands of tourists and amateur sailors, who visit these regions each year. Although, drinking (tap), raw and reclaimed waters are not routinely monitored for the presence of oocysts/cysts of parasites in Poland, a limited number of studies

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monitoring the prevalence of intestinal protozoa in water have been completed recently in several regions of the country (summarized in [2]). *Cryptosporidium* oocysts were detected in the majority of water samples taken from the surface waters in the Poznań area [4, 5]. Additionally, rotifers have been used for monitoring contamination in recreational surface water and parasites were found in these filter feeding organisms [6, 7]. *Giardia* cysts were less prevalent in surface water in the Poznań area [8] but were detected in rotifers. A similar situation was reported from the Gdansk–Gdynia–Sopot area in northern Poland [9]. However, contamination of tap water with *Cryptosporidium* spp. was confirmed in only one case, through a single report from the city of Poznań [10]. In contrast, no evidence of *Giardia* spp. was found in the same set of 12 tap-water samples. To date, no waterborne epidemics of cryptosporidiosis and giardiasis have been identified in Poland [2, 8].

Human cases of *Cryptosporidium* spp. and *Giardia* spp. are notifiable diseases in Poland, laboratory-confirmed cases are reported by physicians to the National Institute of Hygiene [Państwowy Zakład Hygieny (PZH)]. During the last 4 years only single cases of cryptosporidiosis can be found in reports of PZH, following years with no reported cases. The average annual number of reported giardiasis cases is low (around 3000 annually). Diagnosis of giardiasis is routinely performed in clinical laboratories, but *Cryptosporidium* infections often remain undiagnosed as the diagnosis of this parasite is performed primarily in scientific institutions [2]. According to scientific reports, the prevalence of *Giardia* spp. infections in humans in Poland is similar to that noted in other European countries: 1–9% in children, 3–7% in adults. *Cryptosporidium* infections are found in high percentages of patients in the high-risk groups [2]. Although rare in immunocompetent individuals, this parasite is common in patients with diarrhoea (up to 43%) or with primary and secondary immunodeficiencies (23–36%) [2].

The main aim of the present study was to determine the prevalence of two intestinal protozoa in various surface waters including recreational water bodies (i.e. the Mazurian lakes) and drinking-water sources, and the effectiveness of water treatment for the removal of (oo)cysts. To complete this aim, several water treatment plants situated throughout Poland were sampled for the first time, and in each case samples were taken from the surface body of water serving as a water source for the plant; intake (raw) water samples and finally, treated water samples from the same water plant. Additional aims were (1) the development of an effective method of sampling and (2) molecular characterization of protozoan parasites in different types of water over an area of Poland.

MATERIAL AND METHODS

Water samples

Samples ($n=94$) with volumes of 30 l (surface water) to over 1000 l for tap water, were taken in autumn 2008, spring and summer 2009. Samples were taken using specially constructed equipment BISTYP-HYDRO (Poland). According to standard ISO 155553 guidelines (2006 Water quality – isolation and identification of *Cryptosporidium* oocyts and *Giardia* cysts from water) Filta-Max filters (IDEXX, USA) were used for sample collection. Equipment was adapted for filtration of surface and treated water, collected from open, low-pressure or pressure systems. [11]. In total, we screened 33 samples of surface water (lakes and rivers; Table 1); 17 samples of raw (intake) water from water treatment plants (including nine following infiltration) and 31 samples of treated water (Table 2). Of these 31 samples, 23 were final products of water treatment (tap water), following disinfection with UV, ozone or chlorination. The remaining eight samples were taken following standard filtration. Additionally, to discover if secondary contamination of water existed, seven samples of water were checked from a hospital swimming pool [Children's Memorial Health Institute (CMHI), Warsaw]. Finally, to assess the effectiveness of standard filtration, six samples of rinsing water (water used to rinse the filters) from three water treatment plants were studied.

To test the level of recovery of BISTYP-HYDRO equipment with Filta-Max system, two samples of tap water (volume = 30 l) were spiked with 200 formalin-fixed *C. parvum*-like oocysts isolated from free-living susliks [12]. Following filtration of the spiked sample, filters were washed with tap water, to a final volume of 100 and 1000 l, respectively, and processed by an immunofluorescence assay (IFA) for recovery of oocysts. Tap water used to wash the filters was previously tested as negative for these parasites.

Detection of parasites

An IFA and nested polymerase chain reaction (PCR) were used for the detection of parasites in a final

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Table 1. *Occurrence of protozoa in surface water bodies* (*by immunofluorescence assay*)

Water body	Total *Cryptosporidium* spp. (no. positive/ no. studied)	*Giardia* spp. (no. positive/ no. studied)	Total protozoa (no. positive/ no. studied)
NE Poland			
Śniardwy Lake	2/2	0/2	2/2
Mikołajskie Lake	2/2	1/2	2/2
Łuknajno Lake	0/2	0/2	0/2
Central Poland			
River Wisła	4/6	5/6	5/6
Zegrzyński Lake	5/6 (Cp + Ca)	2/6	5/6
River Pilica	6/6	6/6	6/6
Southern Poland			
River Raba	3/3 (Cp + Ca)	2/2	3/3
River Dunajec	2/2	2/2	2/2
Rożnowskie Lake (River Dunajec)	1/1 (Cp + Ca)	0/1	1/1
Czaniec Lake (River Soła)	3/3	2/3	3/3
Total (*n* = 33)	**28/33 (84·8 %)**	**20/33 (60·6 %)**	**29/33 (87·9 %)**

(Cp + Ca) = either *C. parvum*-like and *C. andersoni*-like oocysts present; all remaining positive samples: only *C. parvum*-like oocysts present.

Table 2. *Occurrence of protozoa in treated water* (*by immunofluorescence assay*)

Tap or treatment plant	Total *Cryptosporidium* spp. (no. positive/ no. studied)	*Giardia* spp. (no. positive/ no. studied)	Total protozoa (no. positive/ no. studied)
(A) Central Poland	2/10 (Cp + Ca)	2/10	2/10
(B) Central Poland	0/3	0/3	0/3
(C) Central Poland	0/6	0/6	0/6
(D) Southern Poland	2/6	1/6	3/6
(E) Southern Poland	1/5	3/5	3/5
(F) Northern Poland (well)	0/1	0/1	0/1
Total (*n* = 31)	**5/31 (16·1 %)**	**6/31 (19·4 %)**	**8/31 (25·8 %)**

(Cp + Ca) = either *C. parvum*-like and *C. andersoni*-like oocysts present; all remaining positive samples – only *C. parvum*-like oocysts present.

volume of 50 ml of the condensed water sample. Following centrifugation, 10 or 20 μl of the sediment was placed on the well and processed by IFA (MeriFluor Cryptosporidium/Giardia; Meridian Diagnostics Inc., USA). Oocysts were classified into two types: round, measuring 3·5–5·5 μm in diameter (*C. parvum*-like) and oval, measuring 6–8 × 5–6·5 μm (*C. andersoni*-like) (as this mammalian gastric species was the most common among species identified in surface water in the USA [13, 14]). Protozoan (oo)-cysts were counted and calculated per 10 l of water sample. DNA extractions were performed on the remaining condensed sample (10–100 μl of the sediment, depending on the available sediment volume) using the QIAamp DNA Stool Mini kit (Qiagen, USA). The extracted DNA was subjected to nested PCR with specific primers, in at least three repeats, using 1 and 3 μl of target DNA. For species identification of *Cryptosporidium*, we used two sets of primers amplifying two regions of the *18S rRNA* gene: an 830-bp fragment following Feltus *et al*. [15] and Xiao *et al*. [16] and a 434-bp fragment following Downey & Graczyk [17] and Jellison *et al*. [18]. For species identification of *Giardia*, we used primers

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amplifying a 530-bp fragment of the *tpi* gene [19] and primers amplifying a 310-bp fragment of 5·8 rRNA and the *its* gene fragment [20]. Two sets of controls, positive and negative, were incorporated in each PCR run. The reaction was performed under conditions that have been described previously [16–20]. Sequencing reactions were conducted with the ABI-PRISM 377 automatic DNA sequencer (Applied Biosystems, USA). The resulting sequences were assembled using the program ABI^{TM} BigDye^{TM} and compared with sequences deposited in the GenBank NCBI database.

Statistical methods

Prevalence (percentage of positive samples) was analysed by maximum-likelihood techniques based on log-linear analysis of contingency tables, using the software package, SPSS v. 14 (SPSS Inc., USA). Prevalence of parasite was entered as a binary factor (parasite present = 1, absent = 0) and then water type (1, surface; 2, raw; 3, treated; 4, pool; 5, rinsing) and the presence of other parasite species (parasite present = 1, absent = 0) as factors. Beginning with the most complex model, involving all possible main effects and interactions, those combinations not contributing significantly to explaining variation in the data were eliminated stepwise, beginning with the highest-level interaction [21]. A minimum sufficient model was then obtained, for which the likelihood ratio of χ^2 was not significant, indicating that the model was sufficient for explaining the data. Five statistical models were fitted as part of the overall analysis: for *C. parvum*-like parasites; for *C. andersoni*-like; for total *Cryptosporidium*; for *Giardia* spp., and finally for total protozoan parasites.

Quantitative data reflecting (oo)cyst abundance was expressed as the geometric mean (GM) because the data were highly dispersed. These means reflect the abundance/concentration of parasites as defined by Margolis *et al*. [22] and include all subjects within the specified group, contaminated and not contaminated, for which relevant data was available. Abundance was analysed by multifactorial ANOVA (SPSS v. 14) using models with normal errors after normalization of the data by $\log_{10}(x+1)$ transformation. As with analysis of prevalence we began with full factorial models and simplified these by backward selection to derive the most parsimonious, minimum sufficient models.

RESULTS

Prevalence of *Cryptosporidium* and *Giardia* spp. in water by IFA

The dispersive stages of both intestinal protozoa were found in four of the five types of water samples (surface, raw, treated, rinsing water) (Tables 1–3). No parasites were identified in the hospital swimming pool. Overall prevalence of the three parasites (*C. parvum*-like, *C. andersoni*-like, *Giardia*) was 52% in all the combined water samples. The most prevalent were *C. parvum*-like parasites, identified in 45% of water samples, total *Cryptosporidium* spp. were detected in 48% of samples and *Giardia* cysts in 32% of samples (Table 3). The least prevalent were *C. andersoni*-like oocysts, found in only 8·5% of samples. There were statistically significant differences between occurrence of parasites in different water types (Table 3). The prevalence of parasites decreased with increasing level of water treatment. The overall highest prevalence (88%) of dispersive stages was noted in surface waters. Occurrence of parasites in intake water was a third lower (59%), and the lowest percentage of positive samples was identified in treated water samples (26%). Thirty-three percent of rinsing water samples were positive for intestinal protozoa. Similar associations were found for *Cryptosporidium* spp. but the lowest prevalence of *Giardia* cysts was found in intake water samples (Table 3).

Of the ten surface water bodies tested, dispersive stages were found in nine (Table 1). *C. parvum*-like oocysts were observed in nine of the ten surface water bodies, *Giardia* cysts in seven and *C. andersoni*-like oocysts in three surface water bodies.

Among the intake water samples, dispersive stages of intestinal protozoa were identified in four of the six studied water treatment plants with an overall prevalence of 59% (Table 3). *C. parvum*-like oocysts were observed in four of the six water treatment plants, *Giardia* cysts and *C. andersoni*-like oocysts in one raw water sample from the same treatment plant (6%).

Rinsing water samples were available only from two treatment plants (one from Central Poland and one from Southern Poland). Dispersive stages of the parasites (*C. parvum*-like and *Giardia*) were found in one treatment plant.

Of the treated water samples, dispersive stages of intestinal protozoa were identified in three of the six water treatment plants (Table 2) with an overall prevalence of 26%. *C. parvum*-like oocysts were observed in three of the six water treatment plants,

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Table 3. *Prevalence and abundance of intestinal parasites in different water types*

	Species									
	C. parvum-like		*C. andersoni*-like		Total *Cryptosporidium* spp.		*Giardia* spp.		Total *Cryptosporidium* + *Giardia* spp.	
Water type*	Prevalence	Mean abundance	Prevalence	Mean abundance	Prevalence	Mean abundance	Prevalence	Mean abundance	Prevalence	Mean abundance
Surface water ($n=33$)	78·8 %	5·12	18·2 %	1·43	84·8 %	6·14	60·6 %	2·84	87·9 %	8·98
Raw water ($n=17$)	52·9 %	1·55	5·9 %	0·008	58·8 %	1·56	5·9 %	0·01	58·8 %	1·57
Treated water ($n=31$)	16·1 %	0·03	3·2 %	0·002	16·1 %	0·03	19·4 %	0·03	25·8 %	0·06
Swimming pool ($n=7$)	0 %	0	0 %	0	0 %	0	0 %	0	0 %	0
Rinsing water ($n=6$)	33·3 %	60·9	0 %	0	33·3 %	60·9	33·3 %	69·7	33·3 %	128·6
Total ($n=94$)	**44·7 %**	**3·47**	**8·5 %**	**0·37**	**47·9 %**	**3·93**	**31·5 %**	**1·77**	**52·1 %**	**12·63**

Mean abundance = geometric mean/10 litres.

* 'Water type' was significant factor in minimal sufficient models for either prevalence or abundance:
Water type × prevalence of *Giardia*: $\chi^2=14{\cdot}61$, D.F. = 4, $P=0{\cdot}006$.
Water type × prevalence of *C. parvum*-like: $\chi^2=25{\cdot}0$, D.F. = 4, $P<0{\cdot}001$.
Water type × prevalence of total *Cryptosporidium*: $\chi^2=32{\cdot}38$, D.F. = 4, $P<0{\cdot}001$.
Water type × prevalence of total protozoa: $\chi^2=38{\cdot}08$, D.F. = 4, $P<0{\cdot}001$.
Water type × abundance of log *Giardia*: $F_{4,93}=15{\cdot}41$, $P<0{\cdot}001$.
Water type × abundance of log *C. parvum*-like: $F_{4,93}=13{\cdot}84$, $P<0{\cdot}001$.
Water type × abundance of log total *Cryptosporidium*: $F_{4,93}=17{\cdot}32$, $P<0{\cdot}001$.
Water type × abundance of log total protozoa: $F_{4,93}=25{\cdot}32$, $P<0{\cdot}001$.

Table 4. *Comparison of prevalence and abundance of parasites in raw and treated water samples in water treatment plants*

	Intake/raw water samples				Treated/tap water samples			
	Cryptosporidium spp.		*Giardia* spp.		*Cryptosporidium* spp.		*Giardia* spp.	
Treatment plant	Prevalence	Mean abundance GM/10 l (range)	Prevalence	Mean abundance GM/10 l (range)	Prevalence	Mean abundance GM/10 l (range)	Prevalence	Mean abundance GM/10 l (range)
(A) Central Poland	82 %	4·28 (0–27·9)	9 %	0·02 (0–0·23)	20 %	0·01 (0–0·09)	20 %	0·02 (0–0·11)
(B) Central Poland	0 %	0	0 %	0	0 %	0	0 %	0
(C) Central Poland	100 %	23·7 (18·7–33·3)	100 %	23·2 (20–26·7)	0 %	0	0 %	0
(D) Southern Poland	100 %	1106 (880–1493)	66·7 %	44·6 (0–640)	33 %	0·05 (0–0·17)	17 %	0·01 (0–0·09)
(E) Southern Poland	60 %	11·0 (0–40)	40 %	1·97 (0–26·7)	20 %	0·02 (0–0·11)	60 %	0·03 (0–0·07)
(F) Northern Poland (well)	n.d.	n.d.	n.d.	n.d.	0 %	0	0 %	0

GM, Geometric mean; n.d., not done.

Giardia cysts in three plants and *C. andersoni*-like oocysts in one plant.

Mean abundance/concentration of dispersive stages of three parasites (total protozoa) differed significantly between different water types (Table 3). Mean abundance of the parasites was relatively high in surface water samples [GM = 9 (oo)cysts/10 l)], compared to raw [GM = 1·6 (oo)cysts/10 l] and treated [GM = 0·06 (oo)cysts/ 10 l] water (Table 3). The highest abundance was observed in rinsing water [GM = 129 (oo)cysts/10 l]. The same associations were found for *Cryptosporidium* parasites, mean abundance of oocysts declined significantly with the increasing level of water treatment (Table 3). The abundance of *Giardia* cysts was the highest in surface water samples (GM = 2·84 cysts/10 l), and at very low and similar level in intake and treated water samples (GM = 0·01 cysts/10 l and GM = 0·03 cysts/10 l, respectively) (Table 3).

Comparison of the prevalence and abundance of *Cryptosporidium* and *Giardia* at the level of particular water treatment plant is presented in Table 4. In most cases, similar trends were observed as for summarized data in Table 3. In particular, mean abundance of dispersive stages of both parasites decreased rapidly between raw and tap water samples (Table 4).

Either in surface or raw water, the mean abundance of both *Cryptosporidium* types was several times higher than *Giardia* abundance (Tables 3, 4). In treated water, the mean abundances of *Cryptosporidium* and *Giardia* were low and similar to each other (Tables 3, 4). There was a positive association between occurrence of *Giardia* cysts and *C. parvum*-like oocysts in water samples (*Giardia* prevalence × *C. parvum* prevalence: $\chi^2 = 19{\cdot}67$, D.F. = 1, $P < 0{\cdot}001$). *Giardia* cysts were found in 59·9 % of water samples also containing *C. parvum*-like oocysts, compared to 7·7 % of *Giardia*-positive samples in the absence of *Cryptosporidium* oocysts.

The level of recovery of *Cryptosporidium* oocysts

The recovery level for water samples spiked with 200 formalin-fixed *C. parvum*-like oocysts varied between samples of different volume. The recovery was 50 % for 1000 l and 78 % for 100 l of filtered water.

Quality of observed oocysts

A comparison of the proportion of intact and destroyed oocysts in surface and treated water samples

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Table 5. *Quality of* C. parvum*-like oocysts* (*by immunofluorescence assay*)

Source of oocysts	No. of observed oocysts	No. of destroyed oocysts	% of intact oocysts
Surface water	20	7 (35%)	65%
Treated water	11	10 (91%)	9%

is presented in Table 5. In surface water samples, the majority of observed oocysts were intact, but in treated water samples only 9% of observed oocysts presented intact walls.

Molecular studies of *Cryptosporidium* and *Giardia* in water

Amplification of the samples by the primers of Downey & Graczyk [17] resulted in five weak bands of the correct size (for *Cryptosporidium* spp.). These five positive samples consisted of two samples of surface water originating from the Zegrzynski Lake and Pilica River, both also positive by IFA; two samples of raw (intake) water from two (Southern and Central) treatment plants, including one positive by IFA and one sample of treated water from one of the Central Poland treatment plants (plant C in Table 2), negative by IFA. Only the last sample produced enough PCR product to enable sufficient to be cleaned for sequencing. This final sequence was compared with sequences in the GenBank database, giving only 72% similarity with other *Cryptosporidium* sequences, therefore it was finally classified as a non-specific product. Products of the remaining four samples could not be sequenced and genotyped, thus the presence of *Cryptosporidium* could not be confirmed by molecular tools.

Amplification of the samples by the primers of Xiao *et al*. [16] and Feltus *et al*. [15] in conditions described previously, resulted in a whole range of products of various sizes (range 100–1000 bp) in the majority of samples, including those samples identified as positive or negative by IFA. Thus the reaction was repeated with an increased annealing temperature (T_a) of three degrees. At this T_a only the positive control produced a positive signal, and therefore the results for these primers were classified as negative for all isolates.

A similar situation was obtained for two primer sets in the amplification of *Giardia* DNA. No positive signals were obtained from the amplification of the *tpi* gene fragment. Using the new primers by Cacciò *et al*. [20], nested PCR resulted in a range of products with a much larger product size than expected (600–800 bp compared to 310 bp) in the majority of samples, including samples both positive and negative by IFA. Thus the reaction was repeated at an increased T_a of 3 °C, with no improvement in specificity. Again the reaction was repeated at the increased T_a; however, the results obtained were not an improvement. A product of the correct size (about 310 bp) was obtained in one sample of surface water from the Pilica River (positive by IFA, GM = 23 cysts/10 l), but it was accompanied by two non-specific products, preventing the sequencing and determination of *Giardia* species/genotype.

DISCUSSION

The main findings of our study are (1) the identification of a high level of contamination of surface water, serving as a source of drinking water, with waterborne protozoa of the genera *Cryptosporidium* and *Giardia*, and (2) confirmation of the success of the water treatment procedures for elimination/significant reduction of such contamination in tap water. Using a compilation of standardized techniques (Filta-Max filtration of significant volumes of water followed by IFA), we obtained satisfactory levels of oocyst recovery (50–78%) and generated results that we consider to be reliable, showing levels of contamination similar to those found in many other developed and developing countries [23, 24]. The rates of positive samples identified in this study are in the range of values identified in research conducted in several states in the USA, where contamination of surface water with *Cryptosporidium* has been identified in 24–100% of samples and in particular 17–27% of tap-water samples [23, 24]. In accordance with previous studies conducted in the USA, in our study *Cryptosporidium* oocyst concentrations were 10- to 100-fold greater than concentration of *Giardia* spp. cysts [24]. Although *Cryptosporidium* oocysts were found in greater numbers than *Giardia* spp. cysts, their occurrences were found to be significantly correlated, as has been previously reported

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[24]. The similarity of findings between our study and studies previously completed supports the usefulness of our method for monitoring of *Cryptosporidium* and *Giardia* spp. in different water samples.

Moreover, in our study the level of contamination depended on the water type, and the degree to which the water had been subjected to treatment procedures. In our study, the level of contamination [either the percentage of positive samples and mean abundance of (oo)cysts] decreased significantly (by 25–30% and a 5–6 times lower mean GM) following infiltration (surface *vs*. raw water) and following standard procedures for water treatment (by 40–70% and a 100–200 times lower mean GM; comparing treated *vs*. surface water; Table 3). Considering the high number of (oo)cysts found in rinsing water and the great majority of destroyed oocysts encountered in tap water, it is evident that the treatment procedures for water are effective in reducing protozoa in drinking water in the different regions of Poland, where treatment plants were studied.

No assessment of (oo)cyst viability was performed as many of the viability assays (i.e. FISH [25]) need oocyst permeabilization and in our opinion this step could have had an adverse impact on detection and estimation of abundance. Instead, the quality of observed oocyst (intact or destroyed wall of oocysts) was assessed by a highly experienced observer with more than 10 years experience in the field.

Disappointingly, we were unsuccessful in genotyping either *Cryptosporidium* or *Giardia* spp. from water samples, and therefore we cannot determine what level of zoonotic species/genotypes may be present in the different water types. Given that during recent similar genotyping studies in the USA, the rate of zoonotic *Cryptosporidium* species/genotypes identified in surface water samples was very low (less than 10–20% of all positive [13]), it would not be unexpected to find that a similar percentage of 'Polish' isolates are also zoonotic, giving a 10× lower level of risk of waterborne cryptosporidiosis/giardiasis in Poland, compared to that derived merely from observation of (oo)cysts present in the water systems.

Despite many efforts allocated to molecular typing of either *Cryptosporidium* or *Giardia* spp. from water samples in this study, no convincing results were obtained. The failure to obtain synonymous, clearly positive results in our study could have been caused more by the highly heterogeneous mixture of different DNAs extracted directly from environmental samples than by the inhibition of PCR reactions. Amplification of non-specific product in the majority of samples for *Cryptospordium* and *Giardia* supports this hypothesis. The results of our unsuccessful genotyping strongly support the need for an additional step of (oo)cyst purification, i.e. by immunomagnetic separation (IMS) prior to DNA extraction.

CONCLUSIONS

(1) The results of our study emphasize the wide occurrence of parasites in surface and raw water in Poland.
(2) Both prevalence and abundance of parasites were significantly lower in treated *vs*. surface or raw (intake) water, demonstrating the effectiveness of water treatment procedures.
(3) The effectiveness of water treatment procedures was additionally supported by the high rate of destroyed oocysts found in tap water and the high abundance of (oo)cysts in rinsing water.
(4) Our study also demonstrated the effectiveness of an applied monitoring method for the detection of protozoa in various types of water.

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DECLARATION OF INTEREST

None.

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