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REVIEW ARTICLE
Prevalence and distribution of *Cryptosporidium* and *Giardia* in wastewater and the surface, drinking and ground waters in the Lower Rhine, Germany

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SUMMARY

Samples from different water sources ($n=396$) were collected during 2009 and 2011. Wastewater (2–5 l) was purified by aluminium sulphate flocculation. Surface, ground and drinking waters (400–6400 l) were collected by filtration. *Cryptosporidium* oocysts and *Giardia* cysts were further concentrated by sucrose centrifugation. (Oo)cysts were identified by IFT (immunofluorescence test), DAPI (4′,6-diamidino-2-phenylindole) staining and DICM (difference interference contrast microscopy). Out of 206 wastewater samples, 134 (65·0%) were found to be positive for *Giardia* cysts and 64 (31·1%) for *Cryptosporidium* oocysts. Parasite numbers ranged from 0 to 2436 cysts/l and 0 to 1745 oocysts/l. Eight (4·2%) surface and drinking water samples ($n=190$) were found to be positive for *Giardia* cysts (0–56000/100 l), and 18 (9·5%) for *Cryptosporidium* oocysts (2400/100 l). The purpose of this study was to establish the prevalence and concentrations of *Giardia lamblia* and *Cryptosporidium* spp. by detecting (oo)cysts from water samples. This study provides substantial evidence that *G. lamblia* cysts and *Cryptosporidium* spp. oocysts are able to enter and circulate in the aquatic environment with negative implications for public health.

Key words: *Cryptosporidium*, *Giardia lamblia*, parasites, water (safe), water-borne infections.

INTRODUCTION

Cryptosporidium and *Giardia* parasites are distributed worldwide and cause diseases of the intestinal tract in vertebrates. Affected hosts include humans [1] and wild [2–4] and domestic [5–8] animals. Infection causes diarrhoea and is self-limiting within a few days [9].

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Cryptosporidium and *Giardia lamblia* are major pathogens in the waterborne transmission of infections and they are able to persist in the environment due to the robustness of the (oo)cysts. Different transmission cycles are possible, and one of the most important is waterborne distribution. The occurrence of *Cryptosporidium* spp. oocysts and *G. lamblia* cysts in different types of water has been confirmed, and a considerable number of waterborne outbreaks have been reported worldwide [10–12].

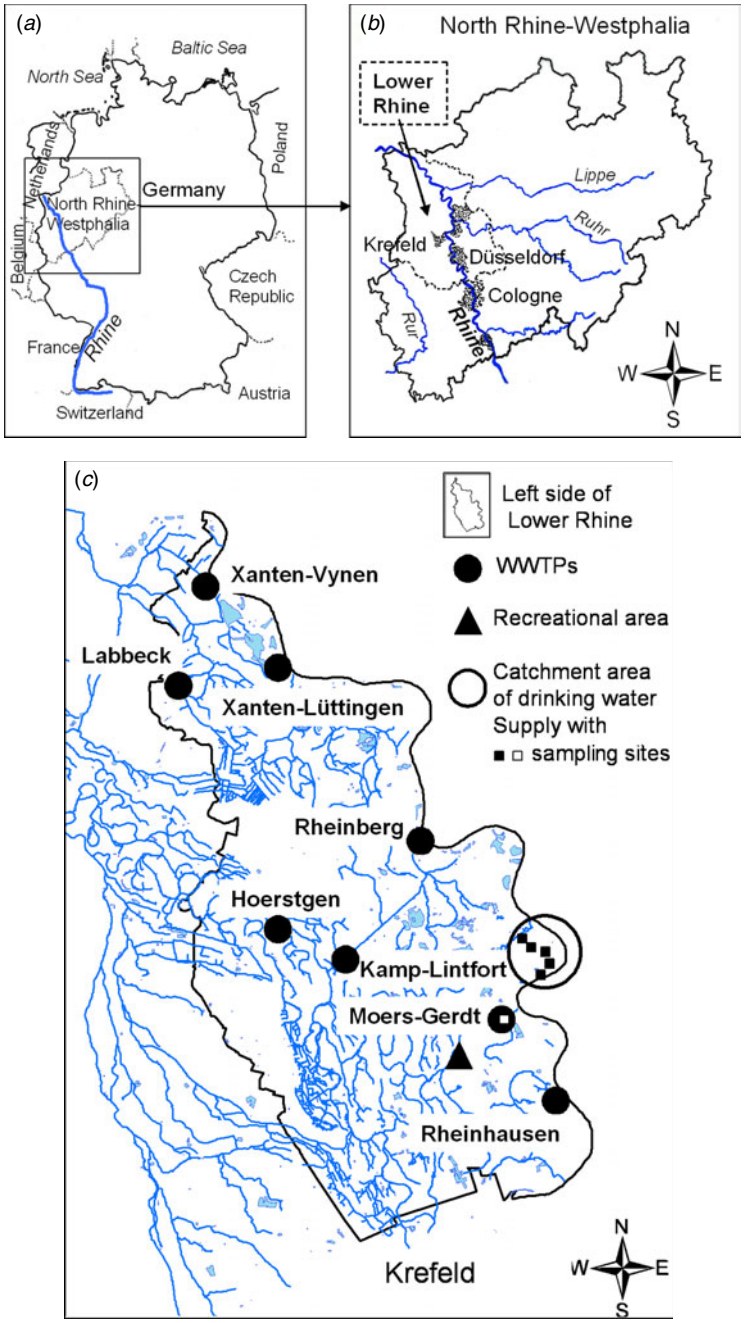

Fig. 1. [*colour online*]. Study area: (*a*) North Rhine-Westphalia in the western part of Germany and (*b*) Lower Rhine area in expanded view. (*c*) Position of the eight municipal wastewater treatment plants (WWTPs), recreational area, and catchment area of drinking water supply (ArcGIS 9, ArcMap version 9.3.1, ESRI, Germany).

Circulation of (oo)cysts from wastewater to surface and ground water and ultimately to drinking water is possible. The presence of the target pathogens in all investigated water sources demonstrates the risk of waterborne transmission for human health. This study reveals the process of pathogens transmission in the hydrological cycle from the originating source to the drinking water.

MATERIALS AND METHODS

Geography and description of the sampling sites

The study area is located in North Rhine-Westphalia on the Lower Rhine in Germany (Fig. 1*a–c*). The River Rhine is one of the longest rivers in Europe, and its tributaries collect different types of materials due to erosion as well as the faeces of wild and domestic

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animals. Deposition of enteric pathogens from the running water discharges from eight municipal sewage water plants (Fig. 1*c*) is possible. The Lower Rhine catchment area contains a drinking water supply (Fig. 1*c*), which provides potable water to a large population [13].

The investigation of the recreational swimming area (Fig. 1*c*) was performed during the 2009 and 2010 bathing seasons. The Aubruch Channel is a small section of running water that passes through a pond. Therefore, the catchment area included residential areas, agricultural landscape and natural and landscaped protection areas influenced by avifauna and mammals. The running water was mainly fed by groundwater. Additionally, it contained the rainwater running off the catchment area including the sealed urban surfaces, and illegal sewage disposal was frequent.

The sampling sites in the catchment area of the drinking water supply was chosen for the investigation of protozoan parasites formed an imaginary line in the direction of the flow of groundwater from the River Rhine to the waterworks situated in the area (Fig. 1*c*). Six sampling sites were examined fortnightly for a period of 1 year. The first sampling site was the surface water from the River Rhine sampled manually near the riverbank. The second, third, and fourth sampling sites were wells 0·25 km, 0·63 km, and 1·5 km away from the River Rhine. The first waterworks was 2·5 km from the river (fifth sampling site). This site represented the water quality before disinfection. The final drinking water of the consumer (sixth sampling site) was collected from a second waterworks situated outside the catchment area that obtained its water from the same aquifer.

Sample collection

On the Lower Rhine in Germany, 396 water samples were collected and investigated for the presence of *Cryptosporidium* oocysts and *Giardia* cysts from 2009 to 2011. Influent and effluent samples from eight wastewater treatment plants (WWTPs) ($n=206$) were taken over a period of 15 months. Fifty-four samples were collected from the recreational swimming area ($n=27$) and the small running water flowing through the pond ($n=27$) during the 2009 and 2010 bathing seasons. A total of 136 samples were collected from the drinking water supply [tap water, $n=24$; raw water (drinking water before disinfection), $n=23$; ground water, $n=66$; and drinking water produced from the River Rhine, $n=23$].

Using permanent samplers (ASP Station 2000, Endress & Hauser, Germany), in- and outflow samples from the WWTPs were extracted as a pooled sample of a 24-h sampling period. The samples were transported to the laboratory within 1 hour in sterile 10-l buckets (Meliseptol, B. Braun Melsungen AG, Germany).

For sample collection, an average volume of 2500 l (min-max 240–6400 l, depending on turbidity) for drinking water and 80 l (min-max 40–408 l, depending on turbidity) for surface water was filtered using polyester microfibre filters with a nominal pore size of 2 μm (ARAD Hungária Kft., Hungary). After filtration, the filter cassette was removed and immediately shipped to the laboratory in a clean plastic bag for further analysis, as described by Plutzer *et al.* [14].

Sample preparation for microscopic examinations

Aluminium sulphate [$Al(SO_4)_3$] flocculation was performed as previously described by Kourenti *et al.* [15]. A sterile glass bottle was filled with 2 l of homogenized influent or 5 l of effluent waste water, $Al_2(SO_4)_3$ solution was added and the pH was adjusted to 5·4–5·8 using sodium hydroxide. On the following day, the supernatant was discarded, and the precipitate was further concentrated by centrifugation (2100 ***g*** for 10 min; Multifuge 3SR+, Thermo Fisher Scientific, Germany). The precipitate was solubilized using acid buffer. The samples were centrifuged again, and the pellets were washed and further analysed for (oo)cyst identification.

The elution of the (oo)cysts from the ARAD filter was performed as described by Inoue *et al.* [16] and modified by Plutzer *et al.* [14]. In brief, the filter was eluted using 300 ml PET solution and scrubbing the surface with a sterile bristle brush. The extracts were collected in 400-ml centrifuge tubes. After centrifugation (3800 ***g*** for 10 min; Multifuge 3SR+, Thermo Fisher Scientific, Germany), the supernatant was carefully aspirated to 2 ml containing the pellet with the (oo)cysts.

The purification of the (oo)cysts was performed as described by Arrowood & Sterling [17] and modified by Kourenti *et al.* [15]. Briefly, Sheather's sugar solution was diluted with 0·1 mol/l PBS, to obtain solutions A (specific gravity 1·11) and B (specific gravity 1·07). A volume of 15 ml from solution B was layered

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over 15 ml solution A in 50-ml sterile polypropylene tubes. The 2-ml pellet was layered over solution B. The samples were then centrifuged at 1200 ***g*** for 30 min (4 °C, brake off) and washed twice with distilled water and centrifuged (2100 ***g*** for 10 min, 4 °C, brake off). After centrifugation the resuspended pellet with an end volume of 2 ml was preserved with 50 μl antibiotics and stored at 4 °C.

The immunofluorescence test (IFT) was performed according to the manufacturer's instructions (Cellabs Pty Ltd, Australia) with minor modifications [15]. Small pellets (100 μl) were incubated in Eppendorf tubes with fluorescently labelled monoclonal antibodies (Waterborne Inc., USA) for 30 min at 37 °C and then with a nucleic acid stain [2-(4-amidinophenyl)-6-indolecarbamidine di-hydrochloride, DAPI; Merck, Germany] for 1 h at room temperature. Microscopic examination was performed with epifluorescent microscopy (Olympus BX51) and difference interference contrast microscopy (DICM). Only samples in which (oo)cysts fulfilled the defined and already published morphological criteria [18] were recorded as positive.

Recovery rates

In this study, pathogens recovery for filtration and flocculation with EasySeed (BTF Pty Ltd, Australia) was performed. The recovery rates ($\pm$S.D.) for filtration were 36·7$\pm$6·2% (*Cryptosporidium* oocysts) and 28·3$\pm$4·7% (*Giardia* cysts) and the flocculation recovery rates were 15·8$\pm$4·2% (*Cryptosporidium* oocysts) and 64·5$\pm$22·7% (*Giardia* cysts).

Statistics

Significance was tested by the comparison of arithmetic average values, given a normal distribution, using a paired sample *t* test. Pearson's correlation coefficient was used to determine the relationship between variables. The statistics were calculated, and the differences in the mean values of the statistical populations were considered to be significant at a confidence level of 0·01.

RESULTS

Results of the examination of WWTPs

Each sampling site of the WWTP was positive at least for one of the two protozoan parasites, with a higher detection frequency for *Giardia* cysts (Table 1). In the WWTPs, the influent numbers ranged from 0 to 1745 *Cryptosporidium* oocysts/l and from 0 to 2436 *Giardia* cysts/l. The mean (min-max) value of effluent numbers ranged from 0 to 36 *Cryptosporidium* oocysts/l and from 0 to 56 *Giardia* cysts/l.

The number of positive samples in the influent was significantly higher than in the effluent (*Giardia* cysts: $t_0 = 6{\cdot}4$, D.F. = 204, $P < 0{\cdot}01$; *Cryptosporidium* oocysts: $t_0 = 10{\cdot}5$, D.F. = 204, $P < 0{\cdot}01$), where t_0 = distances between arithmetic means in relation to standard deviation; D.F. = degrees of freedom; P = level of confidence.

Based on our results, the rates of *Giardia* cysts were significantly higher than the rates of *Cryptosporidium* oocysts (arithmetic average of all influent samples: 48 oocysts/l and 218 cysts/l; effluent samples: 1·7 oocysts/l and 4·4 cysts/l). The distances between arithmetic means in relation to standard deviation of the influent samples was $t_0 = 4{\cdot}2$ (D.F. = 204, $P < 0{\cdot}01$) and of the effluent samples $t_0 = 4{\cdot}3$ (D.F. = 204, $P < 0{\cdot}01$).

In all the WWTPs, a reduction of (oo)cysts in influent compared to effluent water was observed (Table 1). The reduction rate of *Cryptosporidium* oocysts ranged from 73·3% to 100% (arithmetic mean 92·1%) and *Giardia* cysts from 75·3% to 100% (arithmetic mean 92·4%). The average removal efficiency of all the WWTPs was 0·7 $\log_{10}$ for *Cryptosporidium* oocysts and 1·3 $\log_{10}$ for *Giardia* cysts. The average concentrations and reduction rates of *Cryptosporidium* and *Giardia* are shown in Table 1 (the data of the bacterial contamination are not shown).

There were differences in the mean concentrations of *Cryptosporidium* in the influent and effluent samples from WWTPs using different treatments (enhanced secondary treatment or small compact facilities). Higher numbers of *Cryptosporidium* were found in both the influent (mean 53·1 oocysts/l) and effluent (mean 2·0 oocysts/l) of small compact facilities compared to the results of the WWTPs with enhanced secondary treatment. Differences in the mean concentrations of *Giardia* were not observed in influent and effluent water based on a comparison of the two treatment systems (Table 1), i.e. in the smaller WWTPs, the amount of cysts was nearly the same as in the urban plants.

The concentration of (oo)cysts in the influent samples differed throughout the year. *Cryptosporidium* and *Giardia* were more prevalent from late summer

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Table 1. *Occurrence of* Cryptosporidium *oocysts and* Giardia *cysts in the wastewater treatment plants between July 2009 and September 2010*

	Influent *Cryptosporidium* oocysts/l		Effluent *Cryptosporidium* oocysts/l		Reduction rate %	Input *Giardia* cysts/l		Output *Giardia* cysts/l		Reduction rate (%)
	P (T)	Mean (min-max)	P (T)	Mean (min-max)		P (T)	Mean (min-max)	P (T)	Mean (min-max)	
Wastewater treatment plant (WWTP)										
MG*	6 (12)	10 (0–55)	2 (14)	1·5 (0–2)	92·7	9 (12)	260 0–1480)	7 (14)	1·5 (0–40)	98·3
RH*	9 (12)	50 (0–310)	3 (14)	0·3 (0–24)	95·6	9 (12)	174 (0–640)	10 (14)	7·9 (0–56)	90·9
RB*	6 (12)	16 (0–150)	2 (14)	0·7 (0–4)	73·3	10 (12)	186 (0–600)	6 (14)	6·1 (0–28)	86·3
KL*	6 (12)	94 (0–730)	3 (14)	0·8 (0–5)	99·2	11 (12)	273 (0–745)	8 (14)	5·8 (0–26)	97·4
HO†	3 (11)	2·5 (0–10)	1 (13)	0·2 (0–2)	90·0	10 (11)	142 (0–650)	0 (13)	0 (0–0)	100·0
LA†	5 (12)	10 (0–40)	1 (14)	6·3 (0–0)	100	8 (12)	107 (0–450)	7 (14)	6·4 (0–30)	75·3
XL†	6 (12)	183 (0–100)	1 (14)	2·2 (0–36)	86·4	10 (12)	239 (–870)	8 (14)	9 (0–24)	96·4
XV†	5 (12)	13 (0–1745)	5 (14)	5·3 (0–10)	99·4	11 (12)	383 (0–2436)	10 (14)	4·9 (0–14)	94·2
Total	46 (95)			18 (111)		78 (95)			56 (111)	
WWTPs with enhanced secondary treatment*										
Mean		42·4		0·5	90·2		217		5·3	93·2
Percentile 90		150		2·0			643		18·6	
WWTPs – small compact facilities†										
Mean		53·1		2·0	94·0		220		5·0	91·5
Percentile 90		42·0		2·8			444		14·8	
Mean (all WWTPs)					92·1					92·4

MG, Moers-Gerdt; RH, Rheinhausen; RB, Rheinberg; KL, Kamp-Lintfort; HO, Hoerstgen; LA, Labbeck; XL, Xanten-Lüttgen; XV, Xanten-Vynen.
P (T), positive samples (total samples).

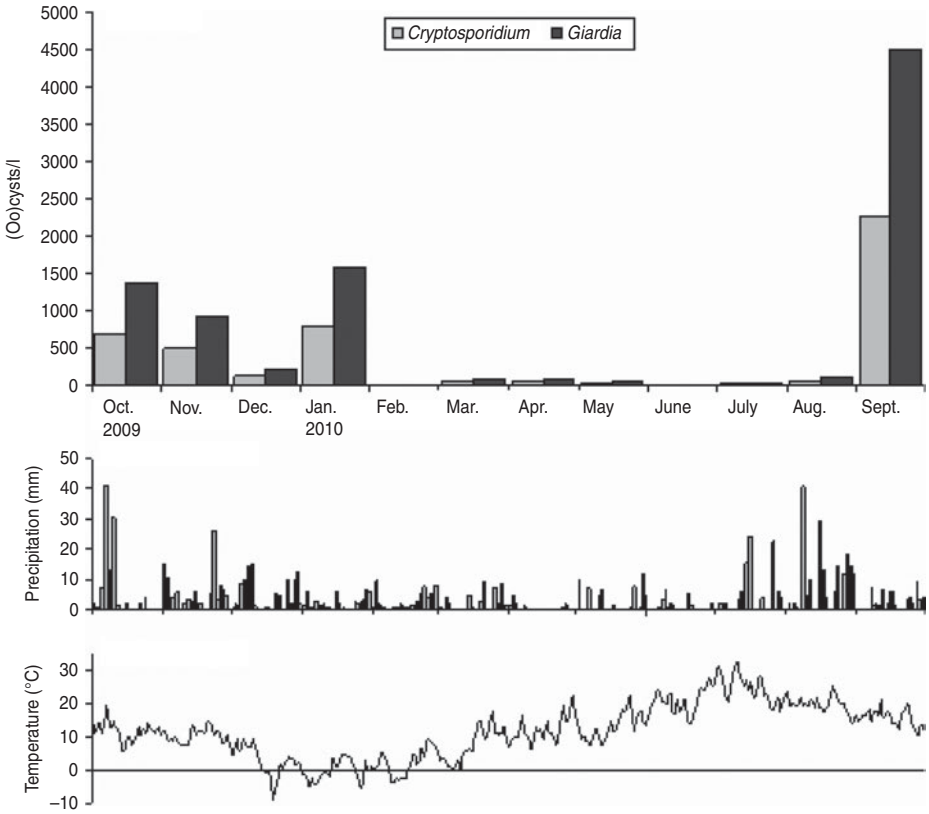

Fig. 2. Numbers of *Cryptosporidium* oocysts and *Giardia* cysts from the influent samples of the wastewater treatment plant, data for precipitation and temperature during October 2009 and September 2010.

throughout winter, depending upon rainfall (Fig. 2). All the WWTPs showed alternating shapes or distinct but smaller peaks in spring and summer (April, May and/or June) for *Giardia* cysts (data not shown).

In plant XV (see Table 1 for names and abbreviations of plants), the highest load of *Giardia* cysts of all the WWTPs during the investigation period was detected on November 2009 (2436 cysts/l), and the highest load of *Cryptosporidium* oocysts on September 2010 (1745 oocysts/l).

Results from the recreational swimming area

Between 2009 and 2011, a total of 77 samples from surface water [recreational sites ($n=54$) and River Rhine ($n=23$)] were investigated.

Cryptosporidium oocysts were detected in nine samples (11·7%). The numbers of oocysts ranged from 20 to 2000 oocysts/100 l (Table 2).

Giardia cysts were detected in eight samples (10·4%). The numbers of cysts ranged from 6·7 to 4000 cysts/l (Table 2).

Results of the examination of the drinking water supply

Of the 113 samples in the catchment area of the drinking water supply during the investigation period of 2009–2010, 14 (12·4%) were contaminated with *Cryptosporidium*.

In the waterworks ~4·5 km away from the riverside, on the side nearest the consumers (final drinking water), three samples were positive for *Cryptosporidium*, with an average of 7·6–16·4 oocysts/1000 l. In raw water, two samples were positive with 3·1–6·5 *Cryptosporidium* oocysts/1000 l. In groundwater, five samples were positive with 4·2–66·4 *Cryptosporidium* oocysts/1000 l. In the water from the Rhine, five samples were also positive with 66·7–250 *Cryptosporidium* oocysts/1000 l (Table 2).

Of the 113 samples in the catchment area of the drinking water supply during the investigation period of 2009–2010, two (1·8%) samples were contaminated with *Giardia*. One groundwater sample had 5·4 cysts/1000 l, and 66·7 cysts/1000 l were detected in one sample collected from the River Rhine.

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Table 2. *Occurrence of* Cryptosporidium *oocysts and* Giardia *cysts in the recreational area, running water, drinking water, raw water, and groundwater between July 2009 and January 2011*

Sampling site (distance from Rhine, km)	Type of water	*Cryptosporidium* spp. oocysts/100 l		*Giardia lamblia* cysts/100 l	
		P (T)	Mean (min-max)	P (T)	Mean (min-max)
Bettenkamper Meer	Recreational area	3 (27)	747 (0–2000)	4 (27)	411 (0–1200)
Aubruch Channel	Running water	1 (27)	20 (0–20)	3 (27)	1673 (0–4000)
River Rhine	Stream	5 (23)	17 (0–25)	1 (23)	6·7 (0–6·7)
Subtotal		9 (77)		8 (77)	
Waterworks 2 (4·5)	Drinking water	3 (24)	1·3 (0–1·6)	0 (24)	0 (0–0)
Waterworks 1 (2·5)	Raw water	2 (23)	0·5 (0–0·7)	0 (23)	0 (0–0)
Radial and vertical well, measure point (1·5, 0·630·25)	Groundwater	5 (66)	2·4 (0–6·6)	1 (66)	0·5 (0–0·5)
P (T)		19 (190)		9 (190)	

P (T), positive samples (total samples).

DISCUSSION

A high variety and combination of methods have been described for the detection of *Cryptosporidium* oocysts and *Giardia* cysts in water samples [14, 18, 19, 20].

In contrast to regular methods, such as USEPA 1623 [18], we chose ARAD filters that offer surface and depth filtration in a compact design, as previously tested [14]. Further advantages include a high filtration rate, easy handling, rapid washing and elution steps, and cost effectiveness. Otherwise, the method needs validation to be accepted as an equivalent of the regular methods or gold standard.

The recovery rates of this study were low in comparison to those reported by other studies (except for the combination *Giardia*/flocculation), although the recovery rates in the literature show a wide range. Recovery rates of $48{\cdot}4 \pm 11{\cdot}8\,\%$ (oocysts) and $57{\cdot}1 \pm 10{\cdot}9\,\%$ (cysts) [21] in filtered tap water have been reported. Inoue *et al.* [16] used the same elution buffer as described in this study with an oocyst recovery rate of $43{\cdot}1 \pm 13{\cdot}9\,\%$. Thus, it may be assumed that the number of (oo)cysts and the number of positive samples was higher than tested and in the future, more specific methods for sampling, preparation and detection must be established.

WWTPs pose an infection risk from both target organisms. As expected, the risk of infection was markedly reduced after the clarifying process, as the number of positive samples in the influent was significantly higher than in the effluent. *Cryptosporidium* oocysts (in six cases) and *Giardia* cysts (in five cases) were higher in the treated than in the raw wastewater samples. The reason for this observation could be the retention time in the WWTPs, which may depend on precipitation. The retention time was not calculated in this study.

The secondary settlement tanks are often frequented by birds, including wild ducks, seagulls, magpies, blackbirds and pigeons. In addition, rodents, particularly mice and rats, are free-living in the catchment area of the WWTPs; therefore, faecal contamination is possible.

In the investigated WWTPs, the average rates of reduction ranged from 73·3% to 100% for *Cryptosporidium* oocysts and from 75·3 and 100% for *Giardia* cysts. In other investigations of six WWTPs from the southwest part of Germany (catchment area of the Swist River), the reduction rates of *Giardia* cysts were slightly higher (86·96–99·97%) [22].

In contrast to the WWTPs with enhanced secondary treatment, the small compact facilities had higher numbers of oocysts in both influent and effluent water, whereas differences in the mean concentrations of the cysts were not observed in influent and effluent water between the two treatment systems (Table 1). The reduction rate for oocysts was more efficient in the compact facilities (94·0%) than in the WWTPs with enhanced secondary treatment (90·2%). The results for *Giardia* cysts were different, with a slightly higher mean reduction rate for the larger plants (93·3%) than for the compact facilities (91·5%). In agreement with this, other authors have reported that the reduction of *Giardia* cysts is higher in plants with tertiary or secondary treatment than in smaller plants with compact facilities [22]. The authors therefore noted the relationship between the removal

Table 3. *Technical data of the wastewater treatment plants. Detailed information concerning design of capacity, retention time, treatment steps and processes, and number of investigated samples*

Wasterwater treatment plant		MG	RH	RB	KL	HO	LA	XL	XV
Catchment area		Urban	Urban	Urban	Urban	Rural	Rural	Rural	Rural
Equivalent of inhabitants in 2009	PE	190 000	175 000	65 000	55 000	1 300	1 800	22 000	3 700
Purified wastewater in 2009	m^3/a	8 801 835	10 519 994	3 527 749	2 411 336	53 185	69 565	1 334 208	248 418
Retention time (min-max)†	h	8–14	10–21	14–27	17–50	5,7–77	7,8–39	12–46	6–38
Primary treatment		Screening and sand-grease separation	Screening and sand-grease separation	Screening and sand-grease separation	Screening and sand-grease separation	Sand-grease separation	Sand-grease separation	Screening and sand-grease separation	Sand-grease separation
Secondary treatment		Activated sludge (2 stages) and sedimentation	Activated sludge (2 stages) and sedimentation	Activated sludge (2 stages) and sedimentation	Activated sludge (2 stages) and sedimentation	Activated sludge (1 stage) and sedimentation	Activated sludge (1 stage) and sedimentation	Activated sludge (1 stage) and sedimentation	Activated sludge, partly membrane bioreactor system (MBR)
Tertiary treatment		None	None	None	None	None	None	None	None
Simultaneous precipitation		Fe salts*	Fe salts*	Fe salts*	Fe salts*	Fe salts	Al salts	Fe salts*	Fe salts
Use of treated water		Discharged to the running water	Discharged to the running water	Discharged to the running water	Discharged to the running water	Discharged to the running water	Discharged to the running water	Discharged to the running water	Discharged to the running water
No. of samples (influent/ effluent)		12/14	12/14	12/14	12/14	11/13	12/14	12/14	12/14

MG, Moers-Gerdt; RH, Rheinhausen; RB, Rheinberg; KL, Kamp-Lintfort; HO, Hoerstgen; LA, Labbeck; XL, Xanten-Lüttgen; XV, Xanten-Vynen.
* In case of dominance of the filamentous Bacteria *Microthrix parvicella* FeCl3 is substituted with Polyaluminate; PE people equivalents; Data from LINEG, Germany, unpublished.
† Depending on precipitation.

efficiencies, the treatment procedures and the size of the WWTPs [22].

The reduction in (oo)cyst number mainly results from precipitation through the activated sludge and not from the disruption or lysis of the (oo)cysts: bacteria are able to build biofilms on surfaces, even on (oo)cyst walls [23]. Most of the bacteria in the activated sludge form flocs, in which (oo)cysts become embedded [24]. After settling, parasites are discarded from the system with the surplus sludge, and flocculation with Al and Fe salts have an additive effect on the sedimentation of flocs and (oo)cysts [25].

In all the WWTPs investigated in this study, Fe or Al salts were added during the treatment process (Table 3). This may lead to a higher reduction rate for flocs and (oo)cysts.

Surplus sludge is treated by additional steps (thickening, anaerobic digestion, drying and heating in a waste incineration plant). The mesophilic fouling process is the state of the art for the stabilization of sewage sludge. Hygienic disinfection is not possible under mesophilic conditions [26]. Therefore, sewage sludge disposal on agricultural areas should be prohibited.

During the investigation period, a typical curve shape was observed for every wastewater plant, with high peaks mostly appearing in autumn and winter, as described for other European countries [27]. The association between the end of the summer and winter holidays with the increase of parasite stages in late summer/autumn and winter could play a role in the spread of disease because diseased travellers returning from endemic areas are more prevalent at these times [28, 29]. Other authors described rising concentrations of (oo)cysts in surface water in spring and summer [30], which may be associated with local conditions or annual variations. This might be the reason that the results from WWTPs RB, KL, and XL showed only single peaks of *Cryptosporidium* spp. over the 1-year period (data not shown).

The Robert Koch Institute in Berlin (RKI, Germany) publishes statistics weekly about the reportable infectious diseases in Germany. In 2009 and 2010, approximately 4000 cases of giardiasis and 1000 cases of cryptosporidiosis were reported. In 2010, the North Rhine-Westphalia region was at the top of the list of the other 15 German states with 720 cases of giardiasis and 187 cases of cryptosporidiosis [31–33]. The weekly number of reported infectious diseases during the period of the study shows an irregular curve shape for giardiasis, whereas an increase in cryptosporidiosis cases was obvious in August 2009 and 2010 (data not shown). Interestingly, this coincides with the observations of the *Cryptosporidium* inflow data from municipal WWTPs.

Jagai *et al*. [34] related the species (genotypes) to seasonal incidences. According to their study, *C. parvum* peaks appear in the late spring, whereas *C. hominis* peaks arise in autumn. The results of Jagai *et al*. [34] are a possible explanation for the different seasonal peaks observed in the present study and should be studied in further investigations.

The concentration of *Giardia* cysts was always higher than that of the *Cryptosporidium* oocysts. This is in agreement with recent studies from other European countries [27, 35].

In an Italian study of the relevant bacterial parameters of the new Drinking Water Directive 98/83/CE [36] and the occurrence of *Cryptosporidium* and *Giardia*, a reciprocal correlation between the levels of the two parasites and intestinal enterococci was found [27]. In the actual study, low or no correlation in wastewater samples was found between *Cryptosporidium* or *Giardia* and other bacterial contaminants [heterotrophic plate count (HPC) 20 °C, HPC 36 °C, total coliforms, *E. coli*, enterococci, *Clostridium perfringens*; data not shown].

The data from the WWTPs demonstrated that effluent water released into the environment, especially to surface water, contains high levels of *Cryptosporidium* spp. and *G. lamblia*. Other sources of pollution, such as the agricultural landscape (spreading of liquid manure or fertilizers from other faecal sources), animal husbandry, and wild and domestic animals, impact the surface running water directly or via leaching, erosion and run-off from adjacent areas.

The amounts of the detected pathogens (up to 2000 *Cryptosporidium* oocysts/100 l and 4000 *Giardia* cysts/100 l) in surface water during our study could cause disease outbreaks due to the low infectious dose of both parasites [$\sim$10 (oo)cysts] [37].

In 2009, 12 samples from the recreational area and its through-running water were collected and processed by flocculation with $Al_2(SO_4)_3$. Despite the low sampling volume of 5 l, two (16·7%) samples were positive for *Cryptosporidium* oocysts and four (33·3%) samples were positive for *Giardia* cysts. During the 2010 bathing season, higher volumes (40–50 l, a total of 42 samples) were filtered, and an increased number of positive results were expected. However, only one sample of the through-running water and two samples from the recreational area were positive for *Cryptosporidium*, although periods

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of heavy rainfall were recorded during the sampling. This was because in March 2010, the regulation of the hydrology of the discharge from rainwater channels was changed by the city of Duisburg before the bathing season (LINEG, unpublished observations). At seven sites, there was discharge from rainwater channels into the running water. At three sites, up to 5 l/s of rainwater drained into the nearby WWTP plant before discharging into the running water. The conditions at one discharge site of the rainwater channel were changed by raising the drainage amount to 10 l/s. The load of contaminants was thereby significantly reduced because the sediments in the rainwater channel were not removed at the same frequency as before March 2010 (LINEG, unpublished observations).

In the catchment area, wastewater from households sometimes flowed into the running water, and it was assumed that parasites could be detected in the whole surface water system. The above-mentioned changes in the regulation of hydrology were successfully and efficiently applied. Hence, neither a higher load nor an increase in the number of positive samples was found.

The River Rhine has a large catchment area and is an important transportation route in Germany. The effluents from numerous WWTPs led directly (plants RH, MG, RB, XL) or indirectly via tributaries (plants HO, KL) into the Rhine. The WWTP MG is the nearest plant that influences the sampling site of the Rhine (distance $\sim$4·5 km). In the River Rhine, five (22%) samples were positive for *Cryptosporidium* and only one (4·3%) for *G. lamblia*. The results are correlated with overflow events. A small-wave flood occurred in December 2010, and another large flood occurred at the beginning of 2011 with the highest water level on 13 January 2011. Increased microbiological loads resulting from heavy rainfall and extreme run-off events were investigated in another study [38].

It has been proven that *Cryptosporidium* oocysts are present in all water sources. As shown in this study, the highest levels were found in running surface waters from the River Rhine. The oocysts were able to infiltrate into the aquifer and pass through the sediments, thereby reaching consumers. From surface water via groundwater to the final drinking water, a reduction of 1–2 orders of magnitude was observed. Karanis *et al*. [39, 40] concluded that water supplies should always follow multiple barrier concepts for the production of drinking water. The technology of the two investigated waterworks consisted of a multi-barrier approach, including UV disinfection resulting in reduction of oocysts. UV light provided an effective reduction in the infectivity of *C. parvum* oocysts [41]. Nevertheless, drinking water is an infection risk to the population, particularly to immunocompromised patients [42–44], especially in cases that are able to decrease the effect of UV radiation, such as massive contamination, turbidity, and precipitation of ferric hydroxide.

G. lamblia cysts were detected in a few samples of surface water and groundwater. The reason for the low number of positive samples is not clear because the effluent from the WWTPs was very large. An explanation for the small number in groundwater and the negative results in the raw and treated drinking water could be the filter effect of the sand and gravel layers. Perhaps filtration by the riverbank is more effective for the retention of *Giardia* cysts but less for *Cryptosporidium* oocysts because the cysts ($\sim$10–12 μm) are twice as large as the oocysts ($\sim$5–6 μm). In a study by Hijnen *et al*. [45], a microbial elimination rate of $>$2 log during soil passage was determined. The authors used column experiments with soil, sand and gravel and showed that the removal efficiency of bacteria and bacterial spores was four- to fivefold that of *Cryptosporidium* oocysts (*Giardia* cysts were not included in these experiments) [45]. Other experiments showed roles for attachment, detachment, and straining in *Cryptosporidium* oocyst retention. Oocyst retention increased from 68% and 79% to 87% when filter columns were filled with defined grain sizes of 710 and 360 to 150 μm, respectively [46]. Monitoring of riverbank-filtrated waters and river waters in Ohio (USA) has been conducted, and *Cryptosporidium* and *Giardia* were occasionally detected in river water but never in well water [47]. Further research is needed to better understand the relationship between the transport of (oo)cysts during passage and the effects of water and sediment characteristics on removal efficiency [47].

There are some original publications from Germany regarding the incidence of *Giardia* and *Cryptosporidium* cases. The prevalence of (oo)cysts in water has been confirmed [22, 48], and a small number of waterborne outbreaks have been reported in Germany [10, 11, 49, 50]. In 2001, 201 (n=450) soldiers were infected with *C. parvum* after field training. Aetiology showed statistical correlations between the consumption of drinking water or various meals and cryptosporidiosis during the field exercise, but the study could not identify the source of infection [49].

A giardiasis outbreak in a small community in Rhineland Palatinate, Germany was reported in 2000. The drinking water caused a remarkable number of *Giardia* infections, and the authors detected inadequate treatment of the drinking water [50]. Karanis *et al*. [39, 40] emphasized the need for further investigations for, 'the determination of the origin of *Giardia* and *Cryptosporidium* in the catchment areas of surface water and ground water supplies from which drinking water is drawn'. This suggestion has been approved, and interesting findings on surface and drinking water contamination via muskrats [51] and wild rodents [52] have arisen: 75·2% of 234 investigated muskrats, 77·5% of 40 cattle and 47·7% of 216 rodents were positive for *Giardia*, and the authors concluded that free-living, grazing, wild and domestic animals are able to contaminate surface waters with (oo)cysts, which could be spread via water.

In a retrospective case-control study, about 700 cases of infection were evaluated by Dreesmann *et al*. in Lower Saxony [53]. This study emphasized 'that the increased regional incidence rate caused by the broader diagnostic activity of this laboratory rather reflects the real occurrence of this infection. Hence, in other regions with lower incidence rates of notified cases underestimation can be presumed' [53].

The index of human cryptosporidiosis and giardiasis in Germany as reported in the statistics of the RKI does not include information about the source of infection [33]. It is expected that the number of cryptosporidiosis and giardiasis cases associated with water contamination is underestimated [11]. This reflects statements already made [11] and should apply to surveillance systems worldwide. Furthermore, this fact reinforces the need for investigations and monitoring programmes to prevent an effect on public health.

There is a lack of research on *Cryptosporidium* and *Giardia* in different water matrices, even though water is a source for the dissemination of these waterborne parasites. Many established detection methods exist that require skilled employees and expensive equipment. We used an easy-to-handle and inexpensive combination method including ARAD filters, aluminium sulphate flocculation and IFT. In the future, other effective and suitable methods may overcome the inhibition threshold to implement statutorily regulated monitoring for parasites. The present study illustrates the prevalence of parasites in different sources of environmental waters. It demonstrates the retention and reduction of (oo)cysts by wastewater treatment, riverbank filtration, passing gravel layers of the aquifer, and raw water treatment by waterworks.

The study gives an overview of the occurrence and distribution of *Cryptosporidium* oocysts and *Giardia* cysts in the water of a large area (650 km²) and can serve as a representative study for other regions worldwide.

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DECLARATION OF INTEREST

None.

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