THE OCCURRENCE OF WATERBORNE PATHOGENIC PROTOZOA IN ENVIRONMENTAL WATER SAMPLES, THEIR REDUCTION BY WASTEWATER TREATMENT AND DISSEMINATION IN THE HYDROLOGICAL CIRCUIT

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vorgelegt von
Carmen Gallas-Lindemann
aus Krefeld

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Berichterstatter: Prof. Dr. Hartmut Arndt

Prof. Dr. Sigrun Korsching

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Abbreviations

a	Year
BMJ	Bundesministerium der Justiz -Federal Ministry of Justice
CFU	Colony-forming units
d	Day
DAPI	4′,6-Diamidino-2-phenylindole
DDW	Double distilled water
df	Degrees of freedom
DICM	Difference interference contrast microscopy
DMSO	Dimethyl sulphoxide
EC	European Commission
EDTA	Ethylenediaminetetraacetate
EU	European Union
FITC	Fluorescein isothiocyanate
h	Hour
HO	Hoerstgen
HPC	Heterotrophic plate count
IFT	Immonofluorecence test
IMS	Immunomagnetic separation
KL	Kamp-Lintfort
LA	Labbeck
LAMP	Loop-mediated isothermal amplification
LANUV NRW	Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen - State Agency for Nature, Environment and Consumer Protection North Rhine Westphalia
LINEG	Linksniederrheinische Entwässerungs-Genossenschaft
MBR	Membrane bioreactor
MG	Moers-Gerdt
n	Number
NABU	Naturschutzbund Deutschland e.V. – Nature and Biodiversity Conservation Union Germany
nPCR	Nested polymerase chain reaction
p	Level of confidence
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction

PE	People equivalents
PET buffer	Pyrrophosphate EDTA Tween buffer
RB	Rheinberg
RH	Rheinhausen
RKI	Robert Koch Institut
RT-PCR	Real-time polymerase chain reaction
t0	Dimension of distances between arithmetic means in relation to standard deviation
UN	United Nations
USEPA	U.S. Environmental Protection Agency
UV	Ultra-violet
WHG	Wasserhaushaltsgesetz - Water Resources Act
WHO	World Health Organisation
WVN	Wasserverbund Niederrhein
WWTP	Wastewater treatment plant
XL	Xanten-Lüttingen
XV	Xanten-Vynen

1 Introduction

1.1 General Introduction

Water plays an important role in the transmission of pathogens. Waterborne pathogens are of high relevance in medicine research since human beings investigate in diseases. Pathogens are roughly categorised in five groups: viruses, bacteria, protozoa, fungi and helminths. Bacterial diseases were researched well in the last two centuries by Robert Koch, when cholera outbreaks were on a high level (Grüntzig et al. 2010, Erer et al. 2010). Extensive investigations in drinking water treatment, sewage treatment, monitoring of drinking water sources regulated by law etc. led to higher hygienic standards. Thus, the problem was widely brought under control in the developed countries. Drinking water is the best investigated foodstuff in industrialized countries.

Nevertheless, 884 million people worldwide live without access to safe drinking water and sanitation (UNICEF and WHO, 2008). Therefore, the human right to water and sanitation has been accepted by the UN resolution in July 2010 (UN, 2010). This was an important step, because waterborne pathogens are relevantly causative agents that induce diarrhoeal illness, which is a main cause of mortality in children under the age of five years and otherwise immunocompomized people in the developing world (Sheth et al., 2010; Parashar et al., 2003).

Cryptosporidium and *Giardia duodenalis* are major pathogens in waterborne transmission of infections. The worldwide annual new infection rate of *G. duodenalis* is 2.8×10^8 and of *Cryptosporidium* it is 300 000, respectively (Lane and Lloyd, 2002; Mead et al., 1999). Studies about the prevalence of *G. duodenalis* and *Cryptosporidium* spp. are rare in the developing countries. The infrastructure for health care is not well established. Laboratories are often under-resourced and there is a lack of skilled employees. Monitoring and safety plans for health care of the population are generally not available.

From deficiency of toilets and WWTPs contamination of the surface water, which serves as reservoir for drinking water, transmission of waterborne pathogens is possible (Snelling et al., 2007; Lim et al., 2007).

Therfore, high prevalence of giardiasis and cryptosporidiosis is significant in developing countries. Especially children, AIDS patients and malnourished people are affected. Diarrhea is the leading cause of deaths; 30 to 50% of childhood mortality is caused by diarrhea (Snelling et al., 2007).

In a Malaysian study wastewater from two WWTPs were investigated. *G. duodenalis* was present in 100% of influent and effluent samples and *Cryptosporidium* in 50 % of the influent and 25% of the effluent samples (Lim et al., 2007).

Compared to the developed countries WWTPs do not have state-of-the-art technology in the developing world. E.g. in Malaysia 50% of the WWTPs have only primary treatment process. In rural areas WWTPs are not available (Lim et al., 2007).

In developing countries necessary funds are rare to invest in WWTPs, sanitation, drinking water treatment and expensive test kits for monitoring programs (Snelling et al., 2007).

Cryptosporidium and *Giardia* are distributed worldwide and cause diseases of the intestinal tract in vertebrates (Mircean et al., 2011). Affected hosts include humans (Thompson and Smith, 2011; Mircean et al., 2011) as well as wild (Ravaszova et al., 2011; Siembieda et al., 2011; Bitto and Aldras, 2009; Levecke et al., 2011) and domestic animals (Budu-Amoako et al., 2012; Ferreira et al., 2011; Coklin et al., 2010; Mark-Carew et al., 2010). Infection causes diarrhea and is self limiting within a few days (Petry et al., 2010). Due to this fact patients usually do not seek medical advice. The actual epidemiological situation can only be estimated. Giardiasis and cryptosporidiosis are life threatening infectious complications that occasionally occur in immunosuppressed patients like children under the age of five years, HIV-infected patients, patients undergoing chemotherapy or organ transplantation and elderly people (Furio and Wordell, 1985). Autoinfection, which has negative effects on health for these patients, is possible (Stürchler 1987). Also extra intestinal infections have been described (Nagazaki et al. 2011). (Oo)cysts, the parasitic stages which are excreted with faeces, are resistant against environmental and chemical influences (e.g. chlorination and UV radiation). The treatment with drugs is possible for giardiasis (Gardner and Hill, 2001; Solaymani-Mohammadi et al., 2010) but not for cryptosporidiosis.

In livestock, economic losses in productivity and animal mortality are often observed, especially in juvenile cattle (Tiranti et al., 2011). The costs of health care and the

non-productive time of employees in case of illness are presumptively enormous (Karanis et al., 2007).

(Oo)cysts are able to persist in the environment for months, different transmission cycles are possible; one of the most important is waterborne distribution. The occurrence of *Cryptosporidium* oocysts and *G. duodenalis* cysts in different types of water has been confirmed, and a considerable number of waterborne outbreaks has been reported worldwide (Karanis et al., 2007; Baldursson and Karanis 2011).

Toxoplasma gondii (Phylum Apicomplexa) is a protozoan pathogen that is phylogenetically closely related to *Cryptosporidium*. *T. gondii* also occurs worldwide and infects humans as well as other vertebrates. During disease progression, tissue cysts are formed, followed by the multiplication of the organism within the host cell cytoplasm (Hutchison et al, 1970). Swelling of the lymph nodes, muscle pain and fever are the most common symptoms of toxoplasmosis, while cysts seldom occur in heart, liver and spleen. Intrauterine infection may exert negative effects on a foetus if the mother is infected for the first time during the third trimester of pregnancy (Kaye, 2011; Olariu et al., 2011). Toxoplasmosis is a self-limiting disease in immunocompetent individuals. Among immunocompromised patients, it often results in morbidity and mortality (Bruck et al., 2010; Nissapatorn, 2009; Utsuki et al., 2011).

T. gondii oocysts are excreted with the faeces of Felidae (Dubey, 1998). Oocysts are able to enter and circulate in terrestrial and aquatic environments. Moreover, these robust parasitic stages are capable of persisting for an extended time in the environment and are highly resistant to various chemicals and disinfection methods that are commonly used by the water supplying industry (e.g. filtration, chlorination, ozonation and radiation) (Dubey, 1998). Water plays an important role in the dissemination of human toxoplasmosis (Dubey, 1998). Therefore, the analysis of *T. gondii* contamination in water samples provides insight into the potential risk of waterborne infections that affect humans and animals.

Several waterborne toxoplasmosis outbreaks have been documented since 1979, including cases in Panama (Benenson et al., 1982), British Columbia (Bowie et al., 1997), Brazil (Keenihan et al., 2002) and four additional outbreaks described in a recent review by Baldursson and Karanis (Baldursson and Karanis, 2011).

There is a rising interest in waterborne diseases and many international scientific investigations are published worldwide. In the past investigations were concentrated on the United States and United Kingdom, but only a few publications are recognized

from Germany. One reason might be the intricate preparation of samples to find parasitic stages in water matrices. The distribution via waterways could be enormous.

The study area of this work is located in Germany and belongs to the administrative district of Düsseldorf in the federal state of North Rhine-Westphalia, which is, in the geographical classification of natural landscapes, a part of the lowland plain of the Lower Rhine (Paffen et al., 1963).

On Lower Rhine area densely populated urban structures exist as well as intensive and extensive used agricultural structures. In the geological history glacial and interglacial changes left a typical lower Rhine terrasse landscape depending on changes of the River Rhine (Schirmer 1994, Schirmer 1990). Many slow running water bodies are distinctive for the Lower Rhine area. Hundreds of kilometres of running water pick up different kinds of material by erosion, losses by surface runoff and other influences. Treated wastewater of eight municipal sewage water plants in the study area drain into them and distribution of parasites could be implicated.

Reasoned by the small difference between the surface of the landscape and the groundwater level, an influence of protozoan parasites on the groundwater has been supposed. This could also be due to riverbank filtration of the River Rhine situated nearby. In addition the catchment area for the drinking water supply is located in close vicinity to the River Rhine.

The purpose of this work was to investigate the occurrence and distribution of parasites on the Lower Rhine in Germany.

Wastewater treatment plants are considered to be a vast source of parasitical contaminations. Immission of protozoan parasites into surface water, groundwater, raw and drinking water is considered probable. The scales of parasites in the different water matrices and their retention should be investigated. Based on the results the risk to human health should be discussed in the relation to drinking water consumption or bathing in surface waters.

Depending on the expected contamination different sampling techniques should be used. The equivalence of the molecular assay PCR and an emerging assay (LAMP) to the conventional Immunofluorescence Test (IFT) and Difference Interference contrast Microscopy (DICM) should be compared.

The LAMP should be further tested for the detection of *T. gondii*. The investigations aimed to raise the attention on the risk of toxoplasmosis outbreaks.

1.2 Introduction of the target parasites

1.2.1 Cryptosporidium

Cryptosporidium is an apicomplexan protozoon (Phylum: Apicomplexa, Class: Sporozoea, Subclass: Coccidia, Order: Eucoccidia, Suborder: Eumeriina, Family Cryptosporidiidae, Genus: *Cryptosporidium*) (Plutzer and Karanis, 2009; Mehlhorn and Piekarski, 1998). To date 21 valid species of the genus *Cryptosporidium* are known (Table 1; Plutzer and Karanis, 2009; Smith et al., 2010). The parasite is capable of infecting humans and other vertebrates. The species and their designated hosts are listed in Table 1. Eight Cryptosporidium species are known as human pathogens (*C. hominis*, *C. parvum*, *C. meleagridis*, *C. felis*, *C. canis*, *C. suis*, *C. muris*, and *C. andersoni*) (Smith et al., 2010). Additionally, five out of the 61 genotypes with uncertain species status (Plutzer and Karanis, 2009) infect immunocompetent and immunocompromized humans (Smith et al., 2010).

Cryptosporidium was firstly described in 1907, but at that time it was not noticed as a pathogen (Tyzzer, 1907). In the first reported outbreak diarrhoeal disease of calves infected by *Cryptosporidium* was mentioned (Panciera et al., 1971). In 1976 a three year old child (Nime et al., 1976) and an immunosuppressed patient (Meisel et al., 1976) were infected by *Cryptosporidium* causing diarrhoeal disease. Later it was recognized that water plays an important role in the dissemination of Cryptosporidium. In 1993 *Cryptosporidium* challenged attention, when the largest documented outbreak of waterborne disease in the United States caused an epidemic with 403 000 infected people and with potentially 112 deaths (MacKenzie et al., 1994). Since then *Cryptosporidium* was of highest interest in scientific research. A worldwide overview of the waterborne outbreaks is given in Karanis et al (2007) and Baldursson and Karanis (2011). Cryptosporidium was the etiological agent in 60.3% of the outbreaks (Baldursson and Karanis, 2011).

	Species	Major host(s)	Minor host(s)	Site of infection
1	C. andersoni	Cattle, Bactrican camel	Sheep	Abomasum
2	C. baileyi	Poultry	Quails, ostriches, ducks	Cloaca, bursa, trachea
3	C. bovis	Cattle	Sheep	Small intestine
4	C. canis	Dogs	Humans	Small intestine
5	C. fayeri	Red kangaroo		Small intestine
6	C. felis	Cats	Humans, cattle	Small intestine
7	C. fragile	Amphibia		Stomach
8	C. galli	Finches, chicken		Proventriculus
9	C. hominis	Humans	Dugong, sheep	Small intestine
10	C. macropodum	Eastern grey kangaroo		Small intestine
11	C. meleagridis	Turkey, humans	Parrots	Small intestine
12	C. molnari	Fish		Stomach (and intestine)
13	C. muris	Rodents	Humans, rock hyrax, mountain goat	Stomach
14	C. parvum	Cattle, livestock, humans	152 mammalian species, deer, mice, pigs	Small intestine
15	C. ryanae	Cattle, Bos taurus		Small intestine
16	C. scophthalmi	Fish		Intestine (and stomach)
17	C. serpentis	Lizards, snakes		Stomach
18	C. suis	Pigs	Humans	Small and large intestine
19	C. varanii syn. saurophilus	Lizards	Snakes	Stomach and small intestine
20	C. wrairi	Guinea pig		Small intestine
21	C. xiaoi	Sheep	Yak, goat	Not known

Table 1: *Cryptosporidium* species with information on organ locations, major and minor hosts. (Modified - Plutzer et al, 2009, Smith and Nichols, 2009).

The robust oocysts (round to ovoid, 5 – 6 µm; Figure 2) are the infective stages of the parasite, which shed into the environment by hosts and are able to persist there for month. Oocysts are extremely virulent; only few oocysts (1 to 10) are capable to infect the host. Mainly, faecal-oral transmission and foodborne and waterborne infections are possible.

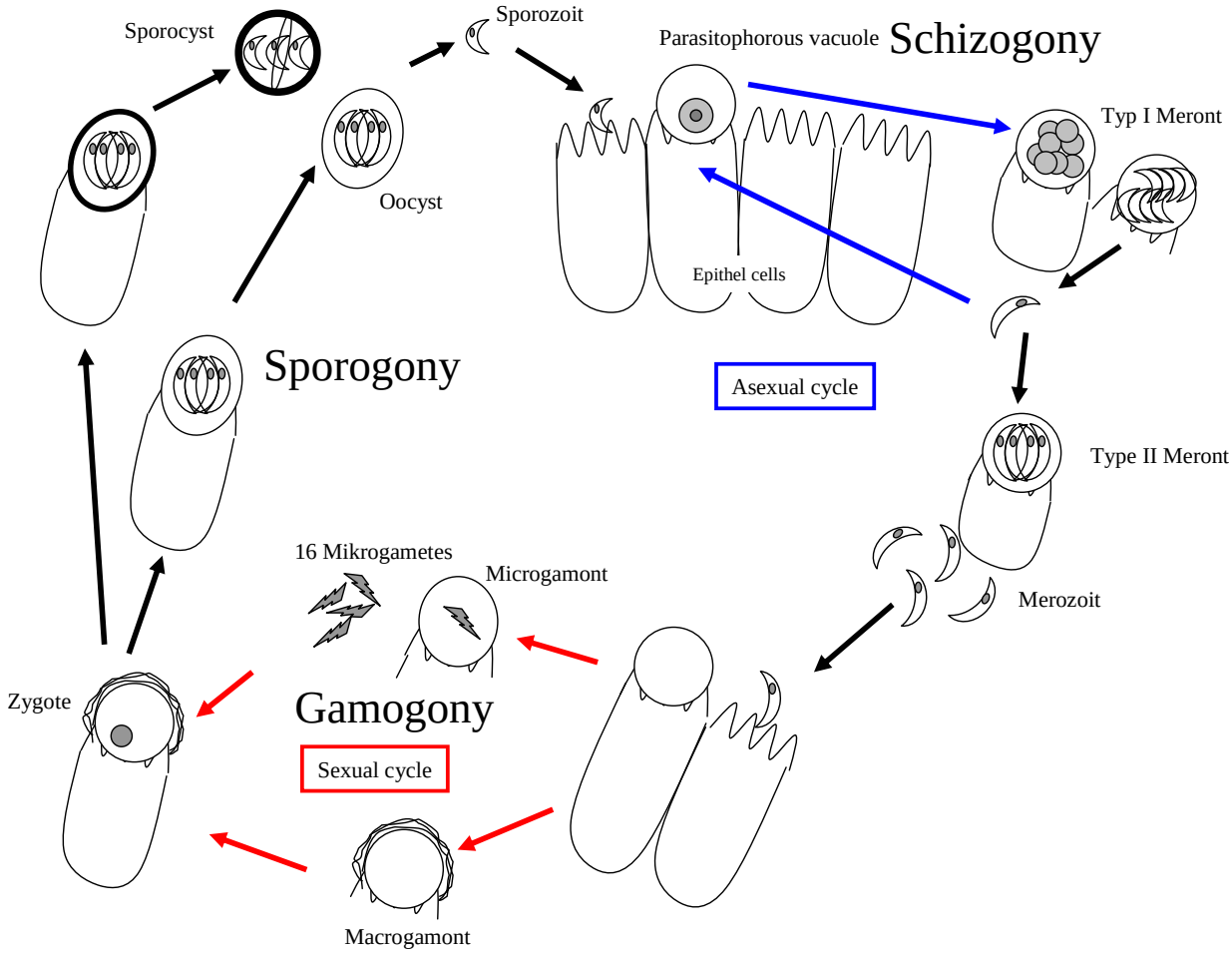

Figure 1: Life cycle of Cryptosporidium parvum (Mehlhorn and Piekarski, 1998, modified).

The monoxenous lifecycle is coccidian-like with division into the three stages schizogony (or merogony), gamogony and sporogony Figure1). The cycle begins with the ingestion of the sporulated oocysts (transmitted into the sporocyst-form). Each sporocyst contains four sporozoites. In the small intestine of the host the sporozoites leave the sporocyst. After that each sporozoite penetrates an epithelial cell, forming an intracellular but extracytoplasmatic parasitophorous vacuole. In the vacuole cell division generating the schizont or meront type I (each including 8 merozoites) is visible. The liberated merozoites are able to penetrate non-infected epithelial cells for further asexual multiplication (schizogony or merogony). In addition the developement of meront types II is possible. Mature type II meronts with four merozoites, which liberate the vacuole and infect new host cells, undergo either further merogony or initiate gamogony (sexual reproduction). For this purpose they invade host cells again and differentiate into the female macrogamont or into the male microgamont. The microgamont develops into a microgametocyte with up to 16

microgametes. Those sperm cell equivalents later fertilize the macrogamonts. A zygote is formed after fertilization and the parasite undergoes asexual multiplication in the sporogony. The sporogony results in the development of sporulated oocysts each containing four sporozoits. The zygote is able to form thin- or thick-walled oocysts. The thin-walled oocysts represent about 20% of all newly sporulated oocysts and are responsible for autoinfection. The thick-walled oocysts are excreted by the faeces in order to infect other hosts. Excretion of sporulated and unsporulated oocysts is possible. The sporulation of this unsporulated form into infective sporocysts then emerges in the environment (Mehlhorn and Piekarski, 1998).

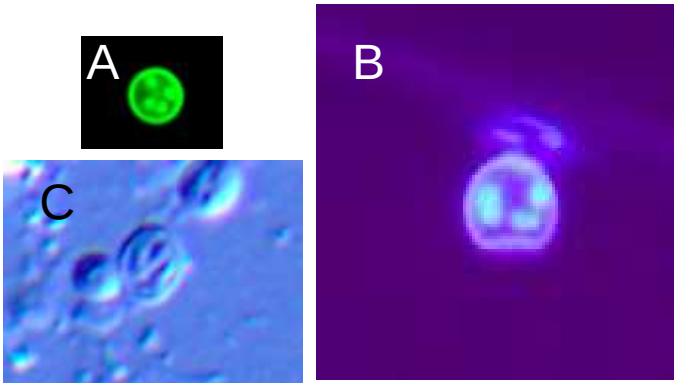

Figure 2: *Cryptosporidium* oocysts fluorescently labelled A: with monoclonal antibodies (fluorescein isothiocyanate, FITC and B: with 2-(4-amidinophenyl)-6-indolecarbamidine di-hydrochloride, DAPI and C. DICM (magnification 1000x).

Symptoms of the disease

The incubation period varies between two and ten days. Clinically asymptomatic run of the cryptosporidiosis in immunocompetent persons is possible, although excretion of oocysts can not be excluded (Arrowood, 1997).

The main symptoms of the disease are watery diarrhea, dehydration, fever, anorexia, weight loss, weakness, abdominal cramps, vomiting, lethargy, general malaise and progressive loss of overall condition (Hunter et al., 2004).

The disease is self-limiting and the symptoms usually last from several days to two weeks. Depending on the immune status symptoms may be more acute in immunocompromised persons often leading to mortality (Clifford et al., 1990). Anti-cryptosporidial drugs are not available and preventive vaccination is not possible.

1.2.2 Toxoplasma gondii

Toxoplasma gondii is an apicomplexan protozoon (Phylum: Apicomplexa, Class: Sporozoea, Subclass: Coccidia, Order: Eucoccidia, Suborder: Eumeriina, Genus: *Toxoplasma*) (Mehlhorn and Piekarski, 1998). *T. gondii* is the only species of this genus.

T. gondii was firstly described after it has been discovered in an African rodent in 1908 (Nicolle and Manceaux, 1909).

The first case of toxoplasmosis of a human being was reported in 1923, when neonatal infection occurred in an 11-month old child (Wolf et al., 1941). Since the 1980s, toxoplasmosis gains higher interest due to the increase of immunosuppressed patients. An outbreak associated with water appeared in 1979 in Panama. The infection has been assumed to be related to the consumption of uncooked creek water contaminated with oocysts by jungle cats; 32 soldiers were affected (AWWA, 1999). The greatest outbreak so far affecting thousands of people was associated with municipal drinking water in British Columbia during September 1994 and March 1995. The contamination was possible due to treatment deficiencies in a water reservoir serving the population with potable water (Bowie et al., 1997).

The robust oocysts (round to ovoid, 9 to 15 µm) are the infective stages of the parasite, which are shed by hosts into the environment and are able to persist for month. Oocysts are extremely virulent; only few oocysts (1 to 10) are enough to infect the host.

The heteroxenous lifecycle is coccidian-like with the division into the three phases schizogony (or merogony), gamogony and sporogony, including facultative change of host (Figure 3; Mehlhorn and Piekarski, 1998).

The asexual replication is possible in almost any warm-blooded animal. Sexual development only occurs in felids (Fritz et al., 2012). The main hosts of *T. gondii* are felidae in which all phases of the coccidian lifecycle appear until the shedding of the oocyts. Infection of the cats is possible by the three stages oocyst, pseudocyst or tissue cyst. After sporulation in the environment infection of a further felid host or another intermediate host is possible. Subsequent to the excretion of the oocysts sporulation of two sporocysts each containing four sporozoites arises.

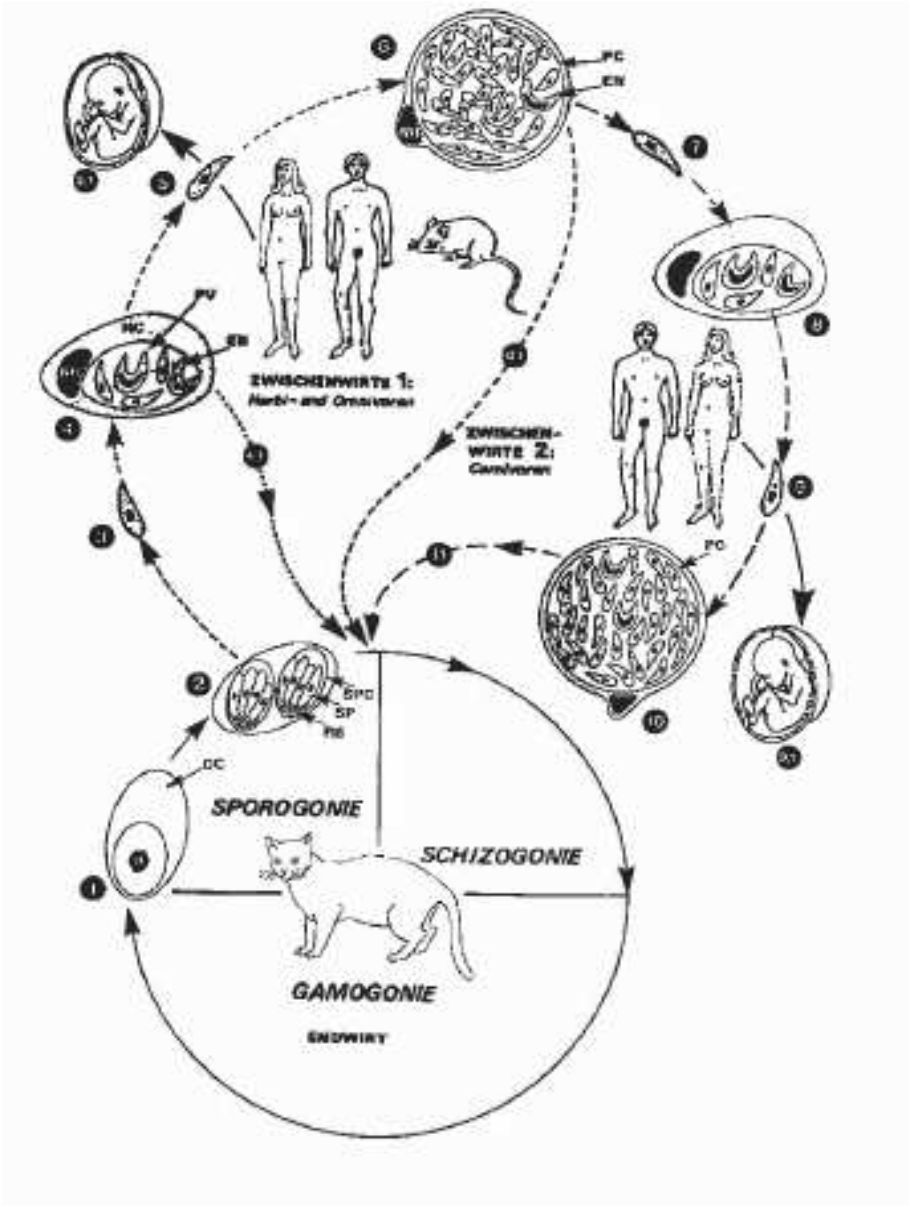

Figure 3: Life cycle *Toxoplasma gondii* (1-11 Cycles of the intermediate hosts, 5.1 and 9.1 Diaplacental transmission, EN = Endodyogeny, HC = Host cell, NH = Nucleus of the host, OC = oocysts, PC = Primary cyst wall, PV = Parasitophorous vacuole, RB = Residual body, SP = Sporozoit, SPC = Sporocyst; Mehlhorn and Piekarski, 1998).

Subsequently the ingestion the sporozoites release the oocysts in the intestine of the intermediate host. Invasion of different tissue cells is possible where the sporozoites penetrate the epithelial cells. Multiple cell divisions (endodyogeny) in the parasitophorous vacuole form a so-called pseudocyst. Additionally tissue cysts mainly in brain and muscle cells of the intermediate host emerge. After multiple endodyogeny the tissue cyst is filled with infective cystmerozoites (dormozoites, bradyzoites). Merozoites of the prey develop to schizonts if they are ingested by the

cat. Altenatively reproduction via pseudocyts is possible, if they are ingested by another carnivore intermediate host.
A characteristical feature in the life cycle of *T. gondii* is the diaplacental transmission and intrauterine infection of foetus. The sexual cycle in the cat's intestine is similar to the gamogony of *Cryptosporidium* in the differentiation of macro- and micro-gametes that form a zygote after fertilization (see above). With the development of the zygote the sporogony is initiated (Mehlhorn and Piekarski, 1998).

Symptoms of the disease

In immunocompetent persons toxoplasmosis generally runs clinically asymptomatic. Swelling of the lymph nodes, muscle pain and fever are the most common symptoms of acute toxoplasmosis, while cysts seldom occur in heart, liver and spleen. Intrauterine infection may exert negative effects on the fetus like hydrocephalus, chorioretinitis and calcifications in the brain if the mother is infected for the first time during the third trimester of pregnancy (Kaye, 2011; Olariu et al., 2011; Mehlhorn and Piekarski, 1998). Toxoplasmosis is a self-limiting disease in immunocompetent individuals. Among immunocompromised patients, it often results in morbidity and mortality (Bruck et al., 2010; Nissapatorn, 2009; Utsuki et al., 2011).
Treatment with drugs is possible in considerable course of the disease or in pregnant women, but preventive vaccination is not available.

1.2.3 Giardia duodenalis

Giardia duodenalis (Syn. *G. lamblia*, *G. intestinalis*) is a diplomonadid flagellated protozoan parasite. New systematical classification is based on genetic, structural and biochemical data: Phylum: Metamonada, Subphylum: Trichozoa, Superclass: Eopharyngia, Class: Trepomonadea, Subclass: Diplozoa, Order: Giardiida, Family: Giardiidae (Cavalier-Smith, 2003).
Six species of the genus exist (*G. duodenalis*, *G. agilis*, *G. muris*, *G. psittaci*, *G. ardae* and *G. microt*i). *G. duodenalis* is further divided in different genotypes, assemblages and subassemblages (A – G), resulting from sequence differences in the genes. Only the assemblages A (subassemblages AI and AII) and B

(subassemblages BIII and BIV) are recognized as human pathogens (Plutzer et al., 2010).

The organism is able to infect humans and other vertebrates. The genus was firstly detected by Anthony van Leuwenhoek (1681), when he microscopically examined his own stool after he had a diarrhoeal sickness. The first detailed description of the trophozoite in 1859 was made by Lambl, and in 1879 Grassi detected robust parasitic stages (cysts) as a part of the lifecycle (Ansari, 1954; Mehlhorn und Piekarski, 1998).

The former name of the genus *Lambl* has been changed to *Giardia*. The whole name (genus and specific epithet) was determined by Stiles 1915 to appreciate the French zoologist Alfred Giard (Ansari, 1954).

The excreted resistant cysts (Figure 4; ovoid, about 10 to 12 µm) are the infectious stages of the parasite and are able to persist in the environment for months (Mehlhorn and Piekarski, 1998). Faecal-oral infection is possible including foodborne and waterborne transmission. The parasite is highly virulent; only 1 to 10 cysts are capable to cause giardiasis (Rendtorff, 1954).

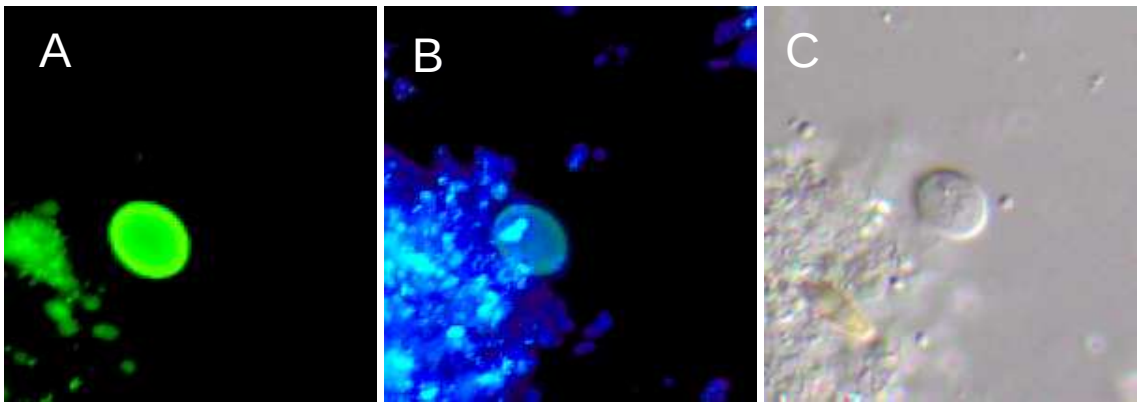

Figure 4: *Giardia duodenalis* cysts fluorescently labelled A: with monoclonal antibodies (fluorescein isothiocyanate, FITC and B: with 2-(4-amidinophenyl)-6-indolecarbamidine di-hydrochloride, DAPI and C. DICM (magnification 1000x).

After oral ingestion rupture of the cysts and duplication of the cell into two trophozoites develops. The trophozoites attach on the epithelium cells of the duodenum with their ventral side. Nutrition by phagocytosis occurs on the dorsal side of the trophozoite. Multiple binary fission of the trophozoites resulting in manifold reproduction leads on to invasive growth of the trophozoites in the intestine.

Trophozoites reaching the rectum form a robust cyst wall, retract the flagella and division of the nucleus follows before the cysts are excreted with the faeces.
While nucleus division already appears in the cysts, the ultimate cell division takes place only after the infection of the new host. In the gut of the host, cysts release trophozoites beginning a new life cycle (Mehlhorn und Piekarski, 1998).

Symptoms of the disease

The incubation period varies between one and twelve days, rarely weeks. Giardiasis is a self-limiting disease in immunocompetent individuals. Clinically asymptomatic run of the giardiasis in immunocompetent persons is possible, although excretion of cysts can not be excluded.
The main symptoms are diarrhoea, bloating, weight-loss, malabsorption, flatulence, abdominal cramps, nausea and vomiting, fatigue, anorexia and chills (Thompson, 2000).
Treatment with drugs is possible in considerable course of the disease or in chronically giardiasis, but preventive vaccination is not available.

1.3 Loop-mediated isothermal amplification (LAMP)

The newly emerging amplification method based on the specific detection of genomic DNA called loop-mediated isothermal amplification (LAMP) has not become common in Germany, yet.
Worldwide LAMP had been utilized for a broad spectrum of applications in the biomedical field including the detection of viruses, bacteria, fungi and parasites, as well as genetically modified organisms, the identification of embryo sex and tumor detection (Karanis and Ongerth, 2009; Fu et al., 2010). LAMP is highly specific, efficient, simple and rapid and the amplification runs under isothermal conditions. No specialized heating equipment is required and the amplification of the target is complete within 60 min (Notomi et al., 2000; Karanis and Ongerth, 2009; Fu et al., 2010).
LAMP is a molecular nucleic acid amplification technique that uses a polymerase with strand displacement activity and there is no need to use heat denaturation of double-stranded DNA products to initiate the next amplification step as stringently

required in the Polymerase Chain Reaction (PCR). LAMP runs under isothermal conditions and the reaction may take place in a waterbath instead of an expensive thermal cycler. LAMP benefits the PCR by the use of four or six primers that identify six or eight distinct regions of the target DNA segments resulting in a higher specifity (Notomi et al., 2000).

In the first step of the LAMP all primers were needed to receive the loop formed structure on both ends of the target. This dumb-bell formed DNA strand converts immediately into a stem-loop DNA which serves as the template of the ultimate reaction (LAMP cycling). Further cycling processes are resulting in a great amount of copies of the target DNA with multiple loops (cauliflower-like structure) (Notomi et al., 2000). The positive LAMP reaction can either be visualized by naked eyes, because of the white magnesium pyrophosphate precipitation in the test tube, or by performing the gel electrophoresis (Goto et al., 2009).

1.4 References

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2 Circulation of Cryptosporidium and Giardia in wastewater and the surface, drinking and ground waters in the Lower Rhine, Germany

Abstract

A total of 396 samples from different water sources were collected in the period from July 2009 to January 2011. In- and outflow samples were collected over 24 h from sewage plants. Wastewater samples (2 l of influent and 5 l of effluent) were collected and purified by aluminium sulphate flocculation. *Cryptosporidium* oocysts and *Giardia* cysts were further concentrated by sucrose centrifugation. Up to 400 l of surface water and 6400 l of ground water and drinking water were collected by micro fibre filtration over a 24-h period for each sample.

(Oo)cysts were identified by IFT in combination with DAPI and DICM at magnifications of 400x and 1000x. Out of 95 raw wastewater samples, 78 (82%) were found to be positive for *Giardia* cysts and 46 (44%) were positive for *Cryptosporidium* oocysts. Out of the 111 samples of treated wastewater, 56 (50%) were positive for cysts and 18 (16%) were positive for oocysts. Parasite numbers ranged from 0-2436 cysts and 0-1745 oocysts /l in influent samples and 0-56 cysts and 0-36 oocysts /l in effluent samples. The elimination rate of (oo)cysts during wastewater treatment was approximately 92%.

Ten out of the 113 drinking water samples were found to be positive for *Cryptosporidium* oocysts (0–6.64 /100 l), and only one sample was found to be positive for *Giardia* cysts (0.54 /100 l). Nine out of the 77 surface water samples were found to be positive for *Cryptosporidium* oocysts (0-2000 /100 l) and eight for *Giardia* cysts (0-4000 /100 l).

This study provides substantial evidence that *Giardia (lamblia) duodenalis* cysts and *Cryptosporidium* spp. oocysts are able to enter and circulate in the aquatic environment with negative implications on public health.

2.1 Introduction

Cryptosporidium and *Giardia* parasites are distributed worldwide and cause diseases of the intestinal tract in vertebrates (Mircean et al., 2011). Affected hosts include humans (Thompson and Smith, 2011; Mircean et al., 2011) and wild (Ravaszova et al., 2011; Siembieda et al., 2011; Bitto and Aldras, 2009; Levecke et al., 2011) and domestic animals (Budu-Amoako et al., 2011; Ferreira et al., 2011; Coklin et al., 2010; Mark-Carew et al., 2010). Infection causes diarrhea and is self limiting within a few days (Petry et al., 2010). In livestock, economic losses in productivity and animal lethality are often observed, especially in juvenile cattle (Tiranti et al., 2011).
Cryptosporidium and *Giardia duodenalis* are major pathogens in the waterborne transmission of infections. Because the robust (oo)cyst form of the pathogens is able to persist in the environment, different transmission cycles are possible, and one of the most important is waterborne distribution. The occurrence of *Cryptosporidium* oocysts and *G. duodenalis* cysts in different types of water has been confirmed, and a considerable number of waterborne outbreaks has been reported worldwide (Karanis et al., 2007; Baldursson and Karanis 2011; Mons et al., 2009).
Circulation of (oo)cysts from waste water 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 pathogen removal in the hydrological cycle from the originating source to the drinking water. The purpose of this work was to investigate the occurrence and distribution of parasites on the Lower Rhine in Germany.

2.2 Materials and methods

2.2.1 Geography and description of the sampling sites

2.2.1.1 The River Rhine

The River Rhine is one of the longest rivers in Europe (Lendering, 2011). The Lower Rhine section (Figure 5A-C) comprises densely populated urban structures and rural regions (NABU -Naturschutzstation e. V, 2011). The River Rhine and its tributaries

collect different types of materials due to erosion and from the faeces of wild and domestic animals. The deposition of enteric pathogens from the running water discharges from municipal sewage water plants is possible (Figure 5C). The retention of pathogens by riverbank filtration or by geological layers is most likely reduced because of the short distance between the groundwater level and landscape surface (depth to water table 0.5 to 1.0 m, partly, LINEG, unpublished observations). The Lower Rhine catchment area contains a drinking water supply (Figure 5C), which provides potable water to a large population (Steinberg, 2009).

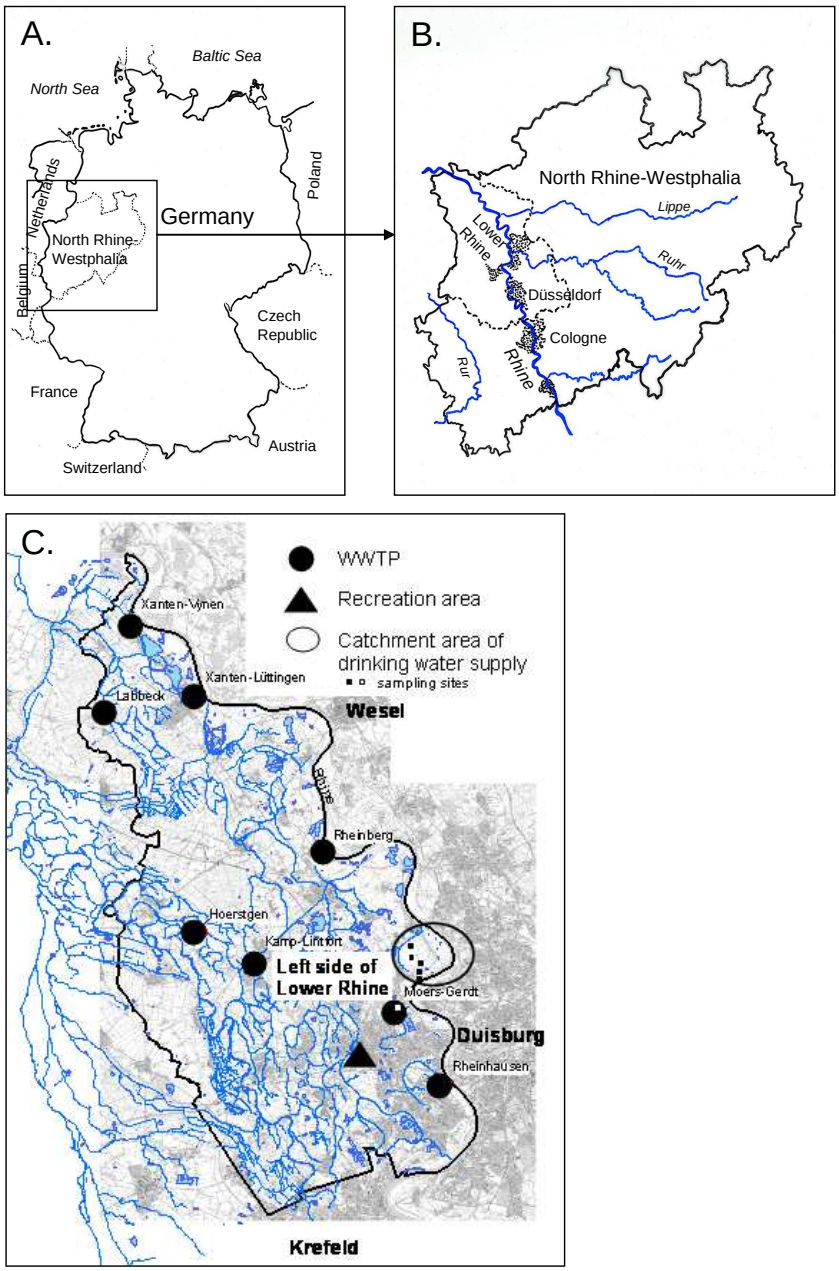

Figure 5: Study area: North Rhine-Westphalia in the western part of Germany (A) and Lower Rhine area as a part of it (B). Position of the eight municipal wastewater treatment plants, recreational area, and catchment area of drinking water supply (C); (ArcGIS 9, ArcMap Version 9.3.1, Esri, Germany).

2.2.1.2 Investigated wastewater treatment plants

Eight municipal wastewater treatment plants (WWTPs) were investigated over a one-year period from 2009-2010 [see description in Table 2; WWTPs: Moers-Gerdt (MG), Rheinhausen (RH), Rheinberg (RB), Kamp-Lintfort (KL), Hoerstgen (HO), Labbeck (LA), Xanten-Lüttingen (XL) and Xanten-Vynen (XV)]. All sewage treatment plants included secondary sewage treatment: four had a two-stage activated sludge treatment process, three had a single-stage activated sludge purification and one had a membrane bioreactor (MBR). Based on German legislation, the WWTPs had state-of-the-art technology, with fixed effluent values only for chemical parameters (LANUV NRW, 1995; 2006; BMJ, 2009; EC, 2000), (Figure 5C) but not for microbiological pollutants.

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

* In case of dominance of the filamentous Bacteria *Microthrix parvicella* $FeCl_3$ is substituted with Polyaluminate; PE people equivalents; Data from LINEG, Germany, unpublished.
** Depending from precipitation.

Table 2: Technical data of the WWTPs. Detailed information concerning design of capacity, retention time, treatment steps and processes, and number of investigated samples.

2.2.1.3 Recreational swimming area

The investigation of the recreational swimming area (Figure 5C) was performed during the 2009 and 2010 bathing seasons. The Aubruch Channel is a small section

of running water that passes through the pond. Therefore, the catchment area included residential areas, agricultural landscape and nature and landscape protection areas influenced by avifauna and mammals, such as rodents, deer, horses, sheep, cattle, dogs and cats. Paths along the running water are popular for walking and other outdoor activities.

The running water was mainly fed laterally by groundwater. Additionally, it contained the rainwater running off the catchment area including the sealed urban surfaces, and illegal sewage disposal was frequent.

Figure 6: Recreational area (area for bathing).

The recreational area (Figure 6) was regularly frequented by approximately 500 persons during the bathing season from March to September. In total ~21000 bathers were registered in 2009 and 2010 (Meurs, 2010).

The recreational area included in this study had a total length of 300 m and a width of 40–50 m. The water depth was 0.6–3.1 m and the sediments were 0.65-1.5 m. The area for bathing was limited to 5000 m^2 (length 100 m, width 50 m).

The swimming pools were under hygienic control by the European Bathing Water Directive (EU, 2006). Monitoring was based on four classes: poor, sufficient, good or excellent. The directive regulates the classification of only two bacterial contaminants, *E. coli* (>900 cfu /100 ml = classification poor) and intestinal

enterococci (>330 cfu /100 ml = classification poor), but not parasites. Although these enteric bacteria indicate the prevalence of faecal contamination, no correlation exists between the counts of these indicator bacteria and the occurrence of *Cryptosporidium* or *Giardia* in recreational areas (Rimhanen-Finne et al., 2004; Mons et al., 2009; Dorevitch et al., 2011). Consequently, regular monitoring of waterborne pathogens of this type of water is important to prevent public disease.

2.2.1.4 Drinking water

The study area (18 km^2) for the drinking water supply was on the left side of the River Rhine in the Field of Binsheim, located between the villages Rheinberg-Orsoy and Duisburg-Baerl (Figure 5C). The Field of Binsheim is used as a local recreational area for walking tours and sports activities, e.g., jogging and biking. Since 1996, the field has been a water protection area with specific water safety plans for waterworks. The restrictions in this area include intensive animal husbandry, use of fertilisers and pesticides, garbage disposal and establishment of WWTPs and gravel pits (WVN, 2011).

In the drinking water catchment area, vertical and radial wells and many groundwater sampling sites have been installed to control water chemistry or to determine the depth to the groundwater (LINEG, 2003).

The sampling sites 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 first waterworks (Figure 5C). Six sampling sites were examined fortnightly for a period of one year. The first sampling site was the surface water from the River Rhine sampled manually near the riverbank (between Rhine kilometres 786 and 787). The second sampling site was a well 250 m away from the River Rhine. The third sampling was taken from a vertical well 630 m away from the riverside, and the fourth sampling was taken from a radial well 1.5 km away from the riverside. 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 Field of Binsheim that obtained its water from the same aquifer. The waterworks technology consisted of oxidation, multilayered filtration (hydro anthracite, quartz gravel and

activated carbon) and UV disinfection (Mikus, 1987; WVN, unpublished observations).

2.2.2 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 2009-2011. Influent and effluent samples from eight WWTPs (n=206) were taken over a period of 15 months. From the recreational swimming area, 54 samples were collected 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 device setting of the auto sampler was ~120 ml /10 min. The homogenised samples were transported to the laboratory within one hour in sterile 10-l buckets (Meliseptol, B. Braun Melsungen AG, Germany). The representative samples collected from the surface water were transported to the lab under the same conditions.

Figure 7: Sampling (waterworks, distance from the River Rhine: 2.5 km); filter apparatus with polyester micro fibre filter).

For sample collection, an average volume of 2500 l (min. 240 l, max. 6400 l, depending on turbidity) of drinking water and 80 l (min. 40 l, max. 408 l, depending on turbidity) of surface water was filtered using polyester micro fibre filters with a nominal pore size of 2 µm (ARAD Hungária Kft., Budapest, Hungary; Figure 7). 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 (2010).

2.2.3 Sample preparation for microscopic examinations

Aluminium sulphate flocculation was performed as previously described by Kourenti et al. (2003). A sterile glass bottle was filled with 2 l of homogenised 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+, ThermoFisher Scientific, Germany). The precipitate was solubilised using acid buffer. The samples were centrifuged again, and the pellet was washed and further analysed for (oo)cyst identification.

The elution of the (oo)cysts from the filter was performed as described by Inoue et al. (2003) and modified by Plutzer et al. (2010). In brief, the filter was eluted using 300 ml PET solution and scrubbing the surface with a sterile nylon bristle brush for 1 min. The extracts were collected in 400-ml centrifuge tubes. After centrifugation (3800 g for 10 min; Multifuge 3SR+, ThermoFisher Scientific, Germany), the supernatant was carefully aspirated to 10 ml above the packed pellet. For a second centrifugation (3800 g for 10 min), the entire sample was transferred into a 50-ml Falcon tube, including the residual PET buffer that was used to rinse the tube walls. The supernatant was discarded, resulting in a final volume of 2 ml containing the pellet with the (oo)cysts.

The purification of the (oo)cysts was performed as described by Arrowood and Sterling (1987) and modified by Kourenti et al. (2003). Briefly, Sheather's sugar solution (500 g sucrose, 6.5 g phenol, 320 ml H_2O) was diluted with 0.1 mol l^{-1} PBS (285 ml 0.1 mol l^{-1} KH_2PO_4, 715 ml 0.1 mol l^{-1} Na_2HPO_4; pH 7.2) to obtain solutions A (Sheather/PBS 1:2, specific gravity 1.11) and B (Seather/PBS 1:4, specific gravity 1.07), each supplemented with a few drops (no more than five) of 1% v/v Tween 80.

A volume of 15 ml from solution B was layered 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 x g for 30 minutes (4 ℃, brake off) and washed twice with distilled water and centrifuged (2100 x g for 10 min, 4 ℃, brake off). The resuspended pellet with an end volume of 2 ml was preserved with 50 µl antibiotic and stored at 4 ℃ until further preparation was ca rried out.

The immunofluorescence test (IFT) was performed as described by the manufacturer of the staining kit (Cellabs Pty Ltd, Australia) with minor modifications (Kourenti et al., 2003). Small pellets (100 µl) were incubated in Eppendorf tubes with fluorescently labelled monoclonal antibodies (fluorescein isothiocyanate, FITC) (Waterborne, Inc, New Orleans, LA) for 30 min at 37℃ 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) using Nomarski polarisation optics. Only samples in which oocysts fulfilled the defined and already published morphological criteria (USEPA, 2001) were recorded as positive.

2.2.4 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.

2.3 Results

2.3.1 Results of the examination of waste water treatment plants

Influent and effluent samples from 8 WWTPs (n=206) were investigated in this study. Each sampling site was positive at least for one of the two protozoan parasites, with

a higher detection frequency for *Giardia* cysts (Table 3). 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 concentration of *Giardia* cysts was always higher than that of the *Cryptosporidium* oocysts.

WWTP	Input *Cryptosporidium* oocysts/l		Output *Cryptosporidium* oocysts/l		Reduction rate %
	P (T)	Mean (min-max)	P (T)	Mean (min-max)	
MG*	6 (12)	10 (0-55)	2 (14)	1.5 (0-2)	92.7
RH*	9 (12)	50 (0-310)	3 (14)	0.3 (0-24)	95.6
RB*	6 (12)	16 (0-150)	2 (14)	0.7 (0-4)	73.3
KL*	6 (12)	94 (0-730)	3 (14)	0.8 (0-5)	99.2
HO**	3 (12)	2.5 (0-10)	1 (14)	0.2 (0-2)	90
LA**	5 (11)	10 (0-40)	1 (13)	6.3 (0-0)	100
XL**	6 (12)	183 (0-100)	1 (14)	2.2 (0-**36**)	86.4
XV**	5 (12)	13 (0-**1745**)	5 (14)	5.3 (0-10)	99.4
Total	46 (95)			18 (111)	
*WWTPs with enhanced secondary treatment:					
Mean		42		0.5	90.2
**WWTPs - small compact facilities:					
Mean		53		2.0	94.0
Mean (all WWTPs)					92.1

WWTP	Input *Giardia* cysts/l		Output *Giardia* cysts/l		Reduction rate [%]
	P (T)	Mean (min-max)	P (T)	Mean (min-max)	
MG*	9 (12)	260 (0-1480)	7 (14)	1.5 (0-40)	98,3
RH*	9 (12)	174 (0-640)	10 (14)	7.9 (0-**56**)	90,9
RB*	10 (12)	186 (0-600)	6 (14)	6.1 (0-28)	86,3
KL*	11 (12)	273 (0-745)	8 (14)	5.8 (0-26)	97,4
HO**	10 (12)	142 (0-650)	0 (14)	0 (0-0)	100
LA**	8 (11)	107 (0-450)	7 (13)	6.4 (0-30)	75,3
XL**	10 (12)	239 (-870)	8 (14)	9 (0-24)	96,4
XV**	11 (12)	383 (0-**2436**)	10 (14)	4.9 (0-14)	94,2
Total	78 (95)			56 (111)	
*WWTPs with enhanced secondary treatment:					
Mean		217		5.3	93.2
**WWTPs - small compact facilities:					
Mean		220		5.0	91.5
Mean (all WWTPs)					92.4

P (T), positive samples (total samples)

Table 3: Occurrence of *Cryptosporidium* oocysts and *Giardia* cysts in the WWTPs between July 2009 and September 2010.

In all the WWTPs, a reduction of (oo)cysts in the influent as compared to the effluent water was observed (Table 3). 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 for *Cryptosporidium* oocysts (0.7 $\log_{10}$) and *Giardia* cysts (1.3 $\log_{10}$) was lower compared to other bacterial pollutants such as heterotrophic plate counts (HPC) 20 ℃ (2.5 $\log_{10}$), HPC 36 ℃ (2.4 $\log_{10}$), total coliforms (2.3 $\log_{10}$), *E. coli* (2.4 $\log_{10}$), enterococci (2.2 $\log_{10}$), and *Clostridium perfringens* (1.7 $\log_{10}$). The average concentrations and reduction rates of *Cryptosporidium* and *Giardia* are shown in Table 3 and Table 4.

Removal (log10)		HPC 20℃	HPC 36℃	*E. coli*	Coliforms	Faecal streptococci	*C. perfringens*	*Giardia duodenalis*	*Cryptospo-ridium*	Median/ WWTP
MG	Median	2.4	2.4	2.5	2.4	2.5	1.6	1.4	0.9	2.4
RH	Median	2.3	2.1	2.0	1.8	2.2	1.1	1.1	0.7	2.0
RB	Median	2.6	2.5	2.1	2.2	2.1	1.7	1.2	**0.4**	2.1
KL	Median	2.4	2.4	2.3	2.2	1.3	1.8	1.3	0.6	2.2
HO	Median	2.7	2.4	2.8	2.8	2.6	2.5	2.1	0.7	2.7
LA	Median	2.8	2.7	2.4	2.5	2.2	2.0	1.0	1.5	2.5
XL	Median	2.4	2.4	2.3	2.2	1.3	1.8	1.3	0.6	2.2
XV	Median	**3.0**	2.8	**3.0**	2.7	2.5	1.5	1.2	1.3	**2.8**
Median/ parameter		**2.5**	2.4	2.4	2.3	2.2	1.7	1.3	**0.7**	2.3

Table 4: Average removal efficiencies ($\log_{10}$ median) of the microbial parameters.

There were differences in the mean concentrations of *Cryptosporidium* oocysts in the influent and effluent samples from WWTPs using different treatments (enhanced secondary treatment or small compact facilities). Higher numbers of *Cryptosporidium* oocysts were found in both the influent (mean 53 oocysts /l) and effluent (2.0 oocysts /l) of small compact facilities. Differences in the mean concentrations of *Giardia* cysts were not observed in the influent and effluent water based on a comparison of the two treatment systems (Table 3), 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* oocysts and *Giardia* cysts were more prevalent from late summer throughout winter, depending on rainfall (Figure 8). All of the WWTPs showed alternating shapes or distinct but smaller peaks in the spring and summer for *Giardia* cysts (data shown in the appendix).

In plant XV (MBR), the highest load of *Giardia* cysts of all the other 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).

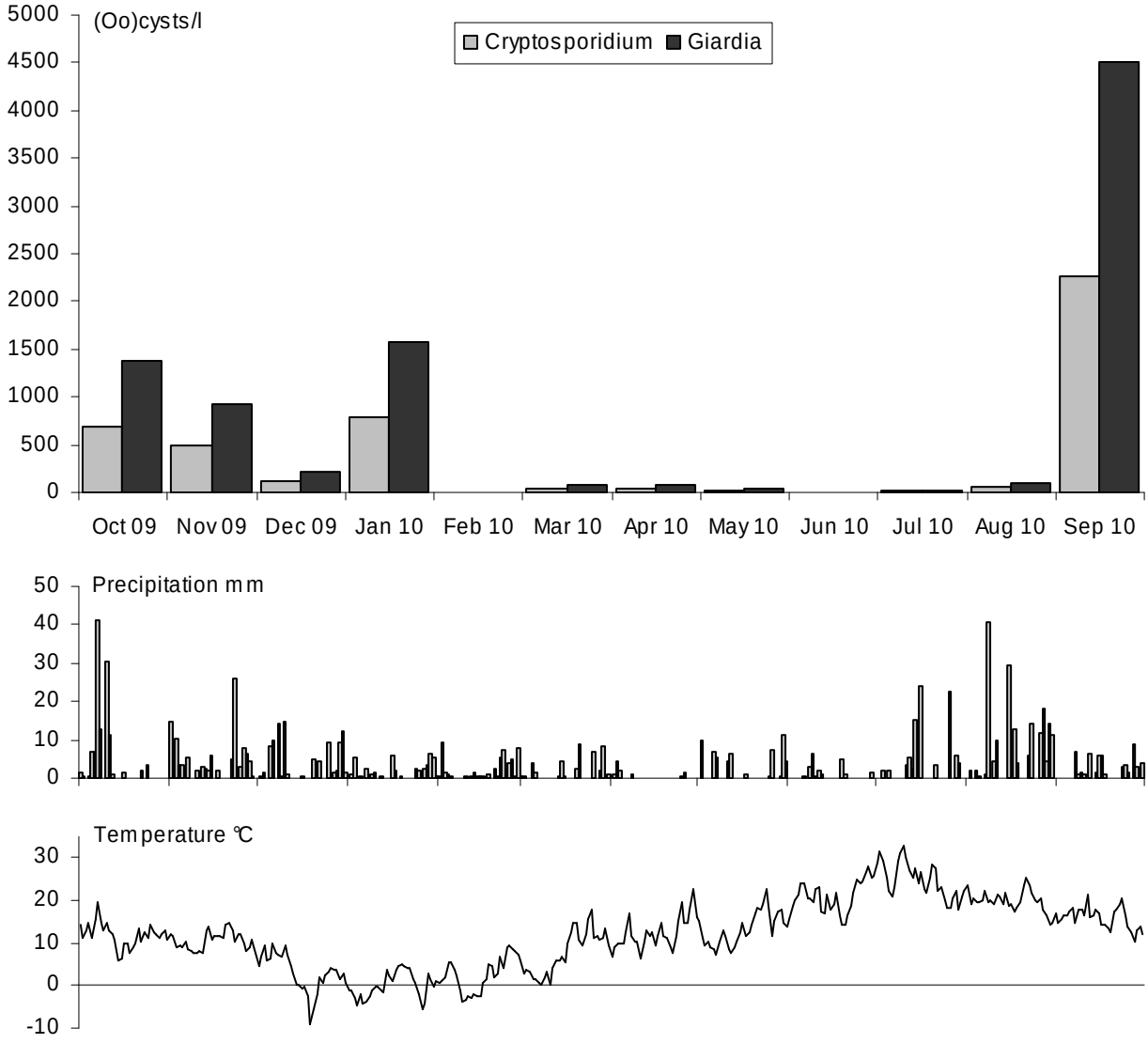

Figure 8: Numbers of *Cryptosporidium* oocysts and *Giardia* cysts from the influent samples of the WWTPs, data of precipitation and temperature during October 2009 and September 2010.

2.3.2 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.

In nine samples (12%), *Cryptosporidium* oocysts were detected by microscopy following IFT and DAPI staining. The numbers ranged from 6.7 to 2000 oocysts /100 l (Table 5).

Similarly to the results from the investigation of *Cryptosporidium*, *Giardia* cysts were detected in eight samples (11%). The numbers of cysts ranged from 6.7 to 4000 cysts /100 l (Table 5).

Sampling site (Distance from Rhine, km)	**Type of water**	***Cryptosporidium* spp. oocysts/100 l**		***Giardia duodenalis* 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)	1.28 (0-1.64)	0 (24)	0 (0-0)
Waterworks 1 (2.5)	Raw water	2 (23)	0.48 (0-0,65)	0 (23)	0 (0-0)
Radial and vertical well, mesure point (1.5; 0.6; 0.25)	Groundwater	5 (66)	2.35 (0-6.64)	1 (66)	0.54 (0-0.54)
P (T)		**19 (190)**		**9 (190)**	

Table 5: 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.

2.3.3 Results of the examination of the drinking water supply

From the 113 samples in the catchment area of the drinking water supply during the investigation period 2009–2010, 10 (8.8%) were contaminated with *Cryptosporidium* oocysts.

In the waterworks approximately 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 0.76-1.64 oocysts /100 l. In the raw water, two samples were positive with 0.31-0.65 *Cryptosporidium* oocysts /100 l. In the groundwater, five samples were positive with 0.42-6.64 *Cryptosporidium* oocysts /100 l. In the drinking water from the Rhine river, five samples were also positive with 6.67-25.0 *Cryptosporidium* oocysts /100 l (Table 5).

From the 113 samples in the catchment area of the drinking water supply during the investigation period 2009–2010, 1 sample (0.88%) was contaminated with *Giardia* cysts. One sample from the groundwater (sampling site vertical well) had 0.54 cysts /100 l, and 6.67 cysts /100 l were detected in one sample collected from the River Rhine.

2.4 Discussion

A high variety and combination of methods have been described for the detection of *Cryptosporidium* oocysts and *Giardia* cysts (USEPA, 2001; Wohlsen et al., 2004; Koompapong et al., 2009; Plutzer et al., 2010).

In contrast to regular methods, such as USEPA 1623 (USEPA, 2001), ARAD filters were chosen that offer surface and depth filtration in a compact design, as previously tested (Plutzer et al., 2010). 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 standards.

In this study, pathogen recovery for filtration and flocculation with EasySeed (BTF Pty. Ltd., Sydney, Australia) was performed. The recovery rates for filtration were 36.7±6.2% (*Cryptosporidium* oocysts) and 28.3±4.7% (*Giardia* cysts) and the flocculation recovery rates were 15.8±4.2% (*Cryptosporidium* oocysts) and 64.5±22.7% (*Giardia* cysts). The recovery rates 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 48.4±11.8% (oocysts) and 57.1±10.9% (cysts) (McCuin and Clancy, 2003) in filtered tap water have been reported. Inoue et al. (2003) used the same elution buffer as described in this study with an oocyst recovery rate of 43.1±13.9%. 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.

Wastewater treatment plants 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 (*Giardia* cysts: t_0=6.37, df=204, p<0.01; *Cryptosporidium* oocysts: t_0=10.5, df=204, p<0.01). *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 the precipitation. The retention time was not calculated in this study. In the WWTP HO, the retention time ranged from 5.7 during heavy rainfalls to 77 hours during dry

weather (Table 1). Therefore, it was difficult to assess when the influent had finally crossed the treatment or when the treated wastewater that had left the plant had been in the inflow for hours.

The secondary settlement tanks are often frequented by birds, including wild ducks, seagulls, magpies, blackbirds and pigeons. In addition, rodents, particularly mice and sometimes 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 (North Rhine-Westphalia and Rhineland Palatinate, catchment area of the Swist river), the reduction rates of *Giardia* cysts were slightly higher (86.96 to 99.97%; Kistemann, 2008).

In contrast to the WWTPs with enhanced secondary treatment, the small compact facilities had higher numbers of oocysts in both the influent and effluent water, whereas differences in the mean concentrations of the cysts were not observed in the influent and effluent water between the two treatment systems (Table 3). 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 bigger plants (93.2%) 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 (Kistemann et al., 2008). The authors therefore noted the relationship between the removal efficiencies, the treatment procedures and the size of the WWTPs (Kistemann et al., 2008).

The reduction in (oo)cyst number mainly results from the 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 (Searcy et al., 2006). Most of the bacteria in the activated sludge form flocs, in which (oo)cysts become embedded (Larsen et al., 2008). 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 (Roels et al., 2002).

In all the WWTPs investigated in this study, Fe or Al salts were added during the treatment process (Table 1). 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 stabilisation of sewage sludge. Although normal medium retention times are 30 days and more, hygienic disinfection is not possible under mesophilic conditions (Klages, 2009). Therefore, sewage sludge disposal on agricultural areas should be prohibited. In the study area, disposal of the drained digested sludge of all eight wastewater plants took place in a nearby waste incineration plant, and the residual water returned to the wastewater treatment process. Hence, further distribution of infectious stages of parasites and other pathogens may be minimised.

During the investigation period, a typical curve shape was observed for every wastewater plant, with high peaks mostly appearing in autumn (or late summer) and winter, as has described for other European countries (Briancesco and Bonadonna, 2005). 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 (Lima, 2001; ten Hove et al., 2009). Other authors described rising concentrations of (oo)cysts in the surface water in spring and summer (Castro-Hermida et al., 2009), 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 one-year period (data are shown in the appendix).

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 (RKI, 2009, 2010, 2011). 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 (Figure 9). Interestingly, this coincides with the observations of the *Cryptosporidium* inflow data from municipal WWTPs.

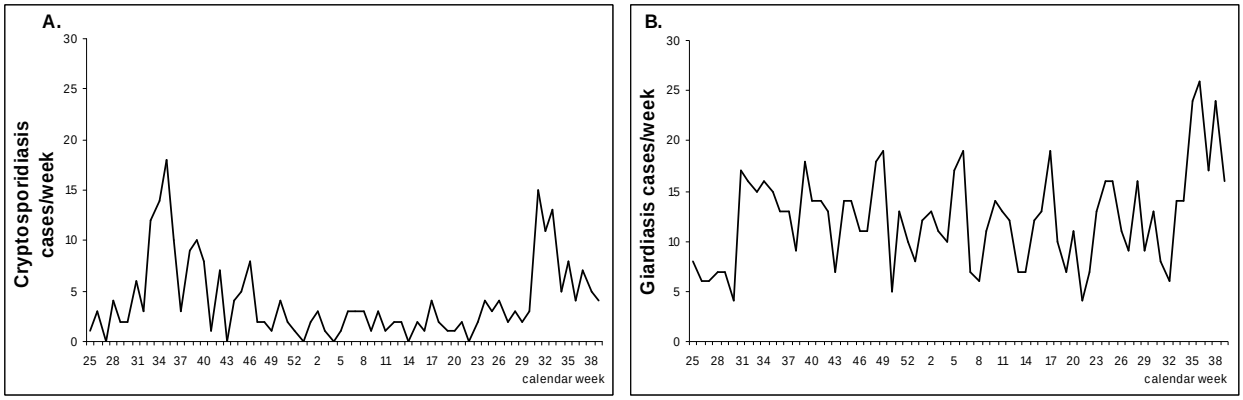

Figure 9: The weekly number of reported infectious diseases during the period of the study 2009 and 2010 (RKI 2009, RKI 2010; RKI 2011).

Jagai et al. (2009) 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. (2009) are a possible explanation for the different seasonal peaks observed in the present study and should be studied in further investigations. Genotyping reveals new insights about the origin and transmission of parasites and also defends against the outbreaks of diseases. The two important human pathogenic species of *Cryptosporidium* (*C. hominis* and *C. parvum*) have differences regarding their host reservoirs [*C. hominis* – humans; *C. parvum* - livestock, e.g., cattle, and humans (Hunter and Thompson, 2005)].

In a study by Jagai et al. (2009), a relationship between *Cryptosporidium* outbreaks and climate conditions was shown. The authors demonstrated that although climatic conditions typically define a pathogen habitat area, meteorological factors affect the timing and intensity of seasonal outbreaks. Therefore, meteorological forecasts can be used to develop focused prevention programs for waterborne cryptosporidiosis. Hence, parameters such as rainfall and temperature seem to be suitable for these predictions (Jagai et al., 2009). In contrast to Jagai et al. (2009), the present study did not find a correlation between ambient temperature and the prevalence of *Cryptosporidium* and *Giardia* (Figure 6). However, the study was mainly focused on cost-effective methods for sampling and detection of parasites in monitoring programs.

Based on the results, the rates of *Giardia* cysts were significantly higher than the rates of *Cryptosporidium* oocysts (arithmetic average of all input samples: 48 oocysts /l and 218 cysts /l; output samples: 1.7 oocysts /l and 5.2 cysts /l). This is in

agreement with recent studies from other European countries (Briancesco and Bonadonna, 2005; Castro-Hermida et al., 2008).

In an Italian study of the relevant bacterial parameters of the new Drinking Water Directive 98/83/CE (European Commission, 1998) and the occurrence of *Cryptosporidium* and *Giardia*, a reciprocal correlation between the levels of the two parasites and intestinal enterococci was found (Briancesco and Bonadonna, 2005). In the actual study, low or no correlation in wastewater samples was found between *Cryptosporidium* or *Giardia* and other bacterial contaminants (HPC 20℃, HPC 36 ℃, total coliforms, *E. coli*, enterococci, *Clolstridium perfringens*; data not shown).

The data from the WWTPs demonstrated that the influent water released to the environment, especially to surface water, contains high levels of *Cryptosporidium* spp. and *G. duodenalis*. Other sources of pollution, such as the agricultural landscape (spreading of liquid manure or fertilisers from other faecal sources), animal husbandry, and wild and domestic animals, impact the surface running water directly or via leaching, erosion and runoff 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 this study could cause disease outbreaks due to the low infection dose of both parasites [~10 (oo)cysts (Grazioli et al., 2006)].

The number of (oo)cysts in river waters from Seine and Marne in France (0.5 - 245.4/ 10 l Cryptosporidium oocysts and 0.5 – 511.5/ 10 l Giardia cysts) were corresponding to the results of the actual study (6,7 - 2000/ 100 l oocysts and 6.7 – 4000/ 100 l cysts), but showed higher detection frequency of 45.7% positive Cryptosporidium samples and 93.8% positive Giardia samples (Monis et al., 2009). However, in the actual study only 8.8% and 11% of the samples were positive for Cryptosporidium and Giardia, respecticely in the surface waters (recreational area and River Rhine).

The Tambre river basin in Spain showed increased results by a factor of ~35 for Giardia (2-1350 oocysts/l) and Cryptosporidium (2-772 cysts/l). The authors held the the large number of farms and high density of cattle reasonable for the increased contamination and calculated a high infectious risk for bather’s accidental swelling water. (Castro-Hermida et al., 2009). Despite to the lower concentration in this study infection by pathogens can not be excluded, considering 500 bathing guests per day in the recreational area investigated here.

In 2009, 12 samples from the recreational area and its through-running water were collected and processed by flocculation with aluminium sulphate. Despite the low sampling volume of 5 l, 2 samples (17%) were positive for *Cryptosporidium* oocysts and 4 samples (33%) 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 was expected. However, only one sample of the through-running water and two samples from the recreational area were positive for *Cryptosporidium*, although periods of heavy rainfall were recorded during the sampling. This is 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). At seven sites, there was discharge from rainwater channels into the running water. At three sites, up to 5 l/s of rainwater drain into the nearby wastewater treatment 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 were found.

The River Rhine has a large catchment area and is an important transportation route in Germany (Uehlinger et al., 2009). The effluents from numerous wastewater treatment plants led directly (plants RH, MG, RB and 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 ~4.5 km). In the River Rhine, five samples (22%) were positive for *Cryptosporidium* and only one (4.3%) for *G. duodenalis*. The results are correlated with overflow events. A small-wave flood occurred in December 2010, and another immense 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 runoff events were formerly investigated in a study by Kistemann et al. (2002).

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, depending on floods. The oocysts were able to infiltrate into the aquifer and pass through the sediments, thereby reaching consumers. From the surface water via groundwater to the final drinking water, a reduction of one to two orders of magnitude was observed. Karanis et al. (1996a, 1998) 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 oocysts reduction. UV light provided an effective reduction in the infectivity of *C. parvum* oocysts (Lee et al., 2008). Nevertheless, drinking water is an infection risk to the population, particularly to immunocompromised patients (McGovan et al., 1993; Ventura et al., 1997; Hunter and Nichols, 2002), especially in cases of the events that are able to decrease the effect of UV radiation, such as massive contamination, turbidity, and precipitation of ferric hydroxide.

G. duodenalis 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 output from the WWTPs was very large. An explanation for the small number in the 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 (~10–12 µm) are twice as large as the oocysts (~5–6 µm). In a study by Hijnen et al. (2005), 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 4- to 5-fold that of *Cryptosporidium* oocysts (*Giardia* cysts were not included in these experiments; Hijnen et al., 2005). 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 (Bradford and Bettahar, 2005). Monitoring of the 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 (Weiss et al., 2005). 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 (Weiss et al., 2005).

There are few original German publications regarding the incidence of *Giardia* and *Cryptosporidium* cases. The prevalence of (oo)cysts in water has been confirmed (Kistemann et al., 2008; Redder et al., 2009), and a small number of waterborne outbreaks has been reported in Germany (Karanis et al., 2007; Baldursson and Karanis 2011; Brockmann et al., 2008; Gornik et al., 2001). In 2001, 201 (n=450) soldiers were infected with *Cryptosporidium 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 (Brockmann et al., 2008). 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 (Gornik et al., 2001). Karanis et al. (1996a, 1998) emphasised 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 (Karanis et al., 1996b) and wild rodents (Karanis et al., 1996c) 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, approximately 700 cases of infection were evaluated by Dreesmann et al. in Lower Saxony (2007). This study emphasised “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 and underestimation can be presumed” (Dreesman et al., 2007).

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 (RKI, 2011). It is expected that the number of cryptosporidiosis and giardiasis cases associated with water contamination is underestimated (Baldursson and Karanis, 2011). This reflects statements already made (Baldursson and Karanis, 2011) and

should apply to surveillance systems worldwide. Furthermore, this fact reinforces the need for investigations and monitoring programs 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. An easy-to-handle and inexpensive combination method including ARAD filters was used, aluminium phosphate flocculation and IFT. In the future, other effective and suitable methods may overcome the inhibition threshold to implement the statutorily regulated monitoring for parasites. The present study illustrated the prevalence of parasites in different sources of environmental waters. It demonstrated the retention and reduction of (oo)cysts by wastewater treatment, riverbank filtration, passing the gravel layers of the aquifer, and the raw water treatment by water works.

The study gives an overview of the occurrence and distribution of *Cryptosporidium* oocysts and *Giardia* cysts in the water of a large area (650 km^2) and can serve as a representative study for other regions worldwide.

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3 *Giardia* and *Cryptosporidium* species dissemination during wastewater treatment and comparative detection by IFT, nPCR and LAMP

Abstract

Environmental water samples originating from the Lower Rhine area in Germany have been investigated and they have been comparatively assayed by immunofluorescence test (IFT), nested polymerase chain reaction (nPCR), and loop-mediated isothermal amplification (LAMP) for the presence of *Giardia duodenalis* (n = 185) and *Cryptosporidium* spp. (n =227). The samples were concentrated by filtration or flocculation and (oo)cysts were purified by centrifugation through a sucrose density gradient. For all samples, IFT was performed first followed by DNA extraction for the nPCR and LAMP assays. *G. duodenalis* was detected in 105 samples (56.8%) by IFT, 62 samples (33.5%) by nPCR and 79 samples (42.7%) by LAMP. *Cryptosporidium* spp. were detected in 69 samples (30.4%) by IFT, 95 samples (41.9%) by nPCR and 99 samples (43.6%) by LAMP. According to these results, all three detection methods are complementary for monitoring *Giardia* and *Cryptosporidium* in environmental waters.

3.1 Introduction

G. duodenalis and *Cryptosporidium* spp. are ubiquitously distributed pathogens with extremely high new infection rates of 2.8 x 10^8 *Giardia* cases (Lane and Lloyd, 2002) and 3.0 x 10^5 *Cryptosporidium* cases per year (Fayer et al., 2000). These parasites cause gastrointestinal illness in animals and humans (Stürchler, 1987; Plutzer et al., 2010). Giardia cysts and Cryptosporidium oocysts are excreted with faeces and are resistant to environmental influences (Gardner and Hill, 2001; Solaymani-Mohammadi et al., 2010).

Transmission of (oo)cysts is possible by the faecal-oral route through contaminated foodstuffs or via water-based transmission. Waterborne distribution is estimated to be the main source of infection (Karanis et al., 2007; Baldursson and Karanis, 2011). *G. duodenalis* and *Cryptosporidium* spp. could potentially be encountered by a large section of the population, resulting in epidemics (Ortega and Adam, 1997). Worldwide, many outbreaks have been reported (Karanis et al., 2007; Baldursson and Karanis, 2011).

In Germany, a few outbreaks have occurred in the past (Messner, 2001; Gornik et al., 2001; Karanis et al., 2007; Brockmann et al., 2008; Baldursson and Karanis, 2011). It could be expected that the number of infections with *G. duodenalis* and *Cryptosporidium* spp. associated with water contamination is underestimated. Underestimation of infections and outbreaks is presumed by other authors, too (Baldursson and Karanis, 2011) and reinforces the need for research and monitoring programs to prospectively maintain public health.

For the detection and identification of *Giardia* and *Cryptosporidium*, different methods exist that each show advantages and disadvantages. In the field of water hygiene, the immunofluorescence test (IFT) is generally applied (USEPA, 2001; ISO, 2006). Nevertheless, qualified and skilled technicians are required for the microscopic determination of the cyst structures.

Detection of *G. duodenalis* and *Cryptosporidium* spp. was also achieved using a nested polymerase chain reaction (nPCR). PCR is a well-known tool for the detection of *G. duodenalis* and *Cryptosporidium* spp. (Hopkins et al., 1997; Appelbee et al., 2003; Nichols et al., 2003; Plutzer et al., 2007; Plutzer et al., 2008). However, PCR is an expensive detection technique and needs sophisticated equipment in contrast to microscopic detection.

In the present investigations, two easy-to-handle sampling methods for water sample collection were used: microfibre filtration and aluminium phosphate flocculation. Three detection assays were applied. In addition to the well-established detection methods IFT and nPCR, the recently developed loop-mediated isothermal amplification (LAMP) assay was applied based on different genes that were specific for the detection of the above parasites. Over several years, LAMP had been utilised for a broad spectrum of applications. Applications of LAMP have been summarised in a comprehensive review describing the applications of LAMP in the biomedical field, including for the detection of viruses, bacteria, fungi and parasites (Karanis and Ongerth, 2009). LAMP is highly specific, efficient, simple and rapid and the amplification runs under isothermal conditions. No specialised heating equipment is required, and the amplification of the target is complete within 60 min (Karanis and Ongerth, 2009).

LAMP uses a polymerase with strand displacement activity, and there is no need to use heat denaturation of double-stranded DNA products to initiate the next amplification step as is stringently required in PCR. LAMP runs under isothermal conditions and the reaction may be carried out in a waterbath instead of an expensive thermal cycler. LAMP outperforms PCR by using four or six primers that identify six or eight distinct regions of the target DNA segments, which results in a higher specificity (Notomi et al., 2000).

In the present study, combinations of sampling and detection methods that are easy to handle and time- and cost-effective to aim for greater acceptance of future monitoring programs were used.

3.2 Materials and Methods

The study area (650 km^2) is situated on the Lower Rhine in Germany (Figure 5A-C). A total of 185 samples for the detection of *G. duodenalis* cysts and 227 samples for the detection of *Cryptosporidium* species oocysts from wastewater treatment plants (WWTPs), surface water, a recreational area, groundwater, raw water and tap water were collected and investigated during the period from July 2009 to January 2011. The study area and sampling sites have been previously described in detail (Gallas-Lindemann et al., 2012, submitted).

The origin of samples from different water matrices is shown in Table 7. In brief, a total of 144 influent samples (n = 63/81 *Giardia*/*Cryptosporidium*) and 161 effluent

samples (n = 75/86 *Giardia*/*Cryptosporidium*) from WWTPs were investigated. Furthermore, surface water samples (n = 35/36 *Giardia*/*Cryptosporidium*) and samples from a drinking water supply (n = 12/24 *Giardia*/*Cryptosporidium*) were included.

Five litres of raw and two litres of treated wastewater samples have been collected and purified by aluminium sulphate flocculation. (Oo)cysts were concentrated by sucrose centrifugation. Surface water, groundwater, raw and drinking water have been collected by microfibre filtration (ARAD Hungária Kft., Budapest, Hungary). Elution of the (oo)cysts from the filter was performed as described by Plutzer et al. (2010). Purification of the (oo)cysts with Sheather's sugar solution was carried out as described by Arrowood and Sterling (1987) and modified by Kourenti et al. (2003).

3.2.1 DNA Extraction

DNA extraction was performed following the manufacturer's instruction for the QIAamp Mini Kit (Qiagen, Hilden, Germany) with the modifications described by Plutzer et al. (2010). In brief, 100 µl of the resulting pellet from the purified sample was mixed with 180 µl lysis buffer (buffer ATL) followed by 15 freeze-thaw cycles in liquid nitrogen and a waterbath (65 °C; Köttermann, Germany) to disrupt the (oo)cyst walls. Incubation with 20 µl of Proteinase K for 3 hours and subsequent heating to 70 °C for 10 minutes with 200 µl of buffer AL followed . The DNA was precipitated with 200 µl of 99% ethanol before the whole sample was transferred into the spin column and centrifuged at 8000 rpm for 1 minute. Two washing steps followed with 500 µl of each buffer AW1 and AW2 and intervening centrifugations at 8000 rpm for one minute after each wash. A final centrifugation at 14000 rpm for 3 minutes was performed. To elute the DNA, the column was placed into a new collecting tube and loaded with 100 µl of buffer AE. Then, the tube was centrifuged at 8000 rpm for one minute. The DNA extract was stored in the freezer at -20 °C until needed for further testing.

3.2.2 Detection methods

The samples were analysed by difference interference contrast microscopy (DICM) after performing IFT with DAPI staining followed by the two molecular assays, nPCR and LAMP.

3.2.2.1 Microscopy

The immunofluorescence test (IFT) was performed according to the manufacturer's instruction for the staining kit (Cellabs Pty Ltd., Australia) and referring to U.S. Environmental Protection Agency: Method 1623 (USEPA, 2001) with minor modifications (Kourenti et al., 2003). A detailed description of the sample collection methods and the preparations for further investigation has been given in Gallas-Lindemann et al., 2012 (submitted).

The samples were microscopically examined at 200x and 400x magnification and additional details were noted at 1000x magnification using oil immersion (Microscope BH-2 Olympus, Germany).

Only (oo)cysts that were compliant with the following criteria of the USEP 1623 method were counted: oocysts with brilliant apple-green fluorescence under UV light, typical size (*Giardia* length/width 8-18 µm/5-15 µm and *Cryptosporidium* 4-6 µm) and shape (round to oval), DAPI-staining of the nuclei (sky-blue fluorescence under UV light) and fulfilling internal morphological characteristics by DIC.

3.2.2.2 Molecular detection by nPCR

Giardia: For the detection of *Giardia* cysts, a fragment (length = 292 bp) of the 18S rRNA ribosomal unit was amplified using the protocol described by Appelbee et al. (2003) and Hopkins et al. (1997). The sequences of the initial primers for the first amplification of the nPCR (Gia2029 and Gia2150c, which generated a 497 bp product) and the secondary primers for the final amplification (RH11 and RH4, which generated a 292 bp fragment) are shown in Table 6.

Both PCRs were performed in tubes containing 2 µl of DNA template, 2.5 µl of 10x PCR buffer containing 15 mM $MgCl_2$, 0.5 µl of 25 mM $MgCl_2$, 1 µl of 10 mM dNTPs (Fermentas, Germany), 2 µl of 5 µM forward primer, 2 µl of 5 µM reverse primer, 1.25 µl of DMSO, 0.1 µl of *Taq* polymerase (QIAGEN, Germany; 5 U μl^{-1}) and 13.65 µl of DDW.

Each PCR was carried out in a thermal cycler (iCycler, Bio-Rad, Munich, Germany). The conditions of the first amplification were 1 cycle at 96 ℃ for 4 minutes, 35 cycles of 96 ℃ for 45 seconds, 55 ℃ for 30 seconds and 7 2 ℃ for 45 seconds, and 1 cycle at 72 ℃ for 4 minutes with a final hold at 15 ℃. The conditions of the second amplification were 1 cycle at 96 ℃ for 4 minutes, 25 cycles of 96 ℃ for 45 seconds,

59 ℃ for 30 seconds and 72 ℃ for 30 seconds, and 1 cycle at 72 ℃ for 4 minutes with a final hold at 15 ℃.

A. PCR primers	**Nucleotide sequence**
Giardia (18S-rRNA)	
Gia2029	5′- AAG TGT GGT GCA GAC GAC TC-3′
Gia2150c	5′-CTG CTG CCG TCC TTG GAT GT-3′
RH11	5′-CAT CCG GTC GAT CCT GCC-3′
RH4	5′-AGT CGA ACC CTG ATT CTC CGC CAG G-3′
Cryptosporidium (SSU rRNA)	
N-DIAGF2	5′-CAA TTG GAG GGC AAG TCT GGT GCC AGC-3′
N-DIAGR2	5′-CCT TCC TAT GTC TGG ACC TGG TGA GT-3′
CPB-DIAGF	5′-AAG CTC GTA GTT GGA TTT CTG -3′
CPB-DIAGR	5′-TAA GGT GCT GAA GGA GTA AGG-3′

B. LAMP primers	**Nucleotide sequence**
Giardia (EF1A)	
GL8F3	5'-ATGGACGACGGCCAGG-3'
GL8B3	5'-CCCTCGTACCAGGGCATC-3'
GL8FIP	5'-AGCCGATGTTCTTGAGCTGTCTGTACTCGAAGGAGCGCTACG-3'
GL8BIP	5'-GGAAGAAGGCCGAGGAGTTCGTTGTCGGACCTCTCCATGA-3'
GL8LB	5'-TCATCTCGCCCTTGATCTCG-3'
GL8LF	5'-CTGGACCGGGACAACA-3'
Cryptosporidium (SAM-1)	
SAMCF3	5'-ATTTGATRGACAAAGAAACTAG-3'
SAMCB3	5'-CGATTGACTTTGCAACAAG-3'
SAMCLF	5'-CTGCTGGCCCMCCAATTG-3'
SAMCLB	5'-CATGGRGGTGGTGCATTTAG-3'
SAMCFIP	5'-TTGCGCCCTGTTAATCCAGCATTAATTAATCCATCTGGCAGRTTT-3'
SAMCBIP	5'-TTGTAGATACATACGGAGGATGGGTCTACTTTAGTTGCATCTTTCC-3'

Table 6: A. Primer sets for the PCR targeting the *Giardia* 18S-rRNA (Appelbee et al., 2003; Hopkins et al., 1997) and the *Cryptosporidium* SSU rRNA (Nichols et al., 2003). B. Primer sets for the LAMP targeting the *Giardia* EF1A gene (Plutzer and Karanis, 2009; Plutzer et al., 2010) and the *Cryptosporidium* SAM-1 gene (Karanis et al., 2007b; Bakheit et al., 2008a).

Cryptosporidium: For the PCR-based detection of *Cryptosporidium* oocysts, a nPCR was performed to amplify a 435 bp long polymorphous fragment of the SSU rRNA according to the protocol of Nichols et al. (2003). The primer sets for the nPCR (first

amplification primers N-DIAGF2 and N-DIAGR2; second amplification primers CPB-DIAGF and CPB-DIAGR) are summarised in Table 6.

Both PCRs were performed in tubes containing 1 µl of each primer (10 µM), 1 µl of dNTP (10 mM) (Fermentas, Germany), 5 µl of 10x PCR buffer containing 1.5 mM $MgCl_2$ (Qiagen GmbH, Germany), 3 µl of $MgCl_2$ (25 mM) (Qiagen GmbH), 0.5 µl of HotStarTaq polymerase (5 U μl^{-1}) (Qiagen GmbH, Germany) and 2 µl of BSA (10 mg/ml) (Fermentas, Germany) according to Nichols et al. (2003).

Each PCR was carried out in a thermal cycler (iCycler, Bio-Rad, Munich, Germany). The conditions of the first amplification were 1 cycle at 95 ℃ for 15 minutes, 35 cycles at 94 ℃ for 30 seconds, 68 ℃ for 60 second s and 72 ℃ for 30 seconds, and 1 cycle at 72 ℃ for 10 minutes with a final hold a t 4 ℃. The conditions of the second amplification were 1 cycle at 95 ℃ for 15 minutes, 35 cycles at 94 ℃ for 30 seconds, 60 ℃ for 60 seconds and 72 ℃ for 30 seconds, and 1 cycle at 72 ℃ for 10 minutes with a final hold at 4 ℃.

A positive and a negative control were included in each run.

3.2.2.3 Molecular detection by LAMP

Giardia: The LAMP primer set targeting the elongation factor 1α (EF1A) gene for *G. duodenalis* Assemblage A and B detection described by Plutzer and Karanis (2009) and Plutzer et al. (2010) was used (Table 6).

Cryptosporidium: For the detection of *Cryptosporidium* DNA, the LAMP assay was performed according to Karanis et al. (2007b) and Bakheit et al. (2008a) to target the S-adenosyl-L-methionine synthetase (SAM-1) gene for *C. parvum*, *C. meleagridis* and *C. hominis* (Table 6).

LAMP reactions were separately performed in tubes in a final volume of 25 µl containing 2 µl of DNA template, 1 µl (8 U μl^{-1}) of *Bst* DNA polymerase (New England Biolabs, Germany), 1.3 µl of primer mixture (40 pmol μl^{-1} each of the FIP and BIP primers, 20 pmol μl^{-1} each of the LF and LB primers, 5 pmol μl^{-1} each of the F3 and B3 primers), 8.2 µl of distilled water and 12.5 µl of 2x LAMP buffer (prepared with 4 µl of 1 M Tris-HCl, 0.15 mg of KCl, 0.19 mg of $Mg(SO_4)_2$, 0.23 mg of $(NH_4)_2SO_4$, 0.2 µl of Tween 20, 18.7 mg of Betain, followed by the addition of distilled water to a volume of 90 µl and the subsequent addition of 10 µl of 25 mM dNTPs). The samples were

incubated at 63 ℃ for 120 minutes for *Giardia* and for 60 minutes for *Cryptosporidium* LAMP.

3.2.3 Gel electrophoresis

Gel electrophoresis was performed after nPCR and LAMP to visualise the results. Gel electrophoresis was carried out in a gel apparatus (Power Pac Basic, Bio-Rad, Munich, Germany) at 120 volts for 20 minutes using a gel composition of 1.6% agarose, 1% TAE buffer and 5 µl of RedSafe™ Nucleic Acid Staining Solution (Intron Biotechnology, Korea) per 100 ml. The results were visualised with UV radiation (PCI-Gel-Imager, Intas, Göttingen, Germany).

3.2.4 Statistics

The evaluation of the average relative performance of any two methods against chosen criteria of equivalence was tested using EN ISO 17994:2004 with the modification that the quantitative results of the IFT had been converted into presence/absence results (Poisson dispersion test).

3.3 Results

On the Lower Rhine in Germany, water samples were collected and investigated for the presence of *G. duodenalis* (n = 185) and *Cryptosporidium* spp. (n = 227) by IFT, nPCR, and LAMP (Table 7). The majority of the samples were from wastewater (*Giardia* n = 138 and *Cryptosporidium* n = 167), and the other samples were from surface, ground, raw and tap waters.

All of the samples were examined by DICM after performing the IFT and DAPI staining for the detection of (oo)cysts resulting in 105 samples (56.8%) that were positive for *G. duodenalis* cysts and 69 samples (30.4%) that were positive for *Cryptosporidium* oocysts. In the samples from WWTPs, *Giardia* cysts were detected in 101 of 138 samples (73.2%) and *Cryptosporidium* oocysts were detected in 60 of 167 samples (35.9%).

	Giardia duodenalis			*Cryptosporidium sp.*		
Sampling site	IFT	PCR	LAMP	IFT	PCR	LAMP
	P (T) %	P (T) %	P (T) %	P (T) %	P (T) %	P (T) %
WWPT - effluent	58 (63) 92.1	25 (63) 39.7	26 (63) 41.3	42 (81) 51.9	14 (81) 50.6	33 (81) 40.7
WWPT - influent	43 (75) 57.3	33 (75) 44.0	34 (75) 45.3	18 (86) 20.9	35 (86) 40.7	26 (86) 30.2
Subtotal WWTP	101 (138) 73.2	58 (138) 42.0	60 (138) 43.5	60 (167) 35.9	76 (167) 45.5	59 (167) 35.3
Recreational area	1 (17) 5.9	1 (17) 5.9	8 (17) 47.1	2 (17) 11.8	7 (17) 41.2	9 (17) 52.9
Running water	3 (15) 20.0	1 (15) 6.7	4 (15) 26.7	1 (16) 6.3	3 (16) 18.8	9 (16) 56.3
Stream	0 (3) 0	0 (3) 0	2 (3) 66.7	2 (3) 66.7	1 (3) 33.3	3 (3) 100
Subtotal surface waters	4 (35) 11.4	2 (35) 5.7	14 (35) 40.0	5 (36) 13.9	11 (36) 30.6	21 (36) 58.3
Groundwater	0 (7) 0	0 (7) 0	3 (7) 42.9	2 (14) 14.3	5 (14) 35.7	11 (14) 78.6
Raw water	0 (3) 0	0 (3) 0	1 (3) 33.3	1 (4) 25.0	1 (4) 25.0	2 (4) 50.0
Tap water	0 (2) 0	2 (2) 100	1 (2) 50.0	1 (6) 16.7	2 (6) 33.3	6 (6) 100
Subtotal - drinking water supply	0 (12) 0	2 (12) 16.7	5 (12) 41.7	4 (24) 16.7	8 (24) 33.3	19 (24) 79.2
Total	**105 (185) 56.8**	**62 (185) 33.5**	**79 (185) 42.7**	**69 (227) 30.4**	**95 (227) 41.9**	**99 (227) 43.6**

P (T) %, positive samples (total samples) percentage of positive samples

Table 7: Efficiency of the detection of *Giardia duodenalis* and *Cryptosporidium* spp. by microscopy after performing IFT, PCR and LAMP in wastewater, surface water, groundwater, raw and tap water between July 2009 and January 2011.

All of the samples had been investigated by nPCR with positive results for *Giardia* DNA in 62 samples (33.5%) and positive results for *Cryptosporidium* DNA in 95 samples (41.9%) (Images of the nPCR are given in Figure 10 and Figure 11).

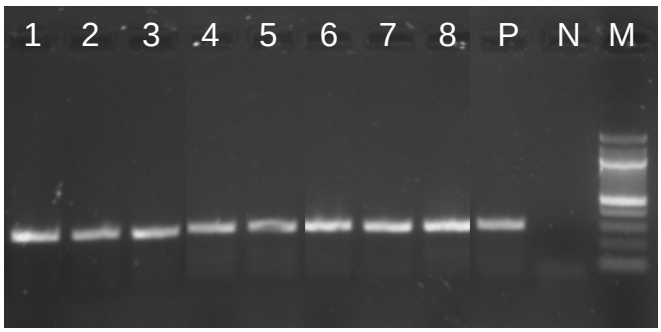

Figure 10: nPCR detection, 18S-rRNA of *Giardia duodenalis*. Lanes 3 and 4: raw wastewater; Lanes 1, 2, and 5-8: treated wastewater; P - positive control; N - negative control; M - 100 bp DNA ladder.

The LAMP assay resulted in 79 samples (42.7%) that were positive for *Giardia* and 99 samples (43.6%) that were positive for *Cryptosporidium* (Images of the LAMP are given in Figure 12 and Figure 13).

In total, 175 samples (94.6%) were positive for *Giardia* and 143 samples (63.0%) were positive for *Cryptosporidium* by at least one assay (Table 8). *Cryptosporidium* oocysts and *Giardia* cysts had been detected in all types of water. As shown in

Table 8, only a small proportion of the samples were positive by all three methods (14.9% of the *Giardia* samples and 9.1% of the *Cryptosporidium* samples).

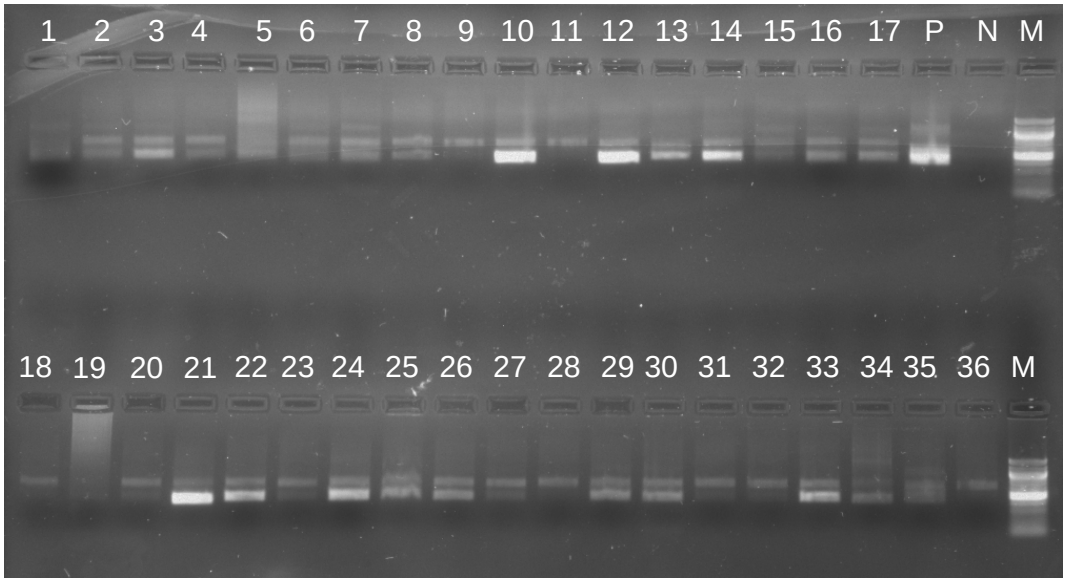

Figure 11: nPCR detection, SSU rRNA of *Cryptosporidium* spp. Lanes 1, 3, 7, 17, 28, and 30–32: tap water; Lanes 5, 29, 34: raw water; Lanes 2, 4 6, 33, and 35–38: groundwater; Lane 8: recreational area; Lanes 21–27 and 39: stream; Lanes 11, 14, and 15: raw wastewater; Lanes 9, 10, 12, 13, and 16: treated wastewater. P - positive control; N - negative control; M - 100 bp DNA ladder.

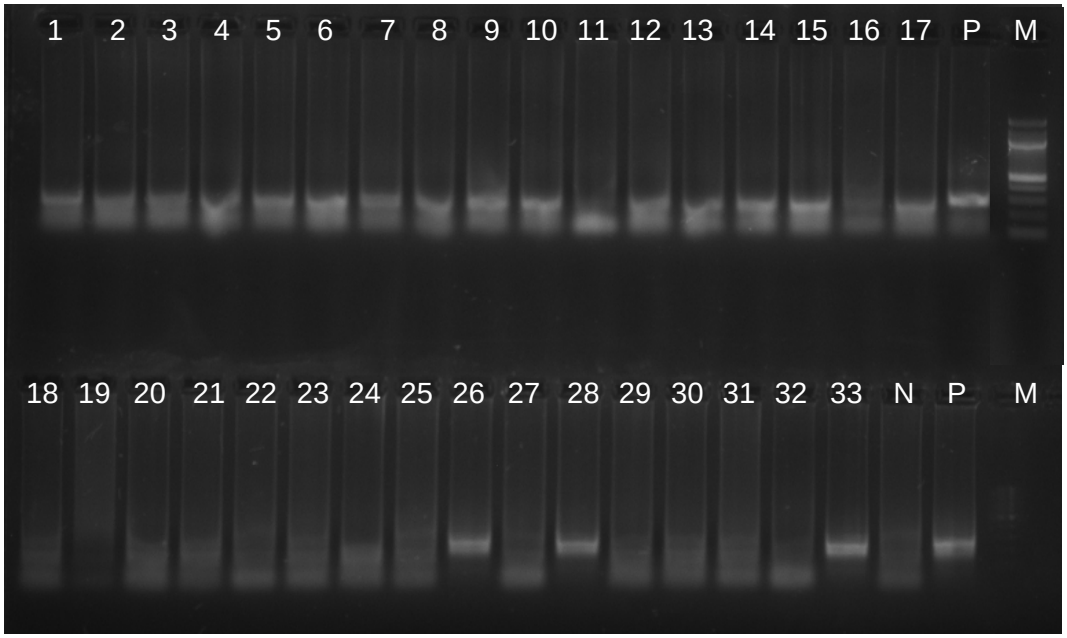

Figure 12: LAMP detection, EF1A gene of *Giardia duodenalis*. Lanes 1, 2, 5, 7, 8, 10-14, 22, 23, 25, 27-31, and 33: treated wastewater; Lanes 3, 4, 6, 9, 15, 16, 21, 24, and 32: raw wastewater; Lane 19: surface water; Lanes 18 and 20: groundwater; Lane 26: raw water. P - positive control; N - negative control; M - 100 bp DNA ladder.

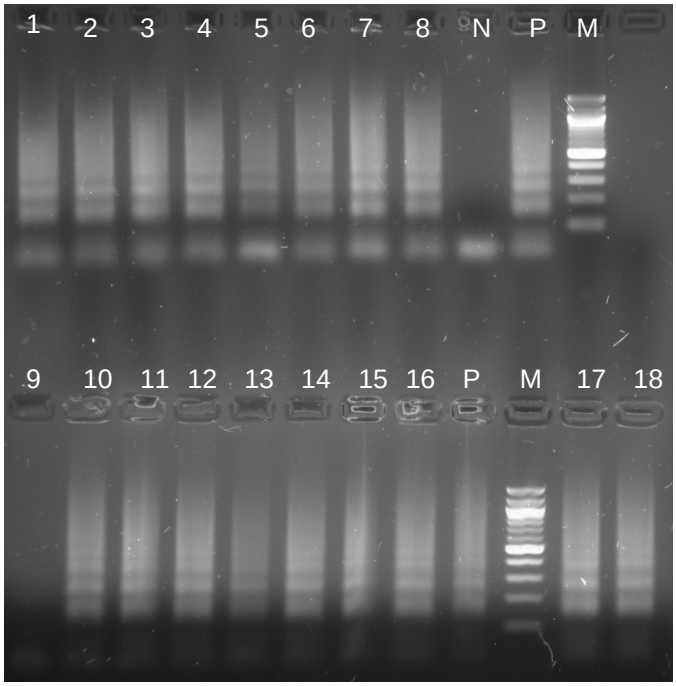

Figure 13: LAMP detection, SAM-1 gene of *Cryptosporidium* spp. Lanes 1, 8, 14, and 18: tap water; Lanes 6 and 12: raw water; Lanes 3, 4, 5, 7, 10, 11, 13, 16, 17 and 19: groundwater; Lanes 2 and 15: stream; Lane 9: empty slot. P - positive control; N - negative control; M - 100 bp DNA ladder.

Comparisons of the three methods for the detection of *Giardia* resulted in ranking IFT over LAMP and LAMP over nPCR (56.8% > 42.7% > 33.5%). For the detection of *Cryptosporidium*, LAMP showed more positive results than nPCR, and nPCR was more sensitive than IFT (43.6% > 41.9% > 30.4%).

Only 28% of the IFT-positive *Cryptosporidium* samples were negative by both LAMP and nPCR. Of the IFT-positive *Giardia* samples, 37% were negative by LAMP and 31% by nPCR (Table 8).

Comparison of methods	*Giardia duodenalis*		*Cryptosporidium sp.*	
	No. (TP)	%	No. (TP)	%
IFT pos and PCR neg	65 (175)	37.1	40 (143)	28.0
IFT neg and PCR pos	19 (175)	10.9	65 (143)	45.5
IFT pos and PCR pos	42 (175)	24.0	30 (143)	21.0
IFT pos and LAMP neg	55 (175)	31.4	40 (143)	28.0
IFT neg and LAMP pos	25 (175)	14.3	69 (143)	48.3
IFT pos and LAMP pos	51 (175)	29.1	29 (143)	20.3
PCR pos and LAMP neg	25 (175)	14.3	53 (143)	37.1
PCR neg and LAMP pos	40 (175)	22.9	56 (143)	39.2
PCR pos and LAMP pos	36 (175)	20.6	37 (143)	25.9
IFT+ PCR+ LAMP pos	26 (175)	14.9	13 (143)	9.1
IFT pos and/or PCR pos and/or LAMP pos	175 (175)	100	143 (143)	100

No. (TP), number of samples (total number of positive samples)

Table 8: Comparative findings of the three methods: IFT, PCR, LAMP.

The results of the Poisson dispersion tests demonstrated that neither molecular assay was equivalent to the standard IFT method, but the statistical comparison of the nPCR and the LAMP methods showed the equivalence of both assays. The chi-squared tests were performed with the results of *Giardia* and *Cryptosporidium* (Table 9).

Giardia duodenalis	A	n_A	B	n_B	$\chi^2 = (n_A-n_B)^2/n_A+n_B$
IFT vs. PCR	+/-	65	-/+	20	23,82
IFT vs. LAMP	+/-	55	-/+	27	9,56
PCR vs. LAMP	+/-	25	-/+	41	**3,88**
n = no. of samples					

Cryptosporidium spp.	A	n_A	B	n_B	$\chi^2 = (n_A-n_B)^2/n_A+n_B$
IFT vs. PCR	+/-	40	-/+	65	5,95
IFT vs. LAMP	+/-	40	-/+	70	8,18
PCR vs. LAMP	+/-	54	-/+	60	**0,32**
n = no. of samples					

Table 9: Statistical evaluation of the average relative performance of two methods against chosen criteria of equivalence (EN ISO 17994:2004; Poisson dispersion test).

3.4 Discussion and outlook

In the present study, water samples with different levels of microbial contamination (wastewater, surface water, groundwater, raw water and drinking water) were investigated in an area of 650 km^2 at the Lower Rhine River in the district of Wesel, North Rhine-Westphalia in Germany. Two different sampling techniques and three detection assays were combined for the detection of *G. duodenalis* and *Cryptosporidium* spp.

Aluminium sulphate flocculation was performed in combination with small sampling volumes of highly contaminated waters (surface, raw and treated wastewater). For waters with presumed low concentrations of (oo)cysts, high volumes of surface water, groundwater, raw water and tap water were filtered.

For the detection of *Giardia*, IFT showed higher results compared to the DNA-based assays, and the LAMP assay was more sensitive than nPCR. For the detection of

Cryptosporidium, the DNA assays had an advantage over IFT, and there was a marginal underestimation of the nPCR assay versus LAMP.

The highest overlaps between the assays occurred when comparing the IFT and LAMP assays for *Giardia* and the nPCR and LAMP assays for *Cryptosporidium*.

Interestingly, the results of the present study (wastewater samples) are in agreement with the findings of a Hungarian investigation (IFT: 67% of samples had *Giardia* cysts and 42% of samples had *Cryptosporidium* oocysts; nPCR: 36% of samples amplified *Giardia* DNA and 28% of samples amplified *Cryptosporidium* DNA) (Plutzer et al., 2008). In contrast, the results of the filtered samples reached only 30 to 50% of the study results from Plutzer et al. (2008). One reason for this difference may be the high filter volumes (up to 6400 l for drinking water) used in this study that exceeded the recently published filter volumes for ARAD filters (Plutzer et al., 2010) in ~ 15 % of the samples (data are shown in the appendix). The discrepancies may also be associated with local conditions or annual variations.

The comparative findings of this study were similar to previously reported results (Plutzer and Karanis, 2009) in which 69% of the samples that were *Giardia*-positive by IFT were also positive by LAMP. Here, 37% of the IFT-positive samples were negative by LAMP.

Developed in the 1990s, immunomagnetic separation (IMS) had been established to overcome inhibition of PCR amplification (Webster et al., 1996; Rochelle et al., 1999; Lowery et al., 2001) and also became an integral part of conventional standard assays for the detection of *Giardia* cysts and *Cryptosporidium* oocysts in water samples (USEPA, 2000; ISO 2006). In this study, IMS was not performed for several reasons. IMS is an expensive tool for concentrating (oo)cysts that uses monoclonal antibodies coated on magnetisable beads (Anceno et al., 2007). The effectiveness of IMS differs between the commercially available test kits, and the targets are limited to a small number of isolates, e.g., *C. parvum* but not *C. hominis* or other species or genotypes (Smith and Nichols, 2010). The turbidity of the samples also has a negative effect on IMS (Zarlenga and Trout, 2004). Finally, (oo)cyst losses reasoned by performing the IMS are possible.

Microscopic examination and IFT are used as the standards for the detection of *Cryptosporidium* and *Giardia* (USEPA, 2001; ISO, 2006) and are considered to be the gold standard. Nevertheless, cross-species identification is possible that may give false positive results (Grazcyk et al., 1996; Zarlenga et al., 2004). Moreover, debris is

able to obscure (oo)cyst observation by microscopy in environmental samples (Nichols, et al., 2003).

Nested PCR is considered to be a sensitive molecular biological assay for the detection of *Giardia* DNA and *Cryptosporidium* DNA (Hopkins et al., 1997; Appelbee et al., 2003; Nichols et al., 2003). The method combining IFT, DAPI staining and DICM (USEPA, 2001) in this study led to higher results (56.8%) for the detection of *G. duodenalis* cysts in the investigated water samples than did nPCR (33.5%). However, in the case of *Cryptosporidium*, the average detection by nPCR (41.9%) was higher than the detection by the IFT/DAPI/DICM method (30.4 %). LAMP showed better results than nPCR for the detection of *Giardia* DNA and LAMP outperformed IFT and nPCR in the detection of *Cryptosporidium* DNA.

In previous studies, several authors have suggested that inhibition of PCR associated with the water matrix is possible (Sluter et al., 1997; Lowery et al., 2000; Monis and Saint, 2001; Loge et al., 2002). Fewer positive PCR results were found in water samples collected from storm drains because of inhibitory compounds in the purified DNA extracts (Loge et al., 2002). Humic-type materials from environmental samples that are co-extracted with the DNA have been identified as a reason for inhibition of PCR (Lowery et al., 2000). Additionally, in these samples, the sensitivity of the *Taq* polymerase was reduced (Lowery et al., 2000). Spiked reagent-grade water samples achieved higher detection rates than untreated source waters that were concentrated by calcium carbonate flocculation and then investigated by RT-PCR. Furthermore, the detection rate could be increased by filtration with Envirocheck filters, which eliminated the PCR inhibitors (Monis and Saint, 2001). This filter type is officially approved for use in standard detection methods (USEPA, 2001; ISO, 2006).

Different approaches have been undertaken to minimise these inhibitory effects. In the study of Sluter et al. (1997), gel and membrane filtration were successfully used to remove PCR inhibitors from lake water (Sluter et al., 1997). Additives for the removal of inhibitors of PCR amplification, such as non-acetylated bovine serum albumin (BSA), dimethyl sulfoxide (DMSO), the T4 gene 32 protein, and polyvinylpyrrolidone, have been described (Jiang et al., 2005, Kreader, 1996) with BSA showing the strongest effect (Jiang et al., 2005). In the present work, DMSO was added to the *Giardia* nPCR assays and BSA to the *Cryptosporidium* nPCR assays to reduce the inhibitory effects. BSA inhibits adherence of the

Taq polymerase with the surface texture of the test tube walls and increases the efficiency of the PCR (Lowery et al., 2000; Kreader, 1996).

Environmental samples include debris with a high number of bacteria and other microorganisms that interfere with the sensitivity of the PCR. Lowery et al. (2000) suggested treatment with sodium hypochlorite for the reduction and inhibition of the attendant organisms, whereas in this study broad-spectrum antibiotics were added (Penicillin – Streptomycin - Amphotericin B) that are fungicidal and antibacterial but do not affect protozoan parasites.

No inhibitory effects are known for the LAMP method. In the original work of Notomi et al. (2000), the authors exclude influences depending on the co-presence of non-target DNA. The method was reinforced by the publications of Karanis et al. (2007b), Plutzer and Karanis, (2009) and Bakheit et al. (2008a) specifically for *Giardia* and *Cryptosporidium* detection. LAMP has been considered highly specific and sensitive (Plutzer and Karanis, 2009; Bakheit et al., 2008a; Sotiriadou and Karanis, 2008; Karanis and Ongerth, 2009; Fu et al., 2010). The sensitivity of the LAMP assay was previously tested by our working group and the detection limits extended as far as the amplified DNA of *C. parvum* oocysts diluted to a minimum concentration equivalent to the DNA of a single oocyst (Karanis et al., 2007b). Additionally, in previously performed sensitivity tests for *G. duodenalis*, the LAMP assay successfully amplified DNA concentrations corresponding to 4 cysts of Assemblage A and 6 cysts of Assemblage B.

Specificity tests including other protozoan parasites such as *Trypanosoma brucei, Theileria parva,* and *Toxoplasma gondii* showed positive LAMP reactions only for *G. duodenalis* Assemblages A and B (Plutzer and Karanis, 2009). In an evaluation of the SAM-1 LAMP for *Cryptosporidium* including *C. parvum*, *C. hominis*, *C. meleagridis*, *C. andersoni*, *Entamoeba histolytica*, *G. duodenalis* Assemblage A, *Cyclospora cayetanensis*, human and bovine DNA, the assay was able to detect *C. parvum*, *C. hominis* and *C. meleagridis* but not *C. andersoni* or any of the other species (Bakheit et al., 2008).

The LAMP assays performed in the present study are specific to *G. duodenalis* Assemblages A and B and for three human *Cryptosporidium* species only (*C. parvum*, *C. meleagridis*, and *C. hominis*) (Bakheit et al., 2008; Plutzer and Karanis, 2009). Given these specificities, the non-comparable findings for the other methods can potentially be explained in a number of ways. *G. duodenalis* Assemblages other

than A and B in the samples could not be detected by LAMP but may be detected by nPCR. *Cryptosporidium* species other than *C. parvum, C. meleagridis* and *C. hominis* could be detected by LAMP but not by nPCR. It is also possible to identify *Cryptosporidium* and *Giardia* on the taxonomic level of their respective genera by IFT, but species or Assemblages of the two parasites cannot be discriminated by this method. Non-human pathogens play an insignificant role in epidemics in human populations and the need for detection of these pathogens to protect public health maybe unnecessary. Therefore, a detection method such as the LAMP assay performed in this study that distinguishes non-human pathogens could be sufficient for water protection.

Overlapping negative results show the differences in the results of the compared methods and illustrate the differences in the detection capabilities of the assays. Therefore, only 14.9% (*Giardia*) and 9.1% (*Cryptosporidium*) of the findings were similarly identified by all three methods (Table 8). Furthermore, the statistical calculation demonstrated that the microscopic analysis could be an effective tool. Interestingly, for the amplification methods nPCR and LAMP, concordance could be established by Poisson dispersion test.

The LAMP assay is a rapid screening tool with advances in cost-effectiveness, sensitivity and specificity. The method was successfully combined with different easy-to-handle sampling techniques for environmental waters with different degrees of contamination. Nevertheless, one must consider that the three methods aim at the same target, the detection of *Giardia* and *Cryptosporidium*, but vary in their basic approaches. IFT detects on the level of the whole organism, does not discriminate between genotypes and allows for the possibility of cross-reaction. The molecular applications are sensitive to fragments of the target DNA but may not include all genotypes. Due to these considerations, direct comparison of the three assays is complicated, although each application on its own is suitable for the detection of *Giardia* and *Cryptosporidium* in water.

The present study provides several indications that *G. duodenalis* and *Cryptosporidium* spp. are frequently present in all water sources, circulate in the water environment and even occur in drinking water systems. In a previous study, the quantified results of 396 microscopically investigated samples showed a considerably reduction of (oo)cysts by wastewater treatment, riverbank filtration, passing through the gravel layer of the aquifer and treatment of the raw water by the waterworks. The

observed reduction could be estimated at two orders of magnitude. As expected, *Cryptosporidium* oocysts and *Giardia* cysts were more prevalent in wastewater than surface, ground and drinking water, and the occurrence in surface water was dependant on flooding (Gallas-Lindemann et al., 2012, submitted).

Physicians should pay particular attention to these protozoan diseases and use surveillance systems to contribute to a better understanding of public health maintenance. The knowledge of the occurrence and behaviour of protozoan parasites in the aquatic environment is still limited. Regular monitoring of *G. duodenalis* and *Cryptosporidium* spp. in the drinking water supply has not been implemented by German legislators, despite the overview about the occurrence of these parasites that it would give and the aid it would offer in terms of prophylactic health protection. In addition, the present study provides confirmation that the newly emerged molecular tools in the field of waterborne parasites, such as LAMP, are sensitive and effective methods for the detection of *G. duodenalis* and *Cryptosporidium* spp., for water monitoring, to support investigations in cases of waterborne disease outbreaks and for tracing the sources of contamination. The present investigations should be the platform for further investigations to develop effective detection of both waterborne pathogens and initiate protective measures for their control during the water-treatment process.

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4 Detection of *Toxoplasma gondii* oocysts in sewage by Loop Mediated Isothermal Amplification (LAMP)

Abstract

Human toxoplasmosis is usually contracted due to consumption of contaminated drinking water and represents an increasing public health risk worldwide. *Toxoplasma gondii* oocysts are resistant to the standard processes that are used by the water supplying industries. Increased awareness of the risk of waterborne toxoplasmosis outbreaks has led to an increase in researcher interest in the detection of oocysts in environmental water. Ninety-five environmental water samples originating from the Lower Rhine area in Germany have been included in the study and were examined for the presence of *Toxoplasma* DNA via a LAMP assay. Water samples were filtered or flocculated by aluminum sulfate and purified by sucrose density gradient. DNA was then extracted, and the DNA samples were then examined by LAMP analysis. *Toxoplasma gondii* DNA were detected in 8 out of 83 (9.6%) influent and effluent samples isolated from wastewater treatment plants. All samples (n = 12) from the surface, ground, raw and tap waters tested negative.

4.1 Introduction

Toxoplamsma gondii (Phylum Apicomplexa) is a protozoan pathogen that infects humans and vertebrates. During disease progression, tissue cysts are formed following multiplication of the organism within the host cell cytoplasm (Hutchison et al, 1970). Swelling of the lymph nodes, muscle pain and fever are the most common symptoms of toxoplasmosis, while cysts seldom occur in heart, liver and spleen. Intrauterine infection may exert negative effects on the fetus if the mother is infected for the first time during the third trimester of pregnancy (Kaye, 2011; Olariu et al., 2011). Toxoplasmosis is a self-limiting disease in immunocompetent individuals. Among immunocompromised patients, it often results in morbidity and mortality (Bruck et al., 2010; Nissapatorn, 2009; Utsuki et al., 2011).

T. gondii oocysts are excreted into the environment by the feces of Felidae (Dubey, 1998). Oocysts are able to enter and circulate in terrestrial and aquatic environments. Moreover, these robust parasitic stages are capable of persisting for an extended time in the environment and are highly resistant to various chemicals and disinfection methods that are commonly used by the water supplying industry (e.g., filtration, chlorination, ozonation and radiation) (Dubey, 1998). Water plays an important role in the dissemination of human toxoplasmosis (Dubey, 1998). Therefore, the analysis of *T. gondii* contamination in water samples provides insight into the potential risk of waterborne infections that affect humans and animals.

Several waterborne toxoplasmosis outbreaks have been documented since 1979, included cases in Panama (Benenson et al., 1982), British Columbia (Bowie et al., 1997), Brazil (Keenihan et al., 2002) and four additional outbreaks described in a recent review by Baldursson and Karanis (Baldursson and Karanis, 2011).

Several methods have been designed for the recovery and detection of *T. gondii* oocysts in contaminated water (Dumètre and Dardé, 2003; Isaac-Renton et al., 1998; Kourenti and Karanis, 2004; Sotiriadou and Karanis, 2008). However, only a few research articles have successfully described the prevalence of *Toxoplasma* in water (Sroka et al., 2006; Villena et al. 2004; Vaudaux et al., 2010).

This investigation provides an analysis of the presence of *Toxoplasma* in water using the Loop Mediated Isothermal Amplification (LAMP) method, which will enhance the attention of the risk of waterborne toxoplasmosis outbreaks and could be effective in preventing and controlling such outbreaks.

4.2 Materials and methods

4.2.1 Study area

The study area is located in North Rhine-Westphalia on the left side of the Lower Rhine in Germany (Fig. 1A-C). A detailed description of the geography of the study area, characterization of the sampling sites, the method of sample collection and sample preparation have been previously described (Gallas-Lindemann et al., 2012).

4.2.2 Sample collection and preparation

Ninety-five environmental water samples originating from the Lower Rhine area in Germany collected over nine months were included in this study. Influent and effluent samples (n = 83) from eight different wastewater treatment plants (WWTPs), surface waters (n = 6), groundwaters (n = 4), raw water (n = 1) and tap water samples (n = 1) were analyzed between January and September 2010. Briefly, water samples from the WWTPs and surface waters were collected and flocculated by $Al_2(SO_4)_3$ as previously described (Karanis et al., 2007; Kourenti and Karanis, 2006; Kourenti et al., 2003). After settlement, the supernatants were discarded and the precipitates were concentrated via centrifugation. The pellets were then incubated with lysis buffer and washed twice with distilled water.

Up to 50 liters of surface water and up to 4450 liters of ground, raw and tap waters were concentrated by microfiber filtration over 24 hours (filter cassettes and filtering apparatus supplied by ARAD Hungaria Kft., Budapest, Hungary). Next, elution of the oocysts from the ARAD filter was performed as previously described (Plutzer et al., 2010). The samples were treated with discontinuous Sheather's sugar gradient solution (Arrowood and Sterling, 1987; Kourenti et al., 2003), and the resulting pellets were transferred to Eppendorf tubes and stored at 4℃ until processed for DNA extraction. Two different sampling methods were utilized due to variations in water matrices, water quality, suspended matter, and expected contamination.

4.2.3 DNA Extraction

DNA was extracted using the QIAamp DNA Mini Kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's description, with the addition of 15 freeze–thaw cycles in liquid nitrogen and water bath incubation (65 ℃; Köttermann, Germany) after resuspending samples in the lysis solution to rupture the *Toxoplasma* oocysts. The DNA was eluted in 100 µl buffer and stored at -20 ℃ until used for the LAMP analysis.

4.2.4 LAMP

A LAMP primer set designed to detect the *Toxoplasma* B1 gene was used as previously described (Sotiriadou and Karanis, 2008). The LAMP reaction was performed as described in the original report by Karanis et al. (2007). LAMP was performed in a 25-µl reaction mixture. The primer mix (0.9 µl) contained 40 pmol FIP, 40 pmol BIP, 20 pmol F3, 20 pmol B3, 12.5 µl reaction buffer (1.6 mol betaine, 40 mmol Tris–HCl [pH 8.8], 20 mmol KCl, 20 mmol liter^{-1}$(NH_4)_2SO_4$, 2.8 mmol desoxyribonucleotide triphosphate (dNTP), 0.2% Tween 20, 16 mmol $MgSO_4$), 1 µl (8 U) B*st* DNA polymerase (Eiken Chemical Co. Ltd.), 1 µl fluorescent detection dye, 2 µl DNA, and 7.6 µl distilled water. The mixture was incubated at 65°C for 1 h and heated at 80℃ for 5 min. Gel electrophoresis was p erformed using a Bio-rad device (Power Pac Basic, Bio-Rad, Munich, Germany) with the following parameters: electrophoresis device settings of 120 volts and 20 min and a gel composition of 1.5 % agarose, TAE buffer 1% and 5 µl RedSafe™ Nucleic Acid Staining Solution (Intron Biotechnology, Korea) per 100 ml reaction. The results were visualized with UV radiation (PCI-Gel-Imager, Intas, Göttingen, Germany). For each LAMP reaction, a positive and negative control was performed to validate the reaction.

4.3 Results and discussion

In total, 95 samples were analyzed by LAMP. *Toxoplasma* DNA was detected in 8 out of 95 (8.4%) water samples (Figure 14).

In particular, 4 out of 45 (8.9%) influent samples of WWTPs tested positive for *Toxoplasma* DNA, whereas 4 out of 38 (10.5%) effluent samples of WWTPs tested

positive. In contrast, all the surface, ground, raw and tap water samples (n=12) tested negative (Table 9).

T. gondii oocysts are highly resistant and can survive for months in the environment. Water has been considered to be an important vehicle for disseminating human toxoplasmosis (Dubey, 1998). To date, only a few reports have described the waterborne route of infection of this parasite, most likely due to the lack of effective research methods (Baldursson and Karanis, 2011; Karanis et al., 2007). In recent years, cases of water-borne toxoplasmosis have been noted worldwide (Isaac-Renton et al., 1998; Alvarado-Esquivel et al., 2010; Vaudaux et al., 2010).

Sampling site		**No. of pos. Samples**	**Total no. of samples**
WWTP no. 1	influent	1	6
	effluent	0	2
WWTP no. 2	influent	0	5
	effluent	1	4
WWTP no. 3	influent	3	4
	effluent	1	4
WWTP no. 4	influent	0	7
	effluent	1	4
WWTP no. 5	influent	0	5
	effluent	0	5
WWTP no. 6	influent	0	5
	effluent	0	7
WWTP no. 7	influent	0	8
	effluent	0	6
WWTP no. 8	influent	0	5
	effluent	1	6
Subtotal	influent	4	45
Subtotal	effluent	4	38
Subtotal (wastewater)		8	83
Groundwater		0	4
Raw water		0	1
Tap water		0	1
River water		0	2
Slow running water		0	2
Pond		0	2
Subtotal		0	12
Total (all samples)		**12**	**95**

[Gauß-Krüger coordinates of the WWTPs (easting / northing): No. 1 (2532720 / 5709740); no. 2 (2536650 / 5707800); no. 3 (2526320 / 5724670); no. 4 (2546280 / 5704350); no. 5 (2541290 / 5715090); no. 6 (2549570 / 5699230); no. 7 (2532600 / 5726000); no. 8 (2528230 / 5730980)]

Tabelle 9: Summarized results of the detection of Toxoplasma gondii by LAMP in WWTPs, groundwaters, raw and tap waters, running waters, and recreational area.

Several cases of waterborne toxoplasmosis outbreaks that were due to the consumption of municipal drinking water or unfiltered water reservoir contaminated by oocysts excreted in the faeces of jungle cats, cougars and/or domestic cats have been documented (Bahia-Oliveira et al., 2003; Benenson et al., 1982; Bowie et al., 1997). In one toxoplasmosis outbreak in Brazil, *T. gondii* was detected in a municipal water reservoir where the water was fed from underground (de Moura et al., 2006).

These events attracted public attention to the problem and resulted in the development of increasingly improved, more sensitive and more effective research methods, including molecular biology techniques.

Depending on the size of the infective stages, the choice of sampling technique plays an important role in the detection of *T. gondii* in water. *Toxoplasma* oocysts ranges from 9 – 15 µm in size (Schares et al., 2008) and are comparable in size to the transmissive stages of other waterborne parasites, such as the diplomonaid flagellata *Giardia duodenalis*. For the analysis, polyester micro fiber filters with a nominal pore size of 2 µm were used, as described by Plutzer et al. for the detection of *G. duodenalis* and *Cryptosporidium spp*. in drinking water (Plutzer et al., 2010), combined with the LAMP assay.

For the investigation of wastewater with a high ratio of settleable solids, filtering is not a suitable technique. The concentration-filtration method is commonly marked by the loss of seeded oocysts, whereas flocculation is simple, inexpensive, and yields high recovery rates in tap water (Kourenti et al., 2003). Sporulated and unsporulated oocysts are recovered more effectively by flocculation than by centrifugation (Kourenti et al., 2003).

The purification step is critical in sample processing as debris co-extracted with oocysts may interfere with downstream applications (Kourenti and Karanis, 2006; Villena et al., 2004).

Recently, Sheather's sugar solutions (Kourenti and Karanis, 2006) and the continuous separation channel centrifugation technique (Borchardt et al., 2009) have shown high recovery efficiencies.

The most readily available method for the isolation of *T. gondii* oocysts from water samples is flocculation or sucrose flotation prior to DNA extraction (Kourenti and Karanis, 2006; Lin et al., 2000; Sroka et al., 2006; Villena et al., 2004). Kourenti and Karanis (Kourenti and Karanis, 2006) concentrated water samples by $Al_2(SO_4)_3$

flocculation followed by purification by discontinuous sucrose gradients, and detected *Toxoplasma* DNA by 18S-rRNA nested PCR.

An immunomagnetic separation (IMS) method has been developed using the monoclonal antibody 4B6 targeting the sporocyst wall of *T. gondii*, *Hammondia hammondi*, *Hammondia heydorni*, and *Neospora caninum* (Dumètre and Dardé, 2007). Due to 4B6 cross-reactions, PCR or LAMP analysis would be useful to further characterize coccidian sporocysts found microscopically (Dumètre and Dardé, 2007). Moreover, LAMP has been described as a high specificity method that avoids cross-reaction (Sotiriadou and Karanis, 2008; Zhang et al., 2007; Zhang et al., 2009).

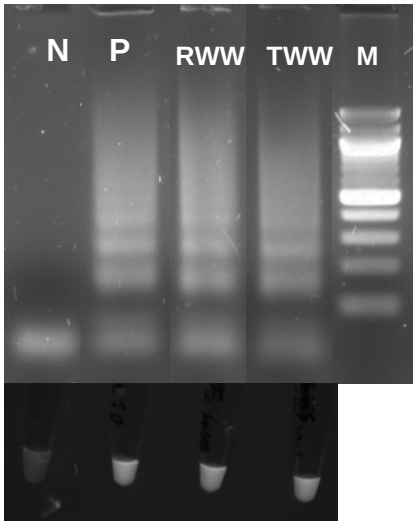

Figure 14: LAMP detection of *Toxoplasma gondii*. N – negative control; P – positive control; RWW – raw wastewater; TWW – treated wastewater; M – 100 bp ladder (below tubes with turbidity derived from magnesium-pyrophosphate).

There are no commercial immunofluorescence kits available for the detection of *Toxoplasma* oocysts; therefore, PCR should allow the detection of low parasite numbers in environmental waters (Aubert and Villena, 2009; Schwab and Devitt, 2003; Sotiriadou and Karanis, 2008). PCR has been shown to be a more sensitive method than the bioassay by mice inoculation (Vaudaux et al., 2010). PCR techniques increase the effectiveness of parasite detection from water samples (Kellogg et al., 2003; Kourenti et al., 2003). Recently, Sotiriadou and Karanis (2008) have developed a LAMP-specific protocol designed to amplify *Toxoplasma* DNA at a particularly higher yield compared to nested PCR. Yang et al. (2009) have used real-time PCR for the detection of *T. gondii* oocysts in concentrates of surface water and

have tested this real-time PCR method in conjunction with a method designed for the direct extraction of inhibitor-free DNA from water (Yang et al., 2009).

In the present study, LAMP as described by Sotiriadou and Karanis (2008) was used. Flocculation and LAMP respectively filtration and LAMP to detect *Toxoplasma* oocysts in sewage water samples from other water sources were combined. In recent years, LAMP had been utilized for a broad spectrum of applications, which primarily involves the diagnosis of toxoplasmosis in human and veterinary medicine (Fu et al., 2010; Krasteva et al., 2009; Lau et al., 2010; Lin et al., 2011; Zhang et al., 2009) but also in environmental soil samples (Du et al., 2011) and water (Sotiriadou and Karanis, 2008). Fu et al. (2010) have summarized the applications of LAMP in a review describing the detection of pathogens from viruses, bacteria, fungi, and parasites (excluding *Toxoplasma*), as well as genetically modified organisms, the identification of embryo sex, and tumor detection (Karanis and Ongerth, 2009; Fu et al., 2010). Veterinary samples have also been examined for the diagnosis of *Toxoplasma* in the lymph nodes of pigs (Zhang et al., 2009), blood samples from pigs and sheep (Lin et al., 2011), as well as in brain, heart, liver, spleen, and kidney samples from mice (Krasteva et al., 2009). Application of the LAMP method for the analysis of human blood samples has been reported by Lau et al. (Lau et al., 2010).

Taken together, LAMP seems to be a promising molecular assay for the detection of *T. gondii*. All authors mentioned above have highlighted and demonstrated the advantages of the LAMP method (Du et al., 2011; Fu et al., 2010; Krasteva et al., 2009; Lau et al., 2010; Lin et al, 2011; Sotiriadou and Karanis, 2008; Zhang et al., 2009). LAMP is highly specific, efficient, simple, and rapid and the amplification runs under isothermal conditions; therefore, no specialized heating equipment is required, and the amplification of the target is complete within a maximum of 60 min.

LAMP specificity for *T. gondii* was tested in comparison with the DNA of other protozoan parasites (Krasteva et al., 2009; Sotiriadou and Karanis, 2008; Zhang et al., 2009; Lau et al., 2010) and other pathogens including *Schistosoma*, *Toxocara cati* and various bacteria (Du et al., 2011). In all studies (Du et al., 2011; Krasteva et al., 2009; Sotiriadou and Karanis, 2008; Zhang et al., 2009; Lau et al., 2010), the negative results of the various targets confirmed the specificity of the LAMP method for *Toxoplasma*.

To support the sensitivity in different studies, the LAMP method has been compared to conventional PCR and RT-PCR (Du et al., 2011; Krasteva et al., 2009; Lau et al.,

2010; Lin et al., 2011; Zhang et al., 2009), resulting in congruent but partly opposed results. Zangh et al. (2009) have pointed out that the LAMP assay shows a slightly higher sensitivity than that of conventional PCR (with detection limits for LAMP at 1 pg of DNA and PCR detection limits at 10 pg of DNA) (Zhang et al., 2009). This result is in agreement with the study by Lau et al. in which the detection limits of LAMP were 10 times more sensitive than the nested PCR method (Lau et al., 2010). In the work by Krasteva et al., the sensitivity rates of LAMP compared to two different conventional PCRs were 100 times higher (Krasteva et al., 2009). Lin et al. have described a slightly higher sensitivity of the RT-PCR method compared to LAMP (detection limits for LAMP at 10 fg DNA and limits for PCR at 1 fg of DNA); although, the variations between the LAMP and RT-PCR techniques were less obvious in the analysis of blood samples from pigs (LAMP 3,17% positive [9/284], RT-PCR 4,22 % positive [12/284]) and sheep (LAMP 17,12% positive [50/292], RT-PCR 17,80 % positive [52/292]) (Lin et al., 2011).

Analysis of environmental soil samples have been performed using a LAMP assay targeting the MIC3 gene as well as two different PCR assays targeting the B1 gene and a 529 bp repetitive fragment of the *T. gondii* genome (Du et al., 2011). The authors found comparable sensitivity rates for the LAMP and PCR/529 assays, whereas the PCR analysis of the B1 gene was less sensitive (Du et al., 2011). Furthermore, they demonstrated higher numbers of positive samples via the LAMP assay (58/252) compared to PCR technique (41/252) (Du et al., 2011).

These findings, and especially those of the environmental soil (Du et al., 2011) and water sample experiments (Sotiriadou and Karanis, 2008), confirm that the LAMP assay is a suitable application for the detection of *T. gondii* in field studies. Moreover, for monitoring contamination, LAMP may be a cost effective application used to address public health concerns.

In addition to the accuracy of molecular investigation, both the choice of sampling method and the modifications of the application of preparation steps also affect the detection of *Toxoplasma* oocysts.

The positive LAMP reactions in this study were exclusively detected in sewage water (influent n = 4; effluent n = 4). In three cases the effluent samples were positive whereas the corresponding influent analysis revealed negative results. The contamination in the effluent samples could be due to several factors, such as the retention time in the WWTPs, which varies depending on the arising amount of raw

wastewater (retention time variation 6 - 77 hours; LINEG, not published). The retention time was not calculated for the sampling of influent and effluent samples in this study (Gallas-Lindemann et al., 2012, submitted). Moreover, subsequent faecal contamination by hosts could not be excluded. The settlement tanks of the WWTPs are located outside and could be frequented by wild and domestic animals (e.g., domestic cats allowed to roam).

In the study by Sroka et al. (Sroka et al., 2006), a total number of 114 drinking water samples were analyzed both from wells and the water supply system. In microscopic and PCR analyses, *T. gondii* oocysts were identified in 15 (13.2%) and 31 (27.2%) samples, respectively. Sotiriadou and Karanis (39) have recently reported the identification of positive samples in 48% of 52 natural water samples using a LAMP-specific protocol targeting the TgOWP and B1 *Toxoplasma* genes. Aubert and Villena (2009) have efficiently detected *Toxoplasma* DNA in 37 of 482 environmental samples (7.7%), including public drinking water, as assessed by PCR analysis. However, none of these samples tested positive by bioassay (Aubert and Villena, 2009). DNA amplification may be due to the detection of dead oocysts (explaining the divergence with the bioassay). Therefore, tests designed for the identification of viable oocysts should be developed in the future.

In conclusion, waterborne parasitic diseases represent a relevant problem. Although methods designed to detect *Cryptosporidium* oocysts and *Giardia* cysts have been available for several years, methods are still in development for the detection of *Toxoplasma* oocysts in environmental samples (especially in water), which represent a possible source of human infection. In Germany, the filtration, disinfection and radiation of discharges from WWTPs is not state of the art; although, Karanis et al. (1996) have suggested a multi-barrier system for the retention or disinfection of waterborne parasites such as *Crptosporidium* and *Giardia duodenalis* found in the effluent of the wastewater treatment plants. The detection of *T. gondii* in 10.5% of WWTP effluent samples suggests that this parasite occurs frequently in the aquatic environment. *T. gondii* has not been detected in water matrices other than wastewater. However, the number of environmental water samples (n = 12) investigated in this study was too low to compare their percentages of *T. gondii* positive samples to those found in the WWTP samples. Furthermore, the limited number of environmental water samples does not supply confirmation for the presence or absence of *T. gondii* in surface water, groundwater, and drinking water.

Felidae are the main source of toxoplasmosis because they shed the infective stages (oocysts) of *T. gondii*. Water input of the investigated WWTPs mainly originates from municipal wastewater but also includes rainwater and water originating from various industries. The contamination of wastewater with *Toxoplasma* oocysts may be due to the disposal of waste from cat litter boxes into toilets.

This work provides evidence that LAMP is a sensitive, specific, rapid and cost effective method for the detection of *T. gondii* and is useful for both the investigations of cases of waterborne outbreaks and for identifying the source of contamination.

This work is the second report describing the LAMP method for the detection of *T. gondii* DNA in various water matrices. Moreover, this is the only investigation of *T. gondii* in water samples in Germany.

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5 Kurzzusammenfassung

Mit der UNO Resolution vom Juli 2010 wurde das Menschenrecht auf Wasser und Sanitätsversorgung anerkannt. Trotzdem leben weltweit 884 Millionen Menschen ohne Zugang zu sauberem Wasser oder sanitärer Grundversorgung. Dabei spielt Wasser eine herausragende Rolle bei der Übertragung von Krankheitserregen. Wasserbedingte Krankheitserreger sind seit jeher von großer humanmedizinischer Bedeutung. Mit der Schaffung geeigneter Instrumentarien wie Abwasserreinigung, Trinkwasseraufbereitung und Überwachung öffentlicher Wasserversorgungsanlagen sind die hygienischen Bedingungen in den Industrieländern bakteriologisch im Allgemeinen unter Kontrolle gebracht. Die parasitäre Belastung bleibt hierbei jedoch unberücksichtigt und die technische Umsetzung von Multibarrierensystemen zur Rückhaltung von Parasiten ist durchaus noch verbesserungswürdig.

Cryptosporidium spp. und *Giardia duodenalis* gehören zu den parasitären Protozoen der Vertebraten. Weltweit kommt es jährlich zu 2,8 x 10^8 Neuinfektionen durch *G. duodenalis* und 3.0 x 10^5 Neuinfektionen durch *Cryptosporidium*. Die Übertragung erfolgt in i.d.R. fäkal-oral durch die mit den Fäzes ausgeschiedenen Dauerstadien der Erreger. Die nahrungsmittel- und wasserassoziierte Übertragung ist weit verbreitet. Wasserbedingte Parasitosen gewinnen zunehmend an Interesse und es existieren zahlreiche wissenschaftliche Veröffentlichungen zu diesem Thema. Über das Vorkommen und die Verbreitung in Deutschland liegt jedoch nur wenig Datenmaterial vor.

In der vorliegenden Arbeit wurden zwischen Juli 2009 und Januar 2011 insgesamt 396 Wasserproben unterschiedlicher Herkunft gesammelt, mit zwei verschiedenen Verfahren aufgearbeitet und mit drei Nachweismethoden vergleichend auf das Vorhandensein von parasitären Protozoen untersucht.

Aus Zu- und Abläufen von acht kommunalen Kläranlagen wurden 206 Proben, aus Oberflächengewässern (ein Badegewässer, ein kleines Fließgewässer und der Rhein) sowie aus einem rheinnahen Trinkwassergewinnungsgebiet mit drei Grundwassermessstellen, einer Rohwassermessstelle und einer Trinkwassermess-stelle insgesamt 190 Proben untersucht.

Die vorliegende Arbeit gibt einen Überblick über das aus den Kläranlagen emittierte Belastungspotenzial an parasitären Krankheitserregern. Sie stellt dar, welche Belastungen in den Oberflächengewässern, im Grundwasser und im Trinkwasser

vorhanden sind. Zusätzlich zur Verbreitung der Parasiten in einem Gebiet von 650 km^2 wurde die Rückhaltung der Parasiten in den Kläranlagen, durch Uferfiltration, durch die Bodenpassage in das Grundwasser und die Trinkwasseraufbereitung anhand des gewonnenen Datenmaterials bewertet.

Im ersten Teil der Arbeit wurden alle Proben mittels Immunofluoreszenz-Test (IFT), 4′,6-Diamidino-2-phenylindol-Färbung und anschließender mikroskopischer Detektion (Epifluoreszenz und Differenz-Interferenz-Konstrast) quantitativ auf das Vorhandensein von *Giardia* Zysten und *Cryptosporidium* Oozysten untersucht.

Parasitenstadien konnten in allen Wasserqualitäten nachgewiesen werden. Die höchsten Raten traten erwartungsgemäß in den Zuläufen von Kläranlagen auf. Nach der Abwasserreinigung und im weiteren Verlauf des Wasserkreislaufs, im Oberflächenwasser und Grundwasser, bis hin zum aufbereiteten Wasser für die Trinkwasserversorgung konnte eine zunehmende Dezimierung der (Oo)zysten beobachtet werden.

Die Betrachtung der Ergebnisse über die Zeit ergaben für die beiden Parasiten jahreszeitliche Schwankungen und typische Jahresgänge v.a. im Zulauf der Kläranlagen. Während *Giardia* Zysten alternierende Kurvenverläufe zeigten, konnten bei *Cryptosporidium* Oozysten saisonale Spitzen beobachtet werden.

Abhängig von der Ausbaugröße, der Verfahrenstechnik und dem Einzugsgebiet traten Unterschiede in den Kläranlagen auf. Eine Korrelation zum Auftreten anderer mikrobiologischer Hygieneparameter konnte jedoch nicht festgestellt werden.

Die Untersuchungen des Badegewässers und des Fließgewässers im Einzugsgebiet wurden während der Badesaison in den Jahren 2009 und 2010 an 54 Proben durchgeführt. Einschließlich der Proben aus dem Rhein waren 11% mit *Cryptosporidien* bzw. 12% mit *Giardien* belastet. Aufgrund von Änderungen im hydraulischen Regime des Gewässersystems ergab sich in der Saison 2010 trotz erhöhten Probenvolumens keine Steigerung der Positivergebnisse.

Aus dem Trinkwassergewinnungsgebiet konnten in 8,8% der 113 Proben *Cryptosporidium* Oozysten und in 0,88% *Giardia* Zysten nachgewiesen werden. Zysten traten jedoch in keiner der Rohwasser- und Trinkwasserproben auf.

Die Arbeit konnte belegen, dass Cryptosporidien in allen Wassermatrizes auftreten und Giardia bei der Trinkwasseraufbereitung besser als Cryptosporidien zurückgehalten wird. Ausgehend von den Kläranlageneinleitungen in das Oberflächenwasser infiltriert ein Teil der Organismen ins Grundwasser. Bis zum

Endverbraucher findet eine Reduzierung der Oozysten um ein bis zwei Größenordnungen statt.

Aufgrund der niedrigen Infektionsdosis von 1 -10 (Oo)zysten stellt das Baden in Oberflächengewässern und der Trinkwasserkonsum ein Infektionsrisiko besonders für immungeschwächte Personen dar. Giardien und Cryptosporidien sollten demnach künftig in die routinemäßige Trinkwasserüberwachung implementiert werden.

Im zweiten Teil der Arbeit wurden 227 Proben mit zwei weiteren molekularbiologischen Methoden (PCR - Polymerase Chain Reaction und LAMP – Loop-mediated Isothermal Amplification) untersucht. Die Vor- und Nachteile der Methoden wurden im Hinblick auf praktische Anwendbarkeit und Effizienz herausgearbeitet und mit den Standardverfahren (USEPA 1623 und ISO 15553) verglichen.

Hieraus ergab sich eine unterschiedliche Rangfolge in der Nachweishäufigkeit der beiden Organismen mit den drei Verfahren. Mit der konventionellen mikroskopischen Untersuchung wurden von *Giardia* mehr Positivergebnisse eruiert als mit der LAMP und der PCR (56.8 % > 42.7% > 33.5%); hingegen war die Rangfolge bei *Cryptosporidium* LAMP, PCR und IFT (43.6% > 41.9% > 30.4%). Die Ursachen sind im Wesentlichen in der Spezifität und Störanfälligkeit der einzelnen Untersuchungsverfahren zu suchen.

Die relative Leistungsfähigkeit der Verfahren wurde mittels statistischer Auswertung mit dem Ergebnis bewertet, dass beide molekularbiologischen Anwendungen keine Gleichwertigkeit zur konventionellen Mikroskopie aufweisen. Das LAMP-Verfahren kann, verglichen mit der PCR, als gleichwertig eingestuft werden.

Der Nachweis von *Toxoplasma gondii* in unterschiedlich stark kontaminierten Wasserproben wird im dritten Teil dieser Arbeit vorgestellt. *T. gongii* gilt ebenfalls als einer der Parasiten, die unter dem Verdacht stehen, über den Wasserweg übertragen zu werden. Für den Nachweis von *T. gondii* existieren keine Standardverfahren, so dass hier das molekularbiologische LAMP-Verfahren, welches auf der Amplifizierung des *Toxoplasma* B1 Gens beruht, für unterschiedlich stark kontaminierte Wasserproben Anwendung fand.

Toxoplasma DNA konnte in 95 und damit in 9,6% der Proben aus den Zu- und Abläufen von Kläranlagen, nicht aber in Oberflächen-, Grund- und Trinkwasser nachgewiesen werden. Dies ist die erste Studie über den Nachweis von *Toxoplasma* Oozysten in Wasserproben aus Deutschland. Die Untersuchung von Belastungen

des Wassers mit *T. gondii* bietet Hinweise auf das Infektionspotenzial wasserassoziierter Parasiten für Mensch und Tier. In der vorliegenden Arbeit wird das LAMP-Verfahren für den Nachweis von *T. gondii* vorgestellt. Ziel war, die Aufmerksamkeit auf das Risiko von Toxoplasmose Ausbrüchen zu erhöhen.

Die Arbeit belegt, dass Parasitenstadien von *G. duodenalis*, *Cryptosporidium* spp. und *T. gondii* im Wasserkreislauf auftreten und über den Wasserweg weiterverbreitet werden. Die Vor- und Nachteile verschiedener Probenahmetechniken und Nachweismethoden konnten dargestellt werden. Außerdem konnte belegt werden, dass IFT, LAMP und PCR für die Hygieneüberwachung des Wassers, zur Vermeidung von Epidemien und zum schnellen Auffinden von Kontaminationsquellen herangezogen werden können. Eine routinemäßige Überwachung insbesondere des Trinkwassers wird gerade im Hinblick auf das Infektionsrisiko immungeschwächter Personen als sinnvoll erachtet.

6 Abstract

In July 2010, the rights of humans to water and sanitation were accepted by a UN resolution. Nevertheless, worldwide 884 million people live without access to safe drinking water and sanitation. This unsafe water plays an important role in the transmission of waterborne parasites, which are an important cause of human disease. Through implementation of techniques such as wastewater treatment, drinking water purification and monitoring of the public water supply, the unhygienic conditions that led to bacterial infections in the developed countries have been largely eliminated. Contamination with parasites has been left out of these considerations, and the implementation of multi-barrier systems for the removal of parasites is needed.

Cryptosporidium spp. and *Giardia duodenalis* are protozoan parasites that cause illness in vertebrates. The worldwide annual new infection rate of *G. duodenalis* is 2.8 x 10^8 infections and *Cryptosporidium* 3.0 x 10^5. Faecal-oral transmission of these parasites usually occurs during shedding of the robust stages of the parasites. Foodborne and waterborne transmission is common. There has been an increasing interest in waterborne parasitosis, and many scientific publications about the topic have been published. However, information about the occurrence and distribution of such infections in Germany is rare.

In total, 396 different water samples with different grades of contamination were investigated between July 2009 and January 2011.

Two different methods for sample preparation and three assays for analysis were combined for the comparative findings reported for the protozoan parasites in this study.

In this work 206 influent and effluent samples of wastewater treatment plants, 190 samples from surface waters (one recreational area, one small stream and the River Rhine) and the catchment area of a drinking water supply situated near the Rhine have been investigated.

This study gives an overview of the pathogens released from wastewater treatment plants. The contamination of surface waters, groundwater and drinking water has been demonstrated. In addition to the prevalence of the parasites in an area of 650 km^2, parasite removal by wastewater treatment plants, by riverbank filtration, by

passing through gravel layers into the groundwater and by drinking water purification have been assessed from these data.

In the first part of the study, all samples have been microscopically examined after performing an immunofluorescence test (IFT) with DAPI staining, and then, the *Giardia* cysts and *Cryptosporidium* oocysts were quantified.

Parasitic stages could be detected in all water matrices. As expected, the highest levels have been detected in the influent samples of wastewater treatment plants (WWTPs). After wastewater treatment and proceeding through the hydrological cycle from surface waters and groundwater through to treated drinking water, a reduction in the number (oo)cysts was observed.

Seasonal variations were obvious for *Cryptosporidium*, particularly for the influent samples of the wastewater treatment plants, while *Giardia* cysts showed irregular curves.

Depending on their design capacity, processing technology and catchment area differences occurred between the wastewater treatment plants. No correlation was found between the prevalence of parasites and other microbial pollutants.

The investigations of the recreational area and of the running water in the catchment area included 54 samples that had been carried out in the bathing seasons of 2009 and 2010. Including the samples from the Rhine River, 11% of the surface waters were contaminated with *Cryptosporidium* spp. and 12% with *Giardia duodenalis*. Due to changes in the hydraulic regime of the surface water system in 2010, no increase in positive results was found despite an increase in the volume of water filtered.

In the area of drinking water supply, out of 113 samples, 8.8% were positive for *Cryptosporidium* oocysts and 0.88% were positive for *Giardia* cysts. However, cysts were not detected in raw water or in drinking water. The study provided evidence that *Cryptosporidium* spp. are present in all types of water and that the removal of *Giardia* during drinking water purification was more successful. Because these parasites originate from wastewater treatment plants, emission into surface water and subsequent infiltration into the aquifer are possible. Prior to reaching the consumer, oocyst reduction of one or two orders of magnitude was detected.

Due to the low infective dose of 1 – 10 (oo)cysts swimming in surface water, consuming drinking water is an infection risk for immunocompromised persons. Therefore, *Giardia* and *Cryptosporidium* should be included in the regular monitoring of drinking water supplies in the future.

In the second part of the study, 227 samples were investigated by two additional molecular assays (nPCR, nested polymerase chain reaction and LAMP, loop-mediated isothermal amplification). The advantages and disadvantages of these methods have been described and compared to standard methods (USEPA 1623 and ISO 15553), particularly with regard to their practical applicability and effectiveness. The detection frequencies for both target organisms varied between the three methods. Conventional microscopy identified more positive *Giardia* results than LAMP and nPCR (56.8%, 42.7% and 33.5%, respectively), whereas for *Cryptosporidium* the results were highest for LAMP followed by nPCR and IFT (43.6%, 41.9% and 30.4%, respectively). The main reasons for these variations are the different specificities and sensitivities of the assays.

The relative efficiencies have been calculated statistically with the result that the molecular assays are considered not equivalent to the conventional microscopy. However, LAMP is as equivalent as nPCR.

The presence of *Toxoplasma gondii* in water samples with different levels of contamination was investigated in the third part of this work.

T. gondii is also considered a parasite that is distributed by water-based transmission. No standards for the detection of *T. gondii* are available. Therefore, the molecular biological LAMP assay, which amplifies the *Toxoplasma* B1 gene, has been performed on water samples with different grades of contamination.

Out of 95 samples, *Toxoplasma* was detected in 9.6% of the influent and effluent samples from wastewater treatment plants but was not detected in surface water, groundwater or tap water samples.

This is the first study to undertake detection of *T. gondii* oocysts in water from Germany. The investigation of *T. gondii* in water samples provides indications of the infection risk from waterborne parasites to humans and animals.

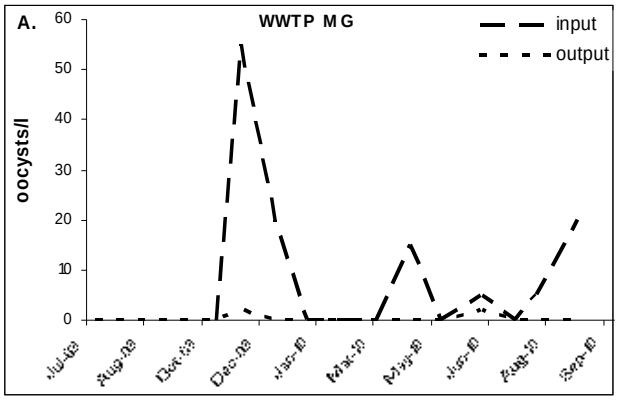
In the present study, the LAMP method was tested for the detection of *T. gondii*. Furthermore, the study aimed to bring attention to the risk of toxoplasmosis outbreaks. The study reveals evidence that parasitic stages of *G. duodenalis*, *Cryptosporidium* spp. and *T. gondii* are present in the hydrological circuit and are distributed by water-based routes. The advantages and disadvantages of the two sampling techniques and the three detection methods were demonstrated. In addition, it was verified that LAMP is equally effective compared to nPCR for the

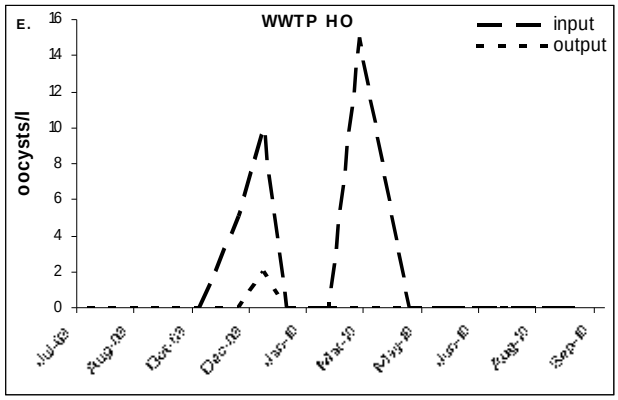
surveillance of drinking water, the prevention of epidemics, and tracing the source of contamination.

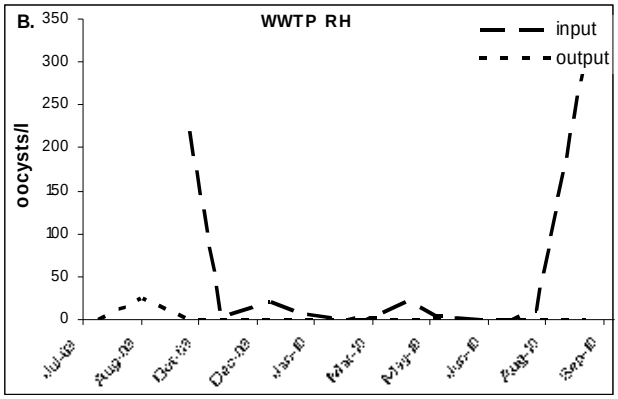
Meaningfully, the addition of *Giardia* and *Cryptosporidium* into the regular monitoring of drinking water supplies is recommended, especially for the health of immunocompromised persons.

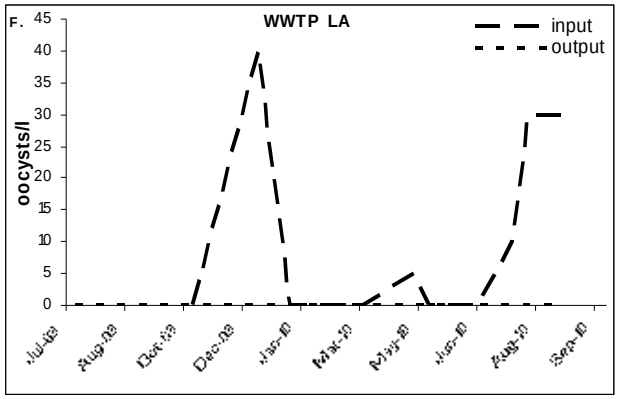
7 Appendix

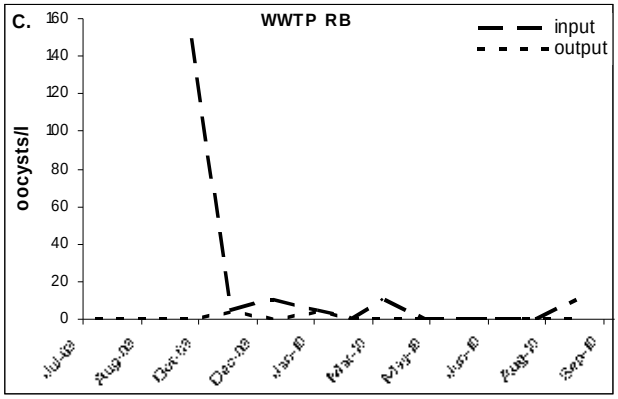
Cryptosporidium oocysts in the WWTPs during 2009 and 2010

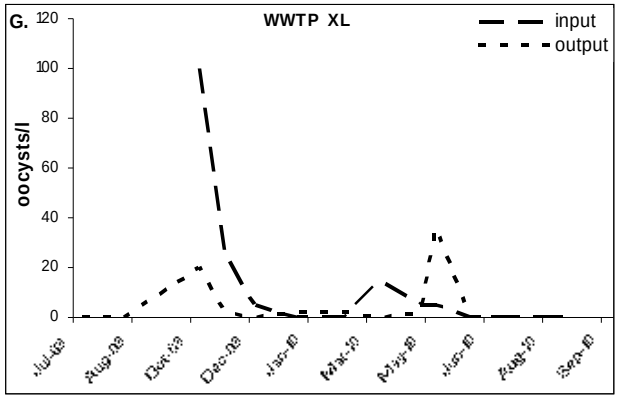


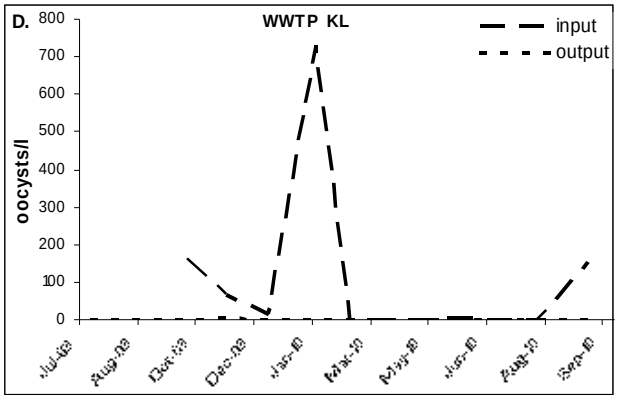


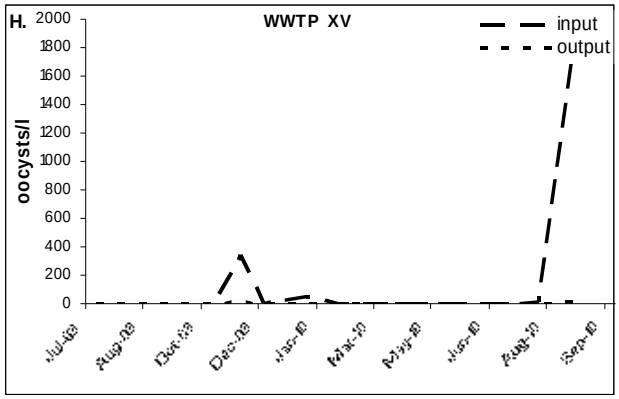




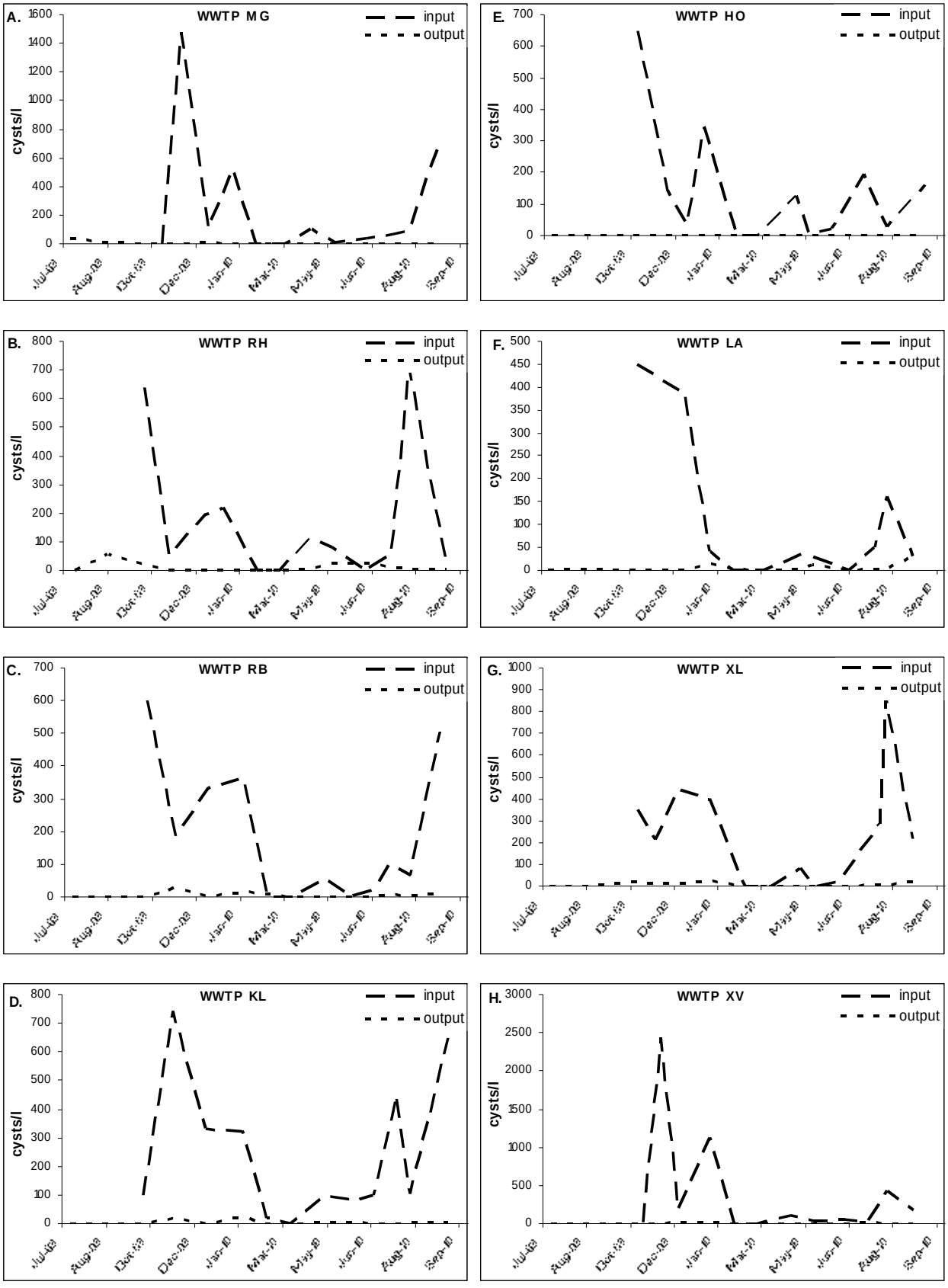








Giradia duodenalis cysts in the WWTPs during 2009 and 2010



Analytical data

WWTP Moers-Gerdt - influent								
Sample no.			126716	127136	127729	129107	130126	130846
Date of sampling (end)			27.10.2009	17.11.2009	17.12.2009	14.01.2010	10.02.2010	15.03.2010
Time of sampling (end)	h:min		07:10	06:35	07:50	08:00	06:40	06:45
Start date of sampling			26.10.2009	16.11.2009	16.12.2009	13.01.2010	09.02.2010	14.03.2010
Start time of sampling	h:min		08:15	07:00	07:50	08:00	07:35	08:15
Duration of sampling	h:min		22:55	23:35	24	24	23:05	22:30
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8	1 330	1270	1570	1600	1460	1480
pH value		DIN 38404 C5 (1984)	7,6	7,5	7,7	7,6	7,5	7,7
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	6 100000	7300000	4850000	5650000	3600000	450000
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	6 800000	7450000	5450000	3700000	4900000	1050000
Coliform Bacteria	/100ml	Colilert-18	> 2400000	24192000	>24200000	>24200000	>24200000	2780000
Escherichia coli	/100ml	Colilert-18	1200000	9208000	13000000	9800000	9800000	860000
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	> 20000	>200000	2500000	2500000	2200000	2700000
Enterococci	/100ml	Enterolert-E						
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	> 200000	400000	500000	300000	200000	2500000
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	55	20	n.n.	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	1480	105	520	n.n.	n.n.

WWTP Moers-Gerdt - influent								
Sample no.			131692	132267	133360	134203	134674	135437
Date of sampling (end)			14.04.2010	10.05.2010	15.06.2010	15.07.2010	02.08.2010	06.09.2010
Time of sampling (end)	h:min		07:45	07:25	07:30	06:15	07:10	06:30
Start date of sampling			13.04.2010	09.05.2010	14.06.2010	14.07.2010	01.08.2010	05.09.2010
Start time of sampling	h:min		07:50	07:50	07:55	07:30	07:50	07:15
Duration of sampling	h:min		23:55	23:35	23:35	22:45	23:20	23:15
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8	1 480	1500	1540	1050	1460	1400
pH value		DIN 38404 C5 (1984)	7,6	7,8	7,6	7,6	7,7	1,5
Heterotrophic Plate Counts 36℃	CFU/ml	TrinkwV 1990 A nl.1 5.	300000		15000000	200000	12100000	7900000
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	1 300000		12000000	<100000	7000000	5600000
Coliform Bacteria	/100ml	Colilert-18	340000	39000000	39000000	9800000	61000000	77000000
Escherichia coli	/100ml	Colilert-18	140000	8400000	10200000	2600000	19000000	30800000
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	2700000	1600000				
Enterococci	/100ml	Enterolert-E			2400000	2200000	2200000	2900000
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	400000	1200000	3900000	200000	100000	5100000
Cryptosporidium spp.	/l	QSA: Parasitenstadien	15	n.n.	5	n.n.	5	20
Giardia duodenalis	/l	QSA: Parasitenstadien	100	10	40	65	85	720

WWTP Moers-Gerdt - effluent									
Sample no.			124896	125256	126717	127137	127730	129108	130127
Date of sampling (end)			13.07.2009	25.08.2009	27.10.2009	17.11.2009	17.12.2009	14.01.2010	10.02.2010
Time of sampling (end)	h:min		08:00	07:35	07:20	06:40	08:00	08:05	06:50
Start date of sampling			12.07.2009	24.08.2009	26.10.2009	16.11.2009	16.12.2009	13.01.2010	09.02.2010
Start time of sampling	h:min		08:00	07:55	08:00	06:55	08:00	08:05	07:30
Duration of sampling	h:min		24	23:40	23:20	23:45	24	24	23:20
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8			1260	1100	1290	1520	1280
pH value		DIN 38404 C5 (1984)			7,6	7,6	7,5	7,1	7,6
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	4 5500	18500	13000	23000	23000	30000	8000
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	5 3000	10400	9300	9000	24000	28000	10000
Coliform Bacteria	/100ml	Colilert-18	155000	>24200	29000	8600	80000	90700	43000
Escherichia coli	/100ml	Colilert-18	51700	14000	10700	23800	33000	36000	12000
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	> 2000	>200	2200	4500	8800	13200	7400
Enterococci	/100ml	Enterolert-E							
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	0	4700	6000	<100000	10100	4400	2100
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	2	n.n.	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	40	8	n.n.	2	6	2	n.n.

WWTP Moers-Gerdt - effluent									
Sample no.			130847	131693	132268	133361	134204	134675	135438
Date of sampling (end)			15.03.2010	14.04.2010	10.05.2010	15.06.2010	15.07.2010	02.08.2010	06.09.2010
Time of sampling (end)	h:min		06:55	07:35	07:20	07:35	06:20	07:20	06:40
Start date of sampling			14.03.2010	13.04.2010	09.05.2010	14.06.2010	14.07.2010	01.08.2010	05.09.2010
Start time of sampling	h:min		08:20	08:00	08:00	07:50	07:40	07:55	07:25
Duration of sampling	h:min		22:35	23:35	23:20	23:45	22:40	23:25	23:15
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8	1 250	1300	1110	1260	970	1120	1220
pH value		DIN 38404 C5 (1984)	7,5	7,4	7,8	7,8	7,8	7,8	7,1
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	4 900	47000		38000	22000	55000	32000
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	7 900	35000		35000	29000	21000	21000
Coliform Bacteria	/100ml	Colilert-18	178200	520000	85000	109000	230000	190000	213000
Escherichia coli	/100ml	Colilert-18	71400	140000	20000	10000	51000	31000	41000
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	14300	0	10000				
Enterococci	/100ml	Enterolert-E				9800	500	5200	8500
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	> 20000	0	20000	20000	1000	10000	40000
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	2	n.n.	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	4	n.n.	n.n.	n.n.	4	n.n.
(n.n. = not detected)									

WWTP Rheinhausen - influent								
Sample no.			126708	126945	127655	128938	130151	130790
Date of sampling (end)			07.10.2009	03.11.2009	14.12.2009	04.01.2010	11.02.2010	09.03.2010
Time of sampling (end)	h:min		06:50	06:50	08:00	07:10	06:40	07:15
Start date of sampling			06.10.2009	02.11.2009	13.12.2009	03.01.2010	10.02.2010	08.03.2010
Start time of sampling	h:min		07:10	07:30	08:10	07:10	07:45	08:00
Duration of sampling	h:min		23:40	23:20	23:50	24	22:55	23:15
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8	1 100	1270	1550	1800	1550	1560
pH value		DIN 38404 C5 (1984)	7,9	7,8	7,8	7,8	8	7,7
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	> 20000	13400000	6950000	2500000	2700000	4300000
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	> 20000	13700000	5762000	2300000	5400000	6200000
Coliform Bacteria	/100ml	Colilert-18	>2420000	24200000	26000000	19400000	23000000	21420000
Escherichia coli	/100ml	Colilert-18	>2420000	10500000	8090000	9300000	8600000	9060000
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	>20000	1640000	1420000	1600000	> 2000000	400000
Enterococci	/100ml	Enterolert-E						
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	4900	> 2000000	200000	1850000	300000	3800000
Cryptosporidium spp.	/l	QSA: Parasitenstadien	220	5	20	10	1	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	640	50	195	220	n.n.	n.n.

WWTP Rheinhausen - influent								
Sample no.			131634	132358	133345	134143	134698	135578
Date of sampling (end)			13.04.2010	06.05.2010	14.06.2010	13.07.2010	03.08.2010	15.09.2010
Time of sampling (end)	h:min		08:00	06:40	07:30	07:10	07:00	07:40
Start date of sampling			12.04.2010	05.05.2010	13.06.2010	12.07.2010	02.08.2010	14.09.2010
Start time of sampling	h:min		08:30	07:50	08:30	07:55	07:50	07:40
Duration of sampling	h:min		23:30	22:50	23	23:15	23:10	24
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8	1 640	1630	1540	1240	1430	1420
pH value		DIN 38404 C5 (1984)	7,8	7,4	7,7	7,7	7,6	7,9
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	7 3000000	9000000	1090000	13000000	31000000	12600000
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	7 000000	10100000	2020000	9000000	16000000	7500000
Coliform Bacteria	/100ml	Colilert-18	3700000	6500	> 24000000	5800	87000000	69000000
Escherichia coli	/100ml	Colilert-18	1100000	880	14000000	1900	22000000	21000000
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	1600000	< 100000				
Enterococci	/100ml	Enterolert-E			> 240000	19000000	4900000	3100000
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	0	300000	780000	110000	30000	160000
Cryptosporidium spp.	/l	QSA: Parasitenstadien	20	5	n.n.	n.n.	10	310
Giardia duodenalis	/l	QSA: Parasitenstadien	115	80	n.n.	55	715	20

WWTP Rheinhausen - effluent									
Sample no.			124901	125257	126709	126946	127656	128939	130152
Date of sampling (end)			20.07.2009	25.08.2009	07.10.2009	03.11.2009	14.12.2009	04.01.2010	11.02.2010
Time of sampling (end)	h:min		08:15	07:15	07:05	07:00	07:55	07:30	06:55
Start date of sampling			19.07.2009	24.08.2009	06.10.2009	02.11.2009	13.12.2009	03.01.2010	10.02.2010
Start time of sampling	h:min		08:15	08:00	07:15	08:00	07:55	07:30	07:55
Duration of sampling	h:min		24	23:15	23:50	23	24	24	23
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8			1020	780	980	1140	1230
pH value		DIN 38404 C5 (1984)			7,6	7,7	7,6	7,5	7,4
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	1 4000	51000	190000	162700	65000	100000	22000
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	9 400	27000	114000	135500	62000	120000	23000
Coliform Bacteria	/100ml	Colilert-18	>240000	24200	>2420000	700000	370000	303000	140000
Escherichia coli	/100ml	Colilert-18	140000	3900	>2420000	220000	98000	97500	40000
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	187	>200	>20000	30000	10000	10000	50000
Enterococci	/100ml	Enterolert-E							
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	25	2100	1900	1000	< 100000	30000	20000
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	24	n.n.	n.n.	n.n.	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	56	20	2	n.n.	n.n.	1

WWTP Rheinhausen - effluent									
Sample no.			130791	131635	132359	133346	134144	134699	135579
Date of sampling (end)			09.03.2010	13.04.2010	06.05.2010	14.06.2010	13.07.2010	03.08.2010	15.09.2010
Time of sampling (end)	h:min		07:10	08:00	06:50	08:00	07:20	06:55	08:00
Start date of sampling			08.03.2010	12.04.2010	05.05.2010	13.06.2010	12.07.2010	02.08.2010	14.09.2010
Start time of sampling	h:min		08:05	08:00	08:00	08:20	07:50	08:05	08:00
Duration of sampling	h:min		23:05	24	22:50	23:40	23:30	22:50	24
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8	1 130	1220	1210	1190	1070	1060	1130
pH value		DIN 38404 C5 (1984)	7,2	7,6	7,1	7,3	7,8	7,4	7,8
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	2 2000	56000	12000	200000	37000	78000	120000
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	3 1000	67000	15000	10000	21000	36000	72000
Coliform Bacteria	/100ml	Colilert-18	1119000	460000	6300	< 100000	270	400000	740000
Escherichia coli	/100ml	Colilert-18	395000	160000	3100	< 100000	52	63000	290000
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	16000	40000	< 10000				
Enterococci	/100ml	Enterolert-E				4100	8500	5200	32000
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	35000	0	<100	< 100000	0	90000	30000
Cryptosporidium spp.	/l	QSA: Parasitenstadien	2	n.n.	2	n.n.	n.n.	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	6	22	24	12	4	4
(n.n. = not detected)									

WWTP Rheinberg - influent								
Sample no.			126712	127031	127737	129251	130293	131068
Date of sampling (end)			08.10.2009	10.11.2009	17.12.2009	25.01.2010	23.02.2010	23.03.2010
Time of sampling (end)	h:min		07:05	07:40	07:00	07:50	07:10	07:55
Start date of sampling			07.10.2009	09.11.2009	16.12.2009	24.01.2010	22.02.2010	22.03.2010
Start time of sampling	h:min		07:05	07:55	07:45	07:50	07:45	07:55
Duration of sampling	h:min		24	23:45	23:15	24	23:25	24
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8	940	1800	1700	3010	1510	2150
pH value		DIN 38404 C5 (1984)	7,8	7,9	7,9	8,1	7,7	7,9
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	2070000	7700000	10800000	3100000	180000	3100000
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	1800000	6900000	7400000	3500000	30000	9600000
Coliform Bacteria	/100ml	Colilert-18	>2420000	410000	58000000	630000	26000000	26000000
Escherichia coli	/100ml	Colilert-18	1120000	200000	18000000	520000	8600000	8130000
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	>200000	500000	2100000	< 10000000	1650000	1400000
Enterococci	/100ml	Enterolert-E						
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	>200000	2500000	600000	< 10000000	320000	0
Cryptosporidium spp.	/l	QSA: Parasitenstadien	150	5	10	5	n.n.	10
Giardia duodenalis	/l	QSA: Parasitenstadien	600	185	330	365	2	n.n.

WWTP Rheinberg - influent								
Sample no.			131946	133807	134449	134126	134707	135455
Date of sampling (end)			28.04.2010	27.05.2010	23.06.2010	12.07.2010	03.08.2010	07.09.2010
Time of sampling (end)	h:min		07:25	07:40	07:50	07:25	07:30	07:50
Start date of sampling			27.04.2010	26.05.2010	22.06.2010	11.07.2010	02.08.2010	06.09.2010
Start time of sampling	h:min		07:50	07:55	08:00	07:25	07:30	07:50
Duration of sampling	h:min		23:35	23:45	23:50	24	24	24
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8	2380	2070	2000	1850	1280	1820
pH value		DIN 38404 C5 (1984)	7,9	7,9	7,9	7,7	7,6	7,9
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	10600000	10820000	12000000	16000000	17000000	18000000
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	12000000	7640000	7100000	10500000	10900000	13000000
Coliform Bacteria	/100ml	Colilert-18	44000000	906000	4600000	8200000	38000000	87000000
Escherichia coli	/100ml	Colilert-18	9060000	300000	760000	2060000	12000000	22000000
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	>2000000	1480000				
Enterococci	/100ml	Enterolert-E			14000000	2060000	2400000	1600000
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	270000	360000	130000	170000	130000	100000
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	10
Giardia duodenalis	/l	QSA: Parasitenstadien	60	n.n.	20	100	65	505

WWTP Rheinberg - effluent									
Sample no.			124898	125240	126713	127032	127738	129252	130294
Date of sampling (end)			16.07.2009	17.08.2009	08.10.2009	10.11.2009	17.12.2009	25.01.2010	23.02.2010
Time of sampling (end)	h:min		07:35	08:15	07:10	07:50	07:10	08:00	07:15
Start date of sampling			15.07.2009	16.08.2009	07.10.2009	09.11.2009	16.12.2009	24.01.2010	22.02.2010
Start time of sampling	h:min		07:55	08:15	07:10	08:00	07:50	08:00	07:50
Duration of sampling	h:min		23:40	24	24	23:50	23:20	24	23:25
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8			860	1000	1130	1400	1500
pH value		DIN 38404 C5 (1984)			7,3	7,3	7,3	7,1	7,1
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	15000	10550	10720	61000	180000	< 5000	76000
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	10500	4700	11100	30200	140000	10000	71000
Coliform Bacteria	/100ml	Colilert-18	37000	17000	205000	150000	460000	<100000	590000
Escherichia coli	/100ml	Colilert-18	8600	2800	46000	53000	110600	<100000	203000
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	1900	1130	18000	< 100000	< 1000000	<10000000	9000
Enterococci	/100ml	Enterolert-E							
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	0	>200	8000	26000	6000	<100000	23000
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	4	n.n.	4	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	28	n.n.	16	7

WWTP Rheinberg - effluent									
Sample no.			131069	131947	133808	134450	134127	134708	135456
Date of sampling (end)			23.03.2010	28.04.2010	27.05.2010	23.06.2010	12.07.2010	03.08.2010	07.09.2010
Time of sampling (end)	h:min		08:00	07:30	07:35	07:45	07:20	07:35	07:55
Start date of sampling			22.03.2010	27.04.2010	26.05.2010	22.06.2010	11.07.2010	02.08.2010	06.09.2010
Start time of sampling	h:min		08:00	08:00	08:00	07:50	07:20	07:35	07:55
Duration of sampling	h:min		24	23:30	23:35	23:55	24	24	24
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8	830	1030	730	1240	1050	1430	1140
pH value		DIN 38404 C5 (1984)	7,2	7,3	7,6	7,5	7,3	7,6	7,5
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	12600	8300	5000	51000	220000	33000	46000
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	27000	8300	2000	28000	150000	17000	19000
Coliform Bacteria	/100ml	Colilert-18	97000	20000	20000	36000	72000	140000	120000
Escherichia coli	/100ml	Colilert-18	31000	10000	<10000	9800	16000	41000	20000
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	10000	0	0				
Enterococci	/100ml	Enterolert-E				190000	20500	7400	14000
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	3000	0	0	2000	1300	2000	40000
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	10	4	8
(n.n. = not detected)									

WWTP Kamp-Lintfort - influent								
Sample no.			126704	127012	127678	129183	130301	131036
Date of sampling (end)			06.10.2009	09.11.2009	15.12.2009	26.01.2010	23.02.2010	22.03.2010
Time of sampling (end)	h:min		07:15	07:55	07:25	07:10	07:35	09:45
Start date of sampling			05.10.2009	08.11.2009	14.12.2009	25.01.2010	22.02.2010	21.03.2010
Start time of sampling	h:min		07:36	07:55	07:25	07:10	07:35	09:45
Duration of sampling	h:min		23:39	24	24	24	24	24
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8	1 870	1960	2770	2230	1110	1830
pH value		DIN 38404 C5 (1984)	7,7	7,7	7,5	7,5	7,8	7,8
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	2 0000	11600000	4850000	6000000	3500000	1100000
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	2 0000	13200000	4750000	4900000	440000	4400000
Coliform Bacteria	/100ml	Colilert-18	242000	24200000	24200000	49000000	13000000	21400000
Escherichia coli	/100ml	Colilert-18	242000	12000000	7300000	9900000	4900000	6800000
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	20000	200000	1610000	1410000	940000	1200000
Enterococci	/100ml	Enterolert-E						
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	20000	2000000	200000	500000	0	700000
Cryptosporidium spp.	/l	QSA: Parasitenstadien	160	65	15	730	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	100	745	330	320	18	n.n.

WWTP Kamp-Lintfort - influent								
Sample no.			131966	132412	133515	134265	134748	135611
Date of sampling (end)			29.04.2010	31.05.2010	23.06.2010	20.07.2010	04.08.2010	16.09.2010
Time of sampling (end)	h:min		07:40	08:10	06:40	07:05	07:15	07:20
Start date of sampling			28.04.2010	30.05.2010	22.06.2010	19.07.2010	03.08.2010	15.09.2010
Start time of sampling	h:min		07:40	08:10	07:45	07:50	07:45	07:20
Duration of sampling	h:min		24	24	22:55	23:15	23:30	24
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8	3 100	1980	1630	2530		1670
pH value		DIN 38404 C5 (1984)	7,2	7,6	7,5	7,5		7,6
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	6 400000	4900000	13000000	5100000	16000000	9000000
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	4 600000	4700000	10500000	2800000	12000000	5900000
Coliform Bacteria	/100ml	Colilert-18	41060000	3300000	7700000	10500000	92000000	41000000
Escherichia coli	/100ml	Colilert-18	7030000	1080000	2600000	3300000	20100000	8100000
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	1250000	242000000	2600000	3900000	24000000	1200000
Enterococci	/100ml	Enterolert-E						
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	400000	3500000	300000	1700000	600000	9800000
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	5	n.n.	n.n.	n.n.	150
Giardia duodenalis	/l	QSA: Parasitenstadien	100	80	100	450	105	650

WWTP Kamp-Lintfort - effluent									
Sample no.			124899	125239	126705	127013	127679	129184	130302
Date of sampling (end)			15.07.2009	17.08.2009	06.10.2009	09.11.2009	15.12.2009	26.01.2010	23.02.2010
Time of sampling (end)	h:min		08:10	08:00	07:10	07:48	07:25	07:20	07:30
Start date of sampling			14.07.2009	16.08.2009	05.10.2009	08.11.2009	14.12.2009	25.01.2010	22.02.2010
Start time of sampling	h:min		08:10	08:00	07:30	07:48	07:25	07:20	07:30
Duration of sampling	h:min		24	24	23:40	24	24	24	24
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8			1520	1440	1930	1720	2030
pH value		DIN 38404 C5 (1984)			7,4	7,3	7,2	7,2	7,3
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	3 0000	19000	22000	11300	19000	21000	43000
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	9 400	6500	17000	11800	15000	30000	61000
Coliform Bacteria	/100ml	Colilert-18	15000	26000	41000	51000	66000	308000	460000
Escherichia coli	/100ml	Colilert-18	3700	5500	13000	16000	22000	86000	140000
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	300	1180	4900	4000	7000	25000	70000
Enterococci	/100ml	Enterolert-E							
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	700	200	4100	22000	0	10000	10000
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	5	n.n.	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	18	n.n.	26	n.n.

WWTP Kamp-Lintfort - effluent									
Sample no.			131037	131967	132413	133516	134266	134749	135612
Date of sampling (end)			22.03.2010	29.04.2010	31.05.2010	23.06.2010	20.07.2010	04.08.2010	16.09.2010
Time of sampling (end)	h:min		09:30	07:30	08:00	06:45	07:10	07:25	07:30
Start date of sampling			21.03.2010	28.04.2010	30.05.2010	22.06.2010	19.07.2010	03.08.2010	15.09.2010
Start time of sampling	h:min		09:30	07:30	08:00	07:20	07:35	07:40	07:30
Duration of sampling	h:min		24	24	24	23:25	23:35	23:45	24
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8	1 220	1480	1490	1450	1170		1240
pH value		DIN 38404 C5 (1984)	7,2	7,3	7,4	7,5	7,5		7,5
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	2 9000	18000	18000	4000	31000	19000	28000
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	3 2000	13000	7000	4000	1000	59000	9000
Coliform Bacteria	/100ml	Colilert-18	96000	84000	1500	860	8800	43000	93000
Escherichia coli	/100ml	Colilert-18	37000	23000	<100	200	1800	17000	16000
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	45000	7000	1400000	1000	2000	10000	5200
Enterococci	/100ml	Enterolert-E							
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	10000	0	3000	0	1000	1000	18000
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	2	n.n.	n.n.	2
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	4	6	2	2	6	6
(n.n. = not detected)									

WWTP Hoerstgen - influent								
Sample no.			126720	127262	127691	128972	130160	130794
Date of sampling (end)			21.10.2009	24.11.2009	16.12.2009	05.01.2010	11.02.2010	09.03.2010
Time of sampling (end)	h:min		07:30	07:40	07:40	07:45	09:50	08:10
Start date of sampling			20.10.2009	23.11.2009	15.12.2009	04.01.2010	10.02.2010	08.03.2010
Start time of sampling	h:min		07:30	10:35	08:38	10:00	09:50	08:10
Duration of sampling	h:min		24	21:05	23:02	21:45	24	24
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8	1 590	860	1510	1390	840	1400
pH value		DIN 38404 C5 (1984)	8	7,8	8	8	7,6	8,4
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	6 500000	5050000	1800000	2600000	2100000	1700000
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	5 800000	7150000	1550000	7200000	2600000	2200000
Coliform Bacteria	/100ml	Colilert-18	>2420000	13000000	>24200000	5400000	16000000	22820000
Escherichia coli	/100ml	Colilert-18	1300000	4100000	9800000	2100000	6600000	12960000
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	>20000	1500000	1430000	1500000	500000	1000000
Enterococci	/100ml	Enterolert-E						
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	>20000	2300000	2800000	600000	100000	1800000
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	5	10	n.n.	n.n.	15
Giardia duodenalis	/l	QSA: Parasitenstadien	650	140	35	345	n.n.	n.n.

WWTP Hoerstgen - influent			131857	132213	133200	134089	134724	135593
Sample no.			21.04.2010	05.05.2010	01.06.2010	08.07.2010	03.08.2010	15.09.2010
Date of sampling (end)			08:20	07:50	08:50	07:40	09:35	10:30
Time of sampling (end)	h:min		20.04.2010	04.05.2010	31.05.2010	07.07.2010	02.08.2010	14.09.2010
Start date of sampling			08:20	07:50	10:55	10:05	09:35	13:26
Start time of sampling	h:min		24	24	21:55	21:35	24	21:04
Duration of sampling	h:min							
			970	1690	1300	1260	770	770
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8	7 ,7	8,2	7,3	7,7	7,6	7,7
pH value		DIN 38404 C5 (1984)						
			3400000	2800000	6800000	3800000	5200000	4400000
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	3 650000	2400000	6600000	2500000	2700000	2900000
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	8 330000	41000	9200000	8700000	46000000	20600000
Coliform Bacteria	/100ml	Colilert-18	2280000	20000	3100000	720000	6600000	6300000
Escherichia coli	/100ml	Colilert-18	1100000	1500000	> 24000000			
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15				560000	310000	2100000
Enterococci	/100ml	Enterolert-E	800000	300000	600000	100	100000	1500000
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5						
			n.n.	n.n.	n.n.	n.n.	n.n.	n.n.
Cryptosporidium spp.	/l	QSA: Parasitenstadien	125	10	20	195	24	160
Giardia duodenalis	/l	QSA: Parasitenstadien						

WWTP Hoerstgen - effluent									
Sample no.			124900	125248	126721	127263	127692	128973	130161
Date of sampling (end)			15.07.2009	18.08.2009	21.10.2009	24.11.2009	16.12.2009	05.01.2010	11.02.2010
Time of sampling (end)	h:min		07:50	07:15	07:25	07:45	07:55	07:40	10:00
Start date of sampling			14.07.2009	17.08.2009	20.10.2009	23.11.2009	15.12.2009	04.01.2010	10.02.2010
Start time of sampling	h:min		07:50	08:00	07:25	10:30	08:34	08:34	10:00
Duration of sampling	h:min		24	23:15	24	21:15	23:21	23:06	24
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8			1250	730	1200	1060	1180
pH value		DIN 38404 C5 (1984)			7,4	7,6	7,4	7,4	7,6
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	1 4000	63000	12000	36000	55000	18000	55000
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	5 800	36000	4000	35000	16000	5000	133000
Coliform Bacteria	/100ml	Colilert-18	55000	39000	14000	210000	130000	12000	> 242000
Escherichia coli	/100ml	Colilert-18	1700	4400	4100	140000	49000	3900	> 242000
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	400	930	1400	16000	6300	2800	> 20000
Enterococci	/100ml	Enterolert-E							
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	300	410	700	> 20000	3000	105000	19000
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	2	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.

WWTP Hoerstgen - effluent									
Sample no.			130795	131858	132214	133201	134090	134725	135594
Date of sampling (end)			09.03.2010	21.04.2010	05.05.2010	01.06.2010	08.07.2010	03.08.2010	15.09.2010
Time of sampling (end)	h:min		08:05	08:15	08:00	08:55	07:45	09:30	10:35
Start date of sampling			08.03.2010	20.04.2010	04.05.2010	31.05.2010	07.07.2010	02.08.2010	14.09.2010
Start time of sampling	h:min		08:05	08:15	08:00	10:50	10:00	09:30	13:25
Duration of sampling	h:min		24	24	24	22:05	21:45	24	21:10
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8	1 270	1130	1140	1110	1190	1140	1080
pH value		DIN 38404 C5 (1984)	7,6	7,7	7,2	7,5	7,6	7,4	
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	1 0000	1000	<1000	3000	13000	59000	49000
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	2 3000	5000	<1000	2000	4300	11000	6000
Coliform Bacteria	/100ml	Colilert-18	127400	2000	100	2900	720	99000	26000
Escherichia coli	/100ml	Colilert-18	77600	< 1000	<10	100	<100	8600	10000
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	27000	<1000	<10	< 100			
Enterococci	/100ml	Enterolert-E					2900	5700	1500
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	37000	< 1000	<10	1000	5000	1000	1000
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.

(n.n. = not detected)

WWTP Labbeck - influent								
Sample no.			126732	127708	129092	130115	130876	131738
Date of sampling (end)			22.10.2009	16.12.2009	13.01.2010	09.02.2010	16.03.2010	28.04.2010
Time of sampling (end)	h:min		08:15	07:50	08:25	06:20	08:15	07:10
Start date of sampling			21.10.2009	15.12.2009	12.01.2010	08.02.2010	15.03.2010	27.04.2010
Start time of sampling	h:min		12:40	07:50	08:30	06:20	08:15	07:10
Duration of sampling	h:min		19:35	24	23:55	24	24	24
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8	1 600	1540	1310	1360	1260	1500
pH value		DIN 38404 C5 (1984)	7,8	8	8,1	8,1	8,6	7,7
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	7 900000	2600000	600000	1000000	130000	8800000
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	1 1300000	2700000	410000	300000	250000	12000000
Coliform Bacteria	/100ml	Colilert-18	>242000	>2420000	>24200000	>24200000	16000000	130000000
Escherichia coli	/100ml	Colilert-18	112000	1120000	3800000	4400000	2500000	19000000
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	>20000	>200000	920000	910000	760000	1680000
Enterococci	/100ml	Enterolert-E						
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	>20000	330000	70000	190000	650000	210000
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	40	n.n.	n.n.	n.n.	5
Giardia duodenalis	/l	QSA: Parasitenstadien	450	385	40	n.n.	n.n.	35

WWTP Labbeck - influent							
Sample no.			132284	133500	134293	134758	135377
Date of sampling (end)			11.05.2010	22.06.2010	21.07.2010	04.08.2010	02.09.2010
Time of sampling (end)	h:min		08:40	06:50	08:10	11:00	07:15
Start date of sampling			10.05.2010	21.06.2010	20.07.2010	03.08.2010	01.09.2010
Start time of sampling	h:min		08:40	07:40	08:10	11:00	07:15
Duration of sampling	h:min		24	23:10	24	24	24
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8	1 570	1610	1330		1610
pH value		DIN 38404 C5 (1984)	8,2	7,6	7,8		7,8
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	7 300000	12300000	1500000	14000000	11118000
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	9 600000	11000000	2000000	8100000	7000000
Coliform Bacteria	/100ml	Colilert-18	35000000	9200000	9200000	140000000	31000000
Escherichia coli	/100ml	Colilert-18	8600000	2600000	1500000	33000000	9600000
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	1410000				
Enterococci	/100ml	Enterolert-E		4320000	3700000	4100000	1313000
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	980000	420000	210000	410000	40000
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	10	30	30
Giardia duodenalis	/l	QSA: Parasitenstadien	30	n.n.	50	160	30

WWTP Labbeck - effluent									
Sample no.			124894	125243	126733	127709	129093	130116	130877
Date of sampling (end)			14.07.2009	19.08.2009	22.10.2009	16.12.2009	13.01.2010	09.02.2010	16.03.2010
Time of sampling (end)	h:min		07:35	07:10	08:25	08:00	08:20	06:25	08:10
Start date of sampling			13.07.2009	18.08.2009	21.10.2009	15.12.2009	12.01.2010	08.02.2010	15.03.2010
Start time of sampling	h:min		07:55	07:10	12:45	08:00	08:20	06:25	08:10
Duration of sampling	h:min		23:40	24	19:40	24	24	24	24
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8			1370	1280	1240	1160	1330
pH value		DIN 38404 C5 (1984)			7,6	7,4	7,5	7,6	7,6
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	3 4000	133000	5000	26300	48000	19000	5300
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	2 6000	89000	7000	22800	31000	19000	10200
Coliform Bacteria	/100ml	Colilert-18	200000	>24200	39000	>242000	820000	920000	920000
Escherichia coli	/100ml	Colilert-18	41000	24200	8000	58000	270000	93000	380000
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	> 2000	30000	1800	9200	17400	11900	84000
Enterococci	/100ml	Enterolert-E							
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	0	1100	2000	1700	2100	1900	70000
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	2	n.n.	n.n.	16	2	n.n.

WWTP Labbeck - effluent								
Sample no.			131739	132285	133501	134294	134759	135378
Date of sampling (end)			28.04.2010	11.05.2010	22.06.2010	21.07.2010	04.08.2010	02.09.2010
Time of sampling (end)	h:min		07:20	08:35	06:55	08:05	11:05	08:45
Start date of sampling			27.04.2010	10.05.2010	21.06.2010	20.07.2010	03.08.2010	01.09.2010
Start time of sampling	h:min		07:20	08:35	08:00	08:05	11:05	07:20
Duration of sampling	h:min		24	24	22:55	24	24	1d 1:25
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8	1 320	1340	1390	1290		1120
pH value		DIN 38404 C5 (1984)	7,6	8,2	7,5	7,4		7,7
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	2 000	9000	5000	8000	18000	13600
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	8 000	15000	3000	3000	12000	3000
Coliform Bacteria	/100ml	Colilert-18	22000	79000	3000	6000	69000	36000
Escherichia coli	/100ml	Colilert-18	1000	15000	410	960	10900	11000
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	0	8000				
Enterococci	/100ml	Enterolert-E			<10000	1000	5200	<1000
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	0	10000	0	0	<1000000	<1000
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	4	12	n.n.	2	4	30
(n.n. = not detected)								

WWTP Xanten-Lüttingen - influent								
Sample no.			126724	127060	127577	129192	130269	131090
Date of sampling (end)			22.10.2009	11.11.2009	08.12.2009	13.01.2010	22.02.2010	24.03.2010
Time of sampling (end)	h:min		07:45	07:55	08:10	08:00	07:50	07:50
Start date of sampling			21.10.2009	10.11.2009	07.12.2009	12.01.2010	21.02.2010	23.03.2010
Start time of sampling	h:min		07:45	07:55	08:10	08:00	07:50	07:50
Duration of sampling	h:min		24	24	24	24	24	24
Conductivity at 25°C (in situ)	µS/cm	DIN EN 27888 C8	1 340	1020	980	1260	1290	1180
pH value		DIN 38404 C5 (1984)	7,9	8	7,8	7,5	7,4	7,6
Counts of colonies 36°C	CFU/ml	TrinkwV 1990 Anl.1 5.	5 500000	3200000	2290000	900000	1900000	3150000
Counts of colonies 20°C	CFU/ml	TrinkwV 1990 Anl.1 5.	9 300000	2400000	3500000	900000	1300000	2400000
Coliform Bacteria	/100ml	Colilert-18	>242000	> 24200000	24200000	1540000	>24200000	16000000
Escherichia coli	/100ml	Colilert-18	170000	9208000	4600000	364000	7300000	5800000
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	>20000	100000	127000	104000	>200000	>200000
Enterococci	/100ml	Enterolert-E						
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	>20000	> 2000000	1000	2000	>200000	>200000
Cryptosporidium spp.	/l	QSA: Parasitenstadien	100	25	5	n.n.	n.n.	15
Giardia duodenalis	/l	QSA: Parasitenstadien	350	210	440	395	2	n.n.

WWTP Xanten-Lüttingen - influent								
Sample no.			131846	132388	133244	134348	134679	135365
Date of sampling (end)			27.04.2010	11.05.2010	08.06.2010	26.07.2010	02.08.2010	02.09.2010
Time of sampling (end)	h:min		08:00	07:55	08:10	07:50	07:10	07:50
Start date of sampling			26.04.2010	10.05.2010	07.06.2010	25.07.2010	01.08.2010	01.09.2010
Start time of sampling	h:min		08:00	07:55	08:10	07:50	07:10	07:50
Duration of sampling	h:min		24	24	24	24	24	24
Conductivity at 25°C (in situ)	µS/cm	DIN EN 27888 C8	1 260	1390	1510	1370	540	1250
pH value		DIN 38404 C5 (1984)	7,5	8,3	7,9	7,7	7,8	7,8
Counts of colonies 36°C	CFU/ml	TrinkwV 1990 Anl.1 5.	3 100000	4600000	6500000	8300000	13000000	8550000
Counts of colonies 20°C	CFU/ml	TrinkwV 1990 Anl.1 5.	2 600000	4700000	5000000	6100000	7100000	5180000
Coliform Bacteria	/100ml	Colilert-18	41100000	24000000	> 24000000	> 24000000	36000000	37000000
Escherichia coli	/100ml	Colilert-18	9330000	8200000	4400000	16000000	12000000	18500000
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	3200000	>300000	> 2400000			
Enterococci	/100ml	Enterolert-E				3100000	1700000	1201000
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	400000	> 200000	> 2000000	6400000	200000	<100000
Cryptosporidium spp.	/l	QSA: Parasitenstadien	5	5	n.n.	n.n.	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	80	n.n.	20	285	870	215

WWTP Xanten-Lüttingen - effluent									
Sample no.			124893	125242	126725	127061	127578	129193	130270
Date of sampling (end)			14.07.2009	19.08.2009	22.10.2009	11.11.2009	08.12.2009	13.01.2010	22.02.2010
Time of sampling (end)	h:min		08:00	07:25	07:50	08:00	08:00	07:55	08:00
Start date of sampling			13.07.2009	18.08.2009	21.10.2009	10.11.2009	07.12.2009	12.01.2010	21.02.2010
Start time of sampling	h:min		08:00	07:25	08:00	08:00	08:00	07:55	08:00
Duration of sampling	h:min		24	24	23:50	24	24	24	24
Conductivity at 25°C (in situ)	µS/cm	DIN EN 27888 C8			1010	780	490	1060	1020
pH value		DIN 38404 C5 (1984)			7	7	6,8	7,3	7,1
Counts of colonies 36°C	CFU/ml	TrinkwV 1990 Anl.1 5.	2 9000	4900	28000	16000	13000	17800	9200
Counts of colonies 20°C	CFU/ml	TrinkwV 1990 Anl.1 5.	2 0000	2400	54000	30000	7600	16400	7600
Coliform Bacteria	/100ml	Colilert-18	200000	>24200	200000	153000	313000	210000	81600
Escherichia coli	/100ml	Colilert-18	61000	6500	34000	48000	24000	86000	36400
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	> 2000	1000	7600	15000	12000	19000	11000
Enterococci	/100ml	Enterolert-E							
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	0	620	3700	18000	4000	6000	2000
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	20	2	n.n.	2	2
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	2	20	12	14	24	n.n.

WWTP Xanten-Lüttingen - effluent									
Sample no.			131091	131847	132389	133245	134349	134680	135366
Date of sampling (end)			24.03.2010	27.04.2010	11.05.2010	08.06.2010	26.07.2010	02.08.2010	02.09.2010
Time of sampling (end)	h:min		08:00	08:05	08:00	08:05	08:00	07:20	08:00
Start date of sampling			23.03.2010	26.04.2010	10.05.2010	07.06.2010	25.07.2010	01.08.2010	01.09.2010
Start time of sampling	h:min		08:00	08:05	08:00	08:05	08:00	07:20	08:00
Duration of sampling	h:min		24	24	24	24	24	24	24
Conductivity at 25°C (in situ)	µS/cm	DIN EN 27888 C8	7 70	890	860	1000	800	830	650
pH value		DIN 38404 C5 (1984)	7,3	7,5	8,2	7,4	7,6	7,1	7,5
Counts of colonies 36°C	CFU/ml	TrinkwV 1990 Anl.1 5.	8 000	17000	13000	3000	31000	61000	16270
Counts of colonies 20°C	CFU/ml	TrinkwV 1990 Anl.1 5.	6 000	16000	16000	10000	16000	20000	11640
Coliform Bacteria	/100ml	Colilert-18	55000	170000	160000	53000	160000	250000	140000
Escherichia coli	/100ml	Colilert-18	203000	36000	39000	15000	39000	130000	24000
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	7000	23000	12300	30500			
Enterococci	/100ml	Enterolert-E					9800	15000	4100
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	2000	3000	8300	8000	2000	3000	3000
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	2	36	n.n.	n.n.	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	2	n.n.	n.n.	8	8	20
(n.n. = not detected)									

WWTP Xanten-Vynen - influent								
Sample no.			126728	127147	127582	129083	130105	130810
Date of sampling (end)			29.10.2009	17.11.2009	08.12.2009	13.01.2010	09.02.2010	10.03.2010
Time of sampling (end)	h:min		08:15	07:50	08:15	07:58	07:15	08:00
Start date of sampling			28.10.2009	16.11.2009	07.12.2009	12.01.2010	08.02.2010	09.03.2010
Start time of sampling	h:min		08:15	07:50	08:15	07:58	07:15	08:00
Duration of sampling	h:min		24	24	24	24	24	24
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8	1 250	1370	210	1250	1330	1210
pH value		DIN 38404 C5 (1984)	7,7	8,6	7,7	7,9	8	8,1
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	3 120000	10600000	900000	510000	1700000	3000000
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	2 150000	9600000	2100000	270000	3200000	4100000
Coliform Bacteria	/100ml	Colilert-18	1259000000	199000000	<1000000	13400000	30900000	30760000
Escherichia coli	/100ml	Colilert-18	426000000	30000000	<1000000	8500000	7400000	23590000
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	> 200000	1880000	390000	990000	1400000	1800000
Enterococci	/100ml	Enterolert-E						
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	< 10000000	2000000	10000	250000	240000	1900000
Cryptosporidium spp.	/l	QSA: Parasitenstadien	60	335	n.n.	45	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	20	2436	185	1125	n.n.	5

WWTP Xanten-Vynen - influent								
Sample no.			131729	132280	133396	134156	134754	135371
Date of sampling (end)			15.04.2010	11.05.2010	16.06.2010	13.07.2010	04.08.2010	02.09.2010
Time of sampling (end)	h:min		07:45	07:25	09:10	10:40	07:40	09:00
Start date of sampling			14.04.2010	10.05.2010	15.06.2010	12.07.2010	03.08.2010	01.09.2010
Start time of sampling	h:min		07:45	07:25	09:10	11:25	07:40	09:00
Duration of sampling	h:min		24	24	24	23:15	24	24
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8	1 320	1350	1470	1330		1240
pH value		DIN 38404 C5 (1984)	7,9	8,2	8,1	7,6		7,8
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	5 800000	6550000	12000000	18000000	15000000	13820000
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	6 200000	7238095	8200000	10500000	8500000	10810000
Coliform Bacteria	/100ml	Colilert-18	3500000	> 24000000	46000000	13000	112000000	112000000
Escherichia coli	/100ml	Colilert-18	850000	19000000	11000000	2100	30800000	14000000
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	1310000	1480000				
Enterococci	/100ml	Enterolert-E			3100000	3080000	2900000	1935000
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	2400	2100000	2200000	1000000	200000	100000
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	10	1745
Giardia duodenalis	/l	QSA: Parasitenstadien	100	35	60	30	425	170

WWTP Xanten-Vynen - effluent									
Sample no.			124897	125241	126729	127148	127583	129084	130106
Date of sampling (end)			16.07.2009	19.08.2009	29.10.2009	17.11.2009	08.12.2009	13.01.2010	09.02.2010
Time of sampling (end)	h:min		07:15	07:05	08:10	07:55	08:10	08:00	07:10
Start date of sampling			15.07.2009	18.08.2009	28.10.2009	16.11.2009	07.12.2009	12.01.2010	08.02.2010
Start time of sampling	h:min		07:15	07:05	08:10	07:55	08:10	08:00	07:10
Duration of sampling	h:min		24	24	24	24	24	24	24
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8			1050	760	560	1060	910
pH value		DIN 38404 C5 (1984)			7,9	7,5	7,1	7,7	7,7
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	1 8000	10000	600	19300	4000	12400	37000
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	3 400	2400	500	16800	8800	17900	98000
Coliform Bacteria	/100ml	Colilert-18	4100	19900	< 1000000	<1000000	1000000	300000	200000
Escherichia coli	/100ml	Colilert-18	1100	4900	< 1000000	<1000000	<1000000	<100000	< 10000
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	170	340	1000	1000	10000	23000	57000
Enterococci	/100ml	Enterolert-E							
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	0	190	< 10000	-	50000	270000	40000
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	2	n.n.	8	n.n.	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	6	14	10	3

WWTP Xanten-Vynen - effluent									
Sample no.			130811	131730	132281	133397	134157	134755	135372
Date of sampling (end)			10.03.2010	15.04.2010	11.05.2010	16.06.2010	13.07.2010	04.08.2010	02.09.2010
Time of sampling (end)	h:min		07:55	07:50	07:30		10:50	07:35	09:00
Start date of sampling			09.03.2010	14.04.2010	10.05.2010	15.06.2010	12.07.2010	03.08.2010	01.09.2010
Start time of sampling	h:min		07:55	07:50	07:30	09:15	11:20	07:35	09:00
Duration of sampling	h:min		24	24	24	-	23:30	24	24
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8	9 90	1080	980	1200	1090		710
pH value		DIN 38404 C5 (1984)	7,1	7,9	8,3	8	7,4		7,6
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	2 000	3000	2000	24000	28000	1000	8200
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	4 000	15000	5000	21000	13000	500	4360
Coliform Bacteria	/100ml	Colilert-18	31400	<1000	17000	130000	1200	209000	22000
Escherichia coli	/100ml	Colilert-18	14600	<1000	4100	17000	270	46000	9000
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	40000	9	<10000				
Enterococci	/100ml	Enterolert-E				< 10000	3000	<10000	<1000
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	28000	100	58000	17000	0	1000	1000
Cryptosporidium spp.	/l	QSA: Parasitenstadien	2	n.n.	2	n.n.	4	n.n.	10
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	2	2	6	10	n.n.	6
(n.n. = not detected)									

Tap water; distance from Rhine 4500 m
Gauß-Krüger coordinate (easting) 2546206
Gauß-Krüger coordinate (northing) 5704447

Tap water; distance from Rhine 4500 m										
Date of sampling			04.02.2010	26.02.2010	16.03.2010	10.04.2010	20.04.2010	05.05.2010	27.05.2010	09.06.2010
Time of sampling time (end)			08:00	09:30	07:30	09:30	06:30	08:00	07:30	07:00
Sample no.			130588	131337	131561	132664	132844	133115	133121	133991
Volume of filtered water	l		2920,7	4452	4,3949	5997,9	3783	40	5511,6	3974
Temperature (in situ)	℃	DIN 38404 C4	11,6	12,7		14,4		17,4	19	
Oxigene (in situ)	mg/l	DIN EN 25814 G22						4,8	5,5	
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8	640	660	680	670		660	657	
pH value		DIN 38404 C5 (1984)	7,6	7,5	7,4	7,5		7,5	7,7	
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	< 5	< 5	< 5	< 5	< 5	17	5300	460
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	< 5	< 5	< 5	< 5	< 5	< 5	4140	> 300
Escherichia coli	/100ml	Colilert-18	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Coliform Bacteria	/100ml	Colilert-18	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	0	0	0	0	0	0	0	0
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	0	0	0	0	0	0	2	
Enterococci	/100ml	Enterolert-E								< 1
Pseudomonas aeruginosa	/100ml	DIN EN ISO 16266 K11	0	0	0	0	5	0	9	1
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.

Tap water; distance from Rhine 4500 m										
Date of sampling			24.06.2010	15.07.2010	26.07.2010	17.08.2010	30.08.2010	13.09.2010	30.09.2010	19.10.2010
Time of sampling time (end)			06:10	07:20	07:00	10:40	15:10	06:30	05:45	07:20
Sample no.			134475	134587	134593	134599	134605	134611	136387	136393
Volume of filtered water	l		6208,3	5157,8	4402,6	4414,6	2024,8	3554,3	5992	3437,2
Temperature (in situ)	℃	DIN 38404 C4	19,7	23,7	23,9	22	20,6	20,4	19,4	20,3
Oxigene (in situ)	mg/l	DIN EN 25814 G22	-	6,3	5,1	7,95	5,6	5,43	6,5	5,5
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8	700	640	630	613	610	589	578	590
pH value		DIN 38404 C5 (1984)	7,5	7,5	7,5	7,6	7,4	7,2	6,8	7,8
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.		< 5	< 5	130	< 5	11	< 5	< 5
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.		< 5	< 5	13	< 5	< 5	< 5	< 5
Escherichia coli	/100ml	Colilert-18	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Coliform Bacteria	/100ml	Colilert-18	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	0	0	0	0	0	0	0	0
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15								
Enterococci	/100ml	Enterolert-E	< 1	< 1	<1	< 1	< 1	< 1	< 1	< 1
Pseudomonas aeruginosa	/100ml	DIN EN ISO 16266 K11	0	0	1	13	0	0	0	0
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.

Tap water; distance from Rhine 4500 m									
Date of sampling			25.10.2010	06.11.2010	22.11.2010	03.12.2010	18.12.2010	14.01.2011	28.01.2011
Time of sampling time (end)			16:00	08:00	10:15	13:30	09:40	08:30	08:10
Sample no.			136398	136404	136410	136416	136422	136428	136434
Volume of filtered water	l		2012,8	4929	6081	1832	1316,8	2847	695
Temperature (in situ)	℃	DIN 38404 C4	16,6	16,8	15	13	12,6	14,7	13
Oxigene (in situ)	mg/l	DIN EN 25814 G22	6	6,3	6,3	4,8	5,8	6,3	6,1
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8	600	600	580	610	610	640	660
pH value		DIN 38404 C5 (1984)	7,7	7,5	7	7,2	7,4	7,6	7,4
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	< 5	< 5	< 5	< 5	34	< 5	< 5
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	< 5	< 5	< 5	< 5	29	< 5	< 5
Escherichia coli	/100ml	Colilert-18	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Coliform Bacteria	/100ml	Colilert-18	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	0	0	0	0	0	0	0
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15							
Enterococci	/100ml	Enterolert-E	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Pseudomonas aeruginosa	/100ml	DIN EN ISO 16266 K11	0	0	0	0	0	5	0
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	0,016	0,008	n.n.	0,014
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.

(n.n. = not detected)

Raw water; distance from Rhine 2500 m
Gauß-Krüger coordinate (easting) 2546931
Gauß-Krüger coordinate (northing 5708984

Raw water; distance from Rhine 2500 m										
Date of sampling			10.02.2010	03.03.2010	19.03.2010	15.04.2010	20.04.2010	18.05.2010	27.05.2010	14.06.2010
Time of sampling time (end)			07:00	07:00	07:00	07:30	12:00	13:00	11:15	13:10
Sample no.			131236	131335	131559	132662	132842	133113	133119	133989
Volume of filtered water	l		3234	3357	3481	2494	2415	2310	2106	4404
Temperature (in situ)	℃	DIN 38404 C4		12,4	12,7	12,5	12,8	12,7	13,1	13,2
Oxigene (in situ)	mg/l	DIN EN 25814 G22		7	7,5	8,6	9,5	7,6	8,1	7,7
Conductivity at 25℃ (in situ)	uS/cm	DIN EN 27888 C8		680	660	680	650	640	550	650
pH value		DIN 38404 C5 (1984)	-	7,3	7,7	7,7	7,8	7,8	7,5	7,9
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	< 5	< 5	-	103	< 5	26	< 5	< 5
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	130	90	-	30	38	85	67	90
Escherichia coli	/100ml	Colilert-18	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Coliform Bacteria	/100ml	Colilert-18	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5								
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	0	0	0	0	0	0	0	
Enterococci	/100ml	Enterolert-E								
Pseudomonas aeruginosa	/100ml	DIN EN ISO 16266 K11	0	0	0	1	0	0	0	0
Cryptosporidium spp.	/l	QSA: Parasitenstadien	0,0031	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.

Raw water; distance from Rhine 2500 m										
Date of sampling			29.06.2010	13.07.2010	27.07.2010	25.08.2010	31.08.2010	14.09.2010	30.09.2010	13.10.2010
Time of sampling time (end)			08:05	07:30	14:40	07:00	07:00	07:45	15:05	07:00
Sample no.			134473	134585	134591	134597	134603	134609	136385	136391
Volume of filtered water	l		2010	2141	7855	2480	1779	3498	2047,3	5170
Temperature (in situ)	℃	DIN 38404 C4	13,7	15		13,8	13,8	15 ,1	14,7	15,2
Oxigene (in situ)	mg/l	DIN EN 25814 G22	7,5	7,9		7,6	6,3	7,3	9,5	7,3
Conductivity at 25℃ (in situ)	uS/cm	DIN EN 27888 C8	650	610	620	604	560	590	570	570
pH value		DIN 38404 C5 (1984)	7,7	7,8	7,9	2,83	7,4	7,8	7,4	7,8
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	< 5	6	29	20	< 5	< 5	< 5	< 5
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	< 5	14	73	14	< 5	< 5	< 5	< 5
Escherichia coli	/100ml	Colilert-18	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Coliform Bacteria	/100ml	Colilert-18	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5								
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15								
Enterococci	/100ml	Enterolert-E								
Pseudomonas aeruginosa	/100ml	DIN EN ISO 16266 K11	0	>200	> 200	0	0	0	0	0
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.

Raw water; distance from Rhine 2500 m									
Date of sampling			27.10.2010	08.11.2010	23.11.2010	07.12.2010	16.12.2010	11.01.2011	25.01.2011
Time of sampling time (end)			07:00	07:35	14:45	09:10	10:15	06:30	12:45
Sample no.			136396	136402	136408	136414	136420	136426	136432
Volume of filtered water	l		2006	1784	1544,7	5001,2	612,4	3140	2341
Temperature (in situ)	℃	DIN 38404 C4	14,6	14,9	14	13,6	13 ,7	13,9	13,2
Oxigene (in situ)	mg/l	DIN EN 25814 G22	9,5	6,4	9,6	6,3	8,9	7,6	7
Conductivity at 25℃ (in situ)	uS/cm	DIN EN 27888 C8	630	590	620	640	620	640	660
pH value		DIN 38404 C5 (1984)	7,4	7,8	7,5	7,5	7,7	7,8	7,6
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	< 5	< 5	< 5	13	10	< 5	> 300
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	< 5	< 5	< 5	< 5	< 5	< 5	9
Escherichia coli	/100ml	Colilert-18	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Coliform Bacteria	/100ml	Colilert-18	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5							
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15							
Enterococci	/100ml	Enterolert-E							
Pseudomonas aeruginosa	/100ml	DIN EN ISO 16266 K11	0	0	0	0	0	0	3
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	0,0065	n.n.	n.n.	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.

(n.n. = not detected)

Groundwater; distance from Rhine 1500 m
Gauß-Krüger coordinate (easting) 2547468
Gauß-Krüger coordinate (northing 5708031

Groundwater; distance from Rhine 1500 m										
Date of sampling			03.02.2010	26.02.2010	18.03.2010	08.04.2010	19.04.2010	17.05.2010	26.05.2010	09.06.2010
Time of sampling time (end)			07:45	06:00	07:30	13:30	14:30	14:00	07:25	14:00
Sample no.			130566	131339	131563	132666	132846	133117	133123	133993
Volume of filtered water	l		2904,6	3997	4937	2880	2001	2554	2760	2403
Temperature (in situ)	℃	DIN 38404 C4	11,4	11,5	11,4	11,6	11,8	11,8	11,5	11,9
Oxigene (in situ)	mg/l	DIN EN 25814 G22		2	2,1	2	3,8	3,7	4,5	2,7
Conductivity at 25℃ (in situ)	uS/cm	DIN EN 27888 C8	740	740	740	740	740	760	730	740
pH value		DIN 38404 C5 (1984)	7	7	7	7	7,2	7,3	7,3	7,3
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
Escherichia coli	/100ml	Colilert-18	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Coliform Bacteria	/100ml	Colilert-18	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	0	0	0	0	0	0	0	0
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	0	0	0	0	0	0	0	
Enterococci	/100ml	Enterolert-E								0
Pseudomonas aeruginosa	/100ml	DIN EN ISO 16266 K11	0	0	0	0	0	0	0	0
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	0,04
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.

Groundwater; distance from Rhine 1500 m										
Date of sampling			30.06.2010	12.07.2010	03.08.2010	23.08.2010	08.09.2010	13.09.2010	29.09.2010	12.10.2010
Time of sampling time (end)			07:30	13:00	07:30	13:15	13:10	13:15	07:45	07:15
Sample no.			134477	134589	134595	134601	134607	134613	136389	136395
Volume of filtered water	l		2814	2141	2891	2135	2105	2801	5470	2570
Temperature (in situ)	℃	DIN 38404 C4	12,4	12,7	11,8	11,9	11,5	12,5	11,6	11,5
Oxigene (in situ)	mg/l	DIN EN 25814 G22	4,9	4	3,7	4	3,9	4,4	4,1	4,1
Conductivity at 25℃ (in situ)	uS/cm	DIN EN 27888 C8	750	740	760	760	750	750	760	770
pH value		DIN 38404 C5 (1984)	7,3	7,5	7,2	7,2	7,2	7,2	7,1	7,2
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
Escherichia coli	/100ml	Colilert-18	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Coliform Bacteria	/100ml	Colilert-18	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	0	0	0	0	0	0	0	0
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15			0					
Enterococci	/100ml	Enterolert-E	0	0		-	< 1	< 1	< 1	< 1
Pseudomonas aeruginosa	/100ml	DIN EN ISO 16266 K11	0	0	0	0	0	0	0	0
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.

Groundwater; distance from Rhine 1500 m									
Date of sampling			03.11.2010	10.11.2010	29.11.2010	09.12.2010	17.12.2010	10.01.2011	25.01.2011
Time of sampling time (end)			14:00	13:00	14:00	11:25	10:15	13:00	07:00
Sample no.			136400	136406	136412	136418	136424	136430	136436
Volume of filtered water	l		2171	2101	1028,7	4708	6463,3	2010	3420
Temperature (in situ)	℃	DIN 38404 C4	11,9	11,5	11	10,3	11	11,5	11,6
Oxigene (in situ)	mg/l	DIN EN 25814 G22	3,5	2,1	3,3	4	3,1	4,7	3,2
Conductivity at 25℃ (in situ)	uS/cm	DIN EN 27888 C8	770	760	760	750	740	770	810
pH value		DIN 38404 C5 (1984)	7,1	7,1	7,1	7,2	7,2	7,2	7,1
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	< 5	< 5	< 5	< 5	< 5	< 5	< 5
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	< 5	< 5	< 5	< 5	< 5	< 5	< 5
Escherichia coli	/100ml	Colilert-18	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Coliform Bacteria	/100ml	Colilert-18	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	0	0	1	0	0	0	0
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15							
Enterococci	/100ml	Enterolert-E	0	-	< 1	< 1	< 1	< 1	< 1
Pseudomonas aeruginosa	/100ml	DIN EN ISO 16266 K11	0	0	0	0	0	0	0
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.

(n.n. = not detected)

Groundwater; distance from Rhine 630 m
Gauß-Krüger coordinate (easting) 2548185
Gauß-Krüger coordinate (northing 5707400

Groundwater; distance from Rhine 630 m										
Date of sampling			11.02.2010	02.03.2010	24.03.2010	16.04.2010	21.04.2010	19.05.2010	31.05.2010	15.06.2010
Time of sampling time (end)			07:20	07:10	07:30	08:00	07:45	07:30	13:10	07:30
Sample no.			131237	131334	131558	132661	132841	133112	133118	133988
Volume of filtered water	l		1654,1	1750	3723	4026	2000	3740	2140	3210
Temperature (in situ)	℃	DIN 38404 C4	11,5	12,1	12,2	13,1	12,3	12,8	12,8	12,2
Oxigene (in situ)	mg/l	DIN EN 25814 G22		9,2	3,8	4,8	3,7	4,6	4,5	3
Conductivity at 25℃ (in situ)	uS/cm	DIN EN 27888 C8	710	650	650	670	650	660	660	650
pH value		DIN 38404 C5 (1984)	7,4	7,2	7,3	7,2	7,2	7,4	7,4	7,5
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	< 5	< 5		< 5	< 5	< 5	17	< 5
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	< 5	< 5		< 5	< 5	< 5	19	< 5
Escherichia coli	/100ml	Colilert-18	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Coliform Bacteria	/100ml	Colilert-18	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	0	0	0	0	0	0	1	0
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	0	0	0	0	0	0	0	
Enterococci	/100ml	Enterolert-E								0
Pseudomonas aeruginosa	/100ml	DIN EN ISO 16266 K11	0	0	0	0	0	0	0	0
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	0,0057	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.

Groundwater; distance from Rhine 630 m										
Date of sampling			30.06.2010	13.07.2010	03.08.2010	25.08.2010	01.09.2010	15.09.2010	01.10.2010	13.10.2010
Time of sampling time (end)			13:30	14:00	14:00		13:40	07:30	08:10	13:30
Sample no.			134472	134584	134590	134596	134602	134608	136384	136390
Volume of filtered water	l		2461	2085	2170	2010	540	3410	4548,5	2110
Temperature (in situ)	℃	DIN 38404 C4	12,2	12,3	12	12,1	14 ,7	14	13,2	13,4
Oxigene (in situ)	mg/l	DIN EN 25814 G22	3,4	3,2	2,8	1,8	2,6	1,5	6,1	2
Conductivity at 25℃ (in situ)	uS/cm	DIN EN 27888 C8	670	640	640	613	580	590	600	600
pH value		DIN 38404 C5 (1984)	7,5	7,5	7,5	7,4	7,4	7,5	7	7,5
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	< 5	< 5	< 5	120	75	< 5	< 5	6
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	< 5	< 5	< 5	7	21	< 5	< 5	< 5
Escherichia coli	/100ml	Colilert-18	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Coliform Bacteria	/100ml	Colilert-18	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	0	0	0	0	0	0	0	0
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15			0					
Enterococci	/100ml	Enterolert-E	0	0		< 1	< 1	< 1	< 1	< 1
Pseudomonas aeruginosa	/100ml	DIN EN ISO 16266 K11	16	28	16	11	0	1	0	0
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	0,018	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.

Groundwater; distance from Rhine 630 m									
Date of sampling			02.11.2010	10.11.2010	25.11.2010	08.12.2010	15.12.2010	11.01.2011	26.01.2011
Time of sampling time (end)			06:20	07:40	11:00	09:45	14:20	13:00	08:45
Sample no.			136401	136407	136413	136419	136425	136431	136437
Volume of filtered water	l		2104	2130	1797,8	2704,1	332,7	1840	3510
Temperature (in situ)	℃	DIN 38404 C4	14,6	14	13,2	12,8	13 ,6	13,9	12,5
Oxigene (in situ)	mg/l	DIN EN 25814 G22	1,1	1,6	5,7	1,7	4	2,2	2,7
Conductivity at 25℃ (in situ)	uS/cm	DIN EN 27888 C8	590	570	590	590	600	630	660
pH value		DIN 38404 C5 (1984)	7,3	7,4	7,4	7,4	7,6	7,4	7,3
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	< 5	< 5	< 5	< 5	< 5	7	< 5
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	< 5	< 5	< 5	< 5	< 5	< 5	< 5
Escherichia coli	/100ml	Colilert-18	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Coliform Bacteria	/100ml	Colilert-18	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	0	0	0	0	0	0	0
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15							
Enterococci	/100ml	Enterolert-E	0	-	< 1	< 1	< 1	< 1	< 1
Pseudomonas aeruginosa	/100ml	DIN EN ISO 16266 K11	0	0	0	0	0	0	0
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	0,0054	n.n.

(n.n. = not detected)

Groundwater; distance from Rhine 250 m
Gauß-Krüger coordinate (easting) 2548069
Gauß-Krüger coordinate (northing 5706925

Groundwater; distance from Rhine 250 m										
Date of sampling			10.02.2010	01.03.2010	18.03.2010	12.04.2010	20.04.2010	07.05.2010	27.05.2010	15.06.2010
Time of sampling time (end)			09:15	10:00	12:45	14:00	14:00	11:00	13:20	11:00
Sample no.			131238	131336	131560	132663	132843	133114	133120	133990
Volume of filtered water	l		240	1990	316	405	401	2150	400	450
Temperature (in situ)	℃	DIN 38404 C4	11,5	10,1	9,5	8,6	8, 3	9	12,1	11,9
Oxigene (in situ)	mg/l	DIN EN 25814 G22		9,2	5,1	3,9	3,1	3,9	2,6	0,3
Conductivity at 25℃ (in situ)	uS/cm	DIN EN 27888 C8	5 80	690	680	620	620	610	640	600
pH value		DIN 38404 C5 (1984)	7,9	7,9	7,9	7,9	7,9	8	8	7,8
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	1 0	11	3	7	35	< 5	270	30
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	1 8	12	29	13	19	< 5	21	< 5
Escherichia coli	/100ml	Colilert-18	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Coliform Bacteria	/100ml	Colilert-18	< 1	< 1	< 1	< 1	< 1	< 1	< 1	1
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	0	0	1	0	0	0	0	6
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	0	0	0	0	0	0	0	
Enterococci	/100ml	Enterolert-E								0
Pseudomonas aeruginosa	/100ml	DIN EN ISO 16266 K11	0	0	1	0	0	1	252	0
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.

Groundwater; distance from Rhine 250 m										
Date of sampling			29.06.2010	09.07.2010	02.08.2010	16.08.2010	06.09.2010	15.09.2010	28.09.2010	12.10.2010
Time of sampling time (end)			11:15	11:15	11:00	13:00	10:20	10:15	09:00	10:00
Sample no.			134474	134586	134592	134598	134604	134610	136386	136392
Volume of filtered water	l		413	435	400	410	1104	408	446	435
Temperature (in situ)	℃	DIN 38404 C4	13,3	16,1	15,3	15,9	19,7	18,8	19,7	18,3
Oxigene (in situ)	mg/l	DIN EN 25814 G22	0,1	1,8	0,1	2,1	0,1	0,2	2,9	1,7
Conductivity at 25℃ (in situ)	uS/cm	DIN EN 27888 C8	5 80	650	570	580	520	520	510	520
pH value		DIN 38404 C5 (1984)	7,8	7,9	7,8	7,9	7,8	7,8	7,8	7,9
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	3 1	119	1200	21	207	130	350	260
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	< 5	12	< 5	111	7	3	770	88
Escherichia coli	/100ml	Colilert-18	< 1	< 1	< 1	2	< 1	< 1	22	< 1
Coliform Bacteria	/100ml	Colilert-18	< 1	< 1	1	>200	< 1	25	201	6
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	0	0	0	2	0	0	3	0
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15								
Enterococci	/100ml	Enterolert-E	0	0	0	< 1	< 1	< 1	4	< 1
Pseudomonas aeruginosa	/100ml	DIN EN ISO 16266 K11	1	1	0	42	2	14	> 200	0
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.

Groundwater; distance from Rhine 250 m						
Date of sampling			02.11.2010	08.11.2010	04.01.2011	24.01.2011
Time of sampling time (end)			10:00	10:30	10:00	10:00
Sample no.			136397	136403	136427	136433
Volume of filtered water	l		408	408	452	2108
Temperature (in situ)	℃	DIN 38404 C4	18,3	17,8	13,7	10,6
Oxigene (in situ)	mg/l	DIN EN 25814 G22	1,7	1,4	5,7	2
Conductivity at 25℃ (in situ)	uS/cm	DIN EN 27888 C8	6 00	580	670	610
pH value		DIN 38404 C5 (1984)	7,9	7,9	8	7,4
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	> 200	130	97	> 300
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	< 5	< 5	< 5	> 300
Escherichia coli	/100ml	Colilert-18	< 1	< 1	< 1	165
Coliform Bacteria	/100ml	Colilert-18	< 1	< 1	< 1	> 201
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	2	0	7	49
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15				
Enterococci	/100ml	Enterolert-E	0	< 1	< 1	32
Pseudomonas aeruginosa	/100ml	DIN EN ISO 16266 K11	2	3	2	13
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	0,066	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.

(n.n. = not detected)

Rhine, surface water
Gauß-Krüger coordinate (easting) 2548085
Gauß-Krüger coordinate (northing) 5706670

Rhine, surface water										
Date of sampling			03.02.2010	23.02.2010	16.03.2010	07.04.2010	13.04.2010	19.04.2010	07.05.2010	26.05.2010
Time of sampling time (end)			15:00	13:00	14:00	13:00	06:55	13:00	11:35	13:20
Sample no.			130589	131338	131562	132665	131664	132845	133116	133122
Volume of filtered water	l		40	40	40	40		40	50	50
Temperature (in situ)	℃	DIN 38404 C4	4,9	6,2	6,1	12,8	11, 4	14,1	13,1	17,4
Oxigene (in situ)	mg/l	DIN EN 25814 G22	11,8	9,2	9,1	9,2	8,3	8,5	7,5	8,7
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8	790	790	690	600	653	670	650	610
pH value		DIN 38404 C5 (1984)	7,8	7,9	7,6	8	8	8	8	8,2
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	18000		460	140	310	110	2000	1400
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	18000		1300	360	820	450	5700	700
Escherichia coli	/100ml	Colilert-18	1600		290	230	180	170	2300	130
Coliform Bacteria	/100ml	Colilert-18	8200		1300	840	860	703	9200	1300
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	490		620	>200		170	1000	110
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	410		210	56		50	224	40
Enterococci	/100ml	Enterolert-E								
Pseudomonas aeruginosa	/100ml	DIN EN ISO 16266 K11	-		> 2000	>200		0	80	50
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	0,25	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.

Rhine, surface water										
Date of sampling			07.06.2010	29.06.2010	13.07.2010	27.07.2010	16.08.2010	06.09.2010	14.09.2010	27.09.2010
Time of sampling time (end)			08:40	12:00	07:15	15:10	10:00	08:05	10:00	08:40
Sample no.			133992	134476	134588	134594	134600	134606	134612	136388
Volume of filtered water	l		50	50	50	100	121	250	401	240
Temperature (in situ)	℃	DIN 38404 C4	19,1	21,7	25,7	22,9	21,6	17,6	18,8	17
Oxigene (in situ)	mg/l	DIN EN 25814 G22	9,2	6,5	6,1	7,5	5,9	9,2	7	6,9
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8	551	540	610	510	600	490	520	660
pH value		DIN 38404 C5 (1984)	7,8	8	8	7,8	7,9	8	8,1	7,9
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	1600	2600	2600	13000	29100	1800	820	2020
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	1400	1600	1060	12000	23100	2000	960	1200
Escherichia coli	/100ml	Colilert-18	200	74	630	4100	10500	170	400	504
Coliform Bacteria	/100ml	Colilert-18	3100	2500	8700	> 24000	24200	> 24000	2200	2900
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	190	70	70	230	270	> 2000	70	60
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15								
Enterococci	/100ml	Enterolert-E	74	10		156	3000	410	92	960
Pseudomonas aeruginosa	/100ml	DIN EN ISO 16266 K11	700	>2000	>2000	7300	>2000	>2000	> 2000	> 2000
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.

Rhine, surface water										
Date of sampling			11.10.2010	26.10.2010	08.11.2010	29.11.2010	13.12.2010	04.01.2011	11.01.2011	26.01.2011
Time of sampling time (end)			11:15	13:00	11:00	10:00	10:30	13:30	13:15	12:30
Sample no.			136394	136399	136405	136411	136423	136429	140338	136435
Volume of filtered water	l		407	200	200		70	408	60	150
Temperature (in situ)	℃	DIN 38404 C4	16,7	11,4	13,3	6,3	3 ,1	11,1		6,1
Oxigene (in situ)	mg/l	DIN EN 25814 G22	6,9	7,6	8,2	10,7	11,5	5,6		10,3
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8	650	680	660	660	560	680	450	580
pH value		DIN 38404 C5 (1984)	8	7,9	7,9	7,7	7,9	8,1	7,5	7,8
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	800	5700	2600	2100	49000	9700		1200
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	540	6200	2200	2300	110000	54000		1300
Escherichia coli	/100ml	Colilert-18	280	330	2600	1010	6500	740		1800
Coliform Bacteria	/100ml	Colilert-18	2040	1780	9200	4400	65000	10400		6900
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	10	210	90	510	1400	9100		50
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15								
Enterococci	/100ml	Enterolert-E	36	63	240	400	3500	860		370
Pseudomonas aeruginosa	/100ml	DIN EN ISO 16266 K11	> 2000	>2000	2300	7	>20000	10100		350
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	0,067	n.n.	0,074	0,333	0,133
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	0,067

(n.n. = not detected)

Aubruch Channel, surface water
Gauß-Krüger coordinate (easting) 2542556
Gauß-Krüger coordinate (northing 5700125

Aubruch Channel, surface water										
Date of sampling			02.07.2009	29.07.2009	24.08.2009	01.09.2009	03.09.2009	17.09.2009	30.03.2010	19.04.2010
Time of sampling time (end)			10:20	10:45	09:50	13:25	09:30	14:25	11:46	09:20
Sample no.			123333	123339	123345	123347	126177	123351	132585	132731
Volume of filtered water	l								40	40
Volume of flocculated water	l		10	5	5	5	5	5		
Temperature (in situ)	℃	DIN 38404 C4	17,9		17,8	17	15,2	16 ,9	11,5	10,9
Oxigene (in situ)	mg/l	DIN EN 25814 G22	7,2				7	9,5		
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8	6 40					630	642	677
pH value		DIN 38404 C5 (1984)	7,6			7,7	7,4	7,3	7,6	7,6
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.			7 50		1000		500	570
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.			1 300		2900		1300	1300
Escherichia coli	/100ml	Colilert-18	390	41	570	220	2100	200	270	402
Coliform Bacteria	/100ml	Colilert-18	6900	2800	5500	4900	12000	660	7700	6900
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5					160	60	62	> 200
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	440	520	490	220		150	92	> 200
Enterococci	/100ml	Enterolert-E								
Pseudomonas aeruginosa	/100ml	DIN EN ISO 16266 K11	0					320	0	0
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	10	40	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.

Aubruch Channel, surface water										
Date of sampling			29.04.2010	03.05.2010	04.05.2010	05.05.2010	06.05.2010	07.05.2010	31.05.2010	21.06.2010
Time of sampling time (end)			13:20	10:10	07:35	07:45	07:50	08:00	10:25	09:10
Sample no.			132735	133097	133101	133105	133157	133161	133830	132755
Volume of filtered water	l		40	40	40	40	40	40	40	50
Temperature (in situ)	℃	DIN 38404 C4	15,6	11,9	10,9	9,5	9 ,7	9,6		13,1
Oxigene (in situ)	mg/l	DIN EN 25814 G22	8,6							8,7
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8	6 87	632	704	707	710	685		696
pH value		DIN 38404 C5 (1984)	7,8	7,5	7,5	7,6	7,6	7,5		7,7
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	4 50	9700	1010	760	1000	800	2080	550
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	1 200	20200	1800	1100	1500	3900	5100	820
Escherichia coli	/100ml	Colilert-18	170	2300	340	301	200	330	960	320
Coliform Bacteria	/100ml	Colilert-18	2600	24200	6500	3300	20000	9800	9200	1400
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	7	470	110	50	100	100	1120	120
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	7	>400	370	480	372	> 400		
Enterococci	/100ml	Enterolert-E							2000	630
Pseudomonas aeruginosa	/100ml	DIN EN ISO 16266 K11	640	>2000	7100	7100	0	0	70	1800
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.

Aubruch Channel, surface water									
Date of sampling			30.06.2010	12.07.2010	15.07.2010	22.07.2010	02.08.2010	10.08.2010	25.08.2010
Time of sampling time (end)			09:30	15:30	12:30	07:20	11:20	08:15	09:25
Sample no.			132763	132759	135168	132767	132771	132775	132779
Volume of filtered water	l		50	50	50	50	50	50	50
Temperature (in situ)	℃	DIN 38404 C4	16,2	19	17,9	17,5	15 ,4	15	14,2
Oxigene (in situ)	mg/l	DIN EN 25814 G22	8		3,8				
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8	6 90	656	466	621	683	657	630
pH-Wert at 20℃ (calculated)		DIN 38404 C5 (1984)	7,5	7 ,4	7,9	7,6	7,7	7,4	7,3
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	1 300	860		3500	860	990	400
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	2 900	5800		6900	1400	960	1000
Escherichia coli	/100ml	Colilert-18	640	1400	6900	1500	270	330	420
Coliform Bacteria	/100ml	Colilert-18	9800	16000	>24200	14000	5500	6100	2400
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	140	50		50	80	60	40
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15							
Enterococci	/100ml	Enterolert-E	1900	1070	17000	1700	520	580	1100
Pseudomonas aeruginosa	/100ml	DIN EN ISO 16266 K11	1900	1380		>2000	1600	360	470
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	0,2	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	n.n.	0,2	n.n.	n.n.	n.n.	n.n.

(n.n. = not detected)

Recreational area, surface water
Gauß-Krüger coordinate (easting) 2542574
Gauß-Krüger coordinate (northing 5700822

Recreational area, surface water										
Date of sampling			02.07.2009	29.07.2009	24.08.2009	01.09.2009	03.09.2009	17.09.2009	30.03.2010	19.04.2010
Time of sampling time (end)			10:35	10:35	09:55	13:15	09:40	14:10	11:28	09:05
Sample no.			123432	123429	123426	123425	126179	123423	132583	132729
Volume of filtered water	l								40	40
Volume of flocculated water	l		10	5	5	5	5	5		
Temperature (in situ)	℃	DIN 38404 C4	21,5		20	18,9	16,8	18 ,2	11,7	12
Oxigene (in situ)	mg/l	DIN EN 25814 G22	10,3				8,9			
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8	6 60					630	638	682
pH value		DIN 38404 C5 (1984)	7,9			8	7,8	7,6	7,6	7,8
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.			3 70		400		450	180
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.			2 60		400		2200	285
Escherichia coli	/100ml	Colilert-18	280	41	74	20	520	130	280	31
Coliform Bacteria	/100ml	Colilert-18	2600	2800	1800	200	1600	860	2900	340
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5					<10	10	59	73
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15	79	850						
Enterococci	/100ml	Enterolert-E								
Pseudomonas aeruginosa	/100ml	DIN EN ISO 16266 K11	10					200	0	0
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	2	20	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	4	n.n.	n.n.	n.n.	12	n.n.	n.n.	n.n.

Recreational area, surface water										
Date of sampling			29.04.2010	03.05.2010	04.05.2010	05.05.2010	06.05.2010	07.05.2010	10.05.2010	31.05.2010
Time of sampling time (end)			13:45	10:00	07:50	07:55	07:05	08:15		10:00
Sample no.			132733	133095	133099	133103	133155	133159	132737	133828
Volume of filtered water	l		40	40	40	40	40	40	40	40
Temperature (in situ)	℃	DIN 38404 C4	16,6	12,3	12,2	11,4	11,4	11		
Oxigene (in situ)	mg/l	DIN EN 25814 G22	7,7							
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8	6 79	686	657	681	688	696		
pH value		DIN 38404 C5 (1984)	7,7	7,6	7,5	7,6	7,7	7,6		
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	3 10	1800	1900	410	310	80	80	370
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	4 90	2700	8400	1400	630	80	300	790
Escherichia coli	/100ml	Colilert-18	10	340	600	220	74	10	10	31
Coliform Bacteria	/100ml	Colilert-18	270	4600	13000	3700	1400	2200	160	3400
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	16	170	140	130	100	100	1600	170
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15								
Enterococci	/100ml	Enterolert-E								250
Pseudomonas aeruginosa	/100ml	DIN EN ISO 16266 K11	130	>2000	>20000	0	0	0	100	860
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	0,25	n.n.	n.n.

Recreational area, surface water										
Date of sampling			21.06.2010	30.06.2010	12.07.2010	15.07.2010	22.07.2010	02.08.2010	10.08.2010	25.08.2010
Time of sampling time (end)			09:00	08:15	15:15	12:15	07:05	11:00	08:00	09:10
Sample no.			132753	132761	132757	135166	132765	132769	132773	132777
Volume of filtered water	l		40	50	50	50	50	50	50	50
Temperature (in situ)	℃	DIN 38404 C4	14,7	19,5	23,1	21,3	20,9	18	14,3	16,7
Oxigene (in situ)	mg/l	DIN EN 25814 G22	9,6	9,2		6,4				
Conductivity at 25℃ (in situ)	µS/cm	DIN EN 27888 C8	6 93	660	645	628	588	653	632	606
pH-Wert at 20℃ (calculated)		DIN 38404 C5 (1984)	7,6	7 ,5	7,7	7,8	7,9	7,6	7,3	7,4
Counts of colonies 36℃	CFU/ml	TrinkwV 1990 Anl.1 5.	3 30	3500	100		1200	440	860	770
Counts of colonies 20℃	CFU/ml	TrinkwV 1990 Anl.1 5.	4 70	6200	620		1900	635	1100	900
Escherichia coli	/100ml	Colilert-18	140	230	63	120	110	340	140	97
Coliform Bacteria	/100ml	Colilert-18	610	7300	5800	6900	4900	250	3300	3100
Clostridium perfringens	/100ml	TrinkwV 2001 Anl.5	30	30	40		10	40	20	60
Enterococci	CFU/ml	DIN EN ISO 7899-2 K15								
Enterococci	/100ml	Enterolert-E	41	10	63	520	41	380	96	52
Pseudomonas aeruginosa	/100ml	DIN EN ISO 16266 K11	690	890	930		530	1500	980	490
Cryptosporidium spp.	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.
Giardia duodenalis	/l	QSA: Parasitenstadien	n.n.	n.n.	n.n.	0,2	n.n.	n.n.	n.n.	n.n.

(n.n. = not detected)

TEILPUBLIKATIONEN

Submitted papers:

- Gallas-Lindemann, C.; Sotiriadou I.; Plutzer J. & Karanis P. Circulation of *Cryptosporidium* and *Giardia* in wastewater and the surface, drinking and ground waters in the Lower Rhine, Germany
- Gallas-Lindemann, C.; Sotiriadou I.; Plutzer J. & Karanis P. *Giardia* and *Cryptosporidium* species dissemination during wastewater treatment and comparative detection by IFT, nPCR and LAMP
- Gallas-Lindemann, C.; Sotiriadou I.; Mahmoodi M. R. & Karanis P. Detection of *Toxoplasma gondii* oocysts in sewage by Loop Mediated Isothermal Amplification (LAMP)

Köln, den 05.04.2012

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Die Bestimmungen der Promotionsordnung sind mir bekannt. Die von mir vorgelegte Dissertation ist von Professor Panagiotis Karanis und Prof. Hartmut Arndt betreut worden.

Carmen Gallas-Lindemann