https://doi.org/10.17590/20220727-111138

Reclaimed wastewater: preventing protozoa on plant foods

Risk assessment of transmission of *Cryptosporidium* spp., *Giardia duodenalis* and *Toxoplasma gondii* to humans

BfR Opinion No 021/2022 issued 27 July 2022

Climatic changes are increasing the pressure on water resources in Germany and Europe. To counteract this pressure, uniform minimum requirements for the use of reclaimed wastewater for agricultural irrigation have been set at European level. They are designed to ensure human and animal health and environmental protection when reusing treated wastewater. Various methods are available for wastewater treatment.

Besides pathogenic bacteria and viruses, protozoa can also be present in reclaimed wastewater. Protozoa are single-celled parasites that can cause illness in humans. Against this background, the German Federal Institute for Risk Assessment (BfR) has evaluated scientific literature on the health risk of using reclaimed wastewater for the irrigation of plants used as food with regard to selected protozoa.

The following protozoa were considered: *Cryptosporidium* spp., *Giardia* (*G.*) *duodenalis* and *Toxoplasma* (*T.*) *gondii*. Infections with cryptosporidia and giardia can be asymptomatic or mild, but can also cause gastrointestinal diseases. Besides diarrhoea, abdominal pain or cramps, nausea and vomiting occur. Depending on age and previous illnesses, the severity of the symptoms can vary, and severe illnesses are possible. Infections with toxoplasma are asymptomatic in the majority of people with an intact immune system. However, in people affected by HIV/AIDS, transplants and chemotherapy, inflammation of the brain and lungs can occur, which is sometimes fatal. If an initial infection with Toxoplasma occurs during pregnancy, this can lead to severe malformations of the unborn child and miscarriages.

Data available on infections with *Cryptosporidium* spp., *G. duodenalis* and *T. gondii resulting from* consumption of plant foods is currently very limited. A conclusive risk characterisation for the use of reclaimed wastewater for plant irrigation intended for consumption with regard to the protozoa mentioned is therefore only possible to a limited extent. However, available data show that the pathogens are robust to common means and procedures for decontamination, which are also used in wastewater treatment. This indicates that they are present in reclaimed wastewater and can get onto plants through irrigation. There they show a high environmental stability. It can be assumed that the protozoa described are infectious on plant foods for a long time and can cause diseases after eating them raw. Only small infectious doses are sufficient for this. In contrast, infected persons excrete very large quantities of *Cryptosporidium spp.* and *G. duodenalis* in their stool, which in turn suggests the possibility of sewage contamination. *T. gondii* can enter municipal wastewater via cat faeces in the environment, disposal via the toilet or through the cleaning of objects and plant foods.

Even if the current data situation is still limited, the BfR recommends, in the interests of consumer health protection, only using irrigation water of a quality comparable to drinking water for watering plants that are usually consumed raw. In this case, it can be assumed that the pathogenic protozoa in it have been reduced as best as possible. The protozoa described are sensitive to heat. Therefore, in the case of plants that are not consumed raw, adverse health effects are not to be expected from irrigation with reclaimed wastewater, as long as they are sufficiently heated before consumption.

	BfR risk profile: Infection with *Cryptosporidium spp.* , *Giardia duodenalis* and *Toxoplasma gondii* after consumption of raw fruit and vegetables irrigated with reclaimed wastewater (Opinion [number/2022])				
A Affected persons	**General population, pregnant women, immunocompromised persons, chronically ill, small children**				
B Likelihood of impairment to health from consumption of raw fruit and vegetables irrigated with reclaimed wastewater	Practically impossible	Unlikely	Possible	Probable	Certain
C Severity of impairment to health from consumption of raw fruit and vegetables irrigated with reclaimed wastewater	No impairment (only general population)	Mild impairment [reversible] (only general population)	Moderate impairment [reversible] (only pregnant women, immunocompromised persons, chronically ill, small children)	Severe impairment [irreversible] (only pregnant women, immunocompromised persons, chronically ill, small children)	
D Validity of available data	High: The most important data are available and are internally consistent	Medium: Some important data are missing or contradictory	Low: A large volume of important data are missing or inconsistent		
E Controllability by the consumer	Controls not needed	Controllable with precautionary measures	Controllable by avoidance	Not controllable	

Explanations

The risk profiles are intended to visualise the risk outlined in the Opinion. The profile is not intended to be used to compare risks. The risk profiles should be read only in conjunction with the Opinion.

[1] Line B – Likelihood of impairment to health
The probability of an adverse health effect is influenced, among other things, by the quantity of pathogens present in the irrigation water, the type of plants irrigated and the irrigation technique. The BfR expects this probability to increase if plants whose parts growing close to or in the ground are intended for raw consumption are irrigated with reclaimed wastewater in Germany.

[2] Line C - Severity of impairment to health
The severity of the impairment can vary depending on the immune status, age and previous illnesses. With regard to cryptosporidia, persons with a severely weakened immune system, especially HIV-infected persons, as well as cancer and transplant patients are particularly at risk. Complications and even death can also occur in these risk groups. With regard to Giardia, the symptoms in young children and immunocompromised persons are usually longer lasting and can have a more severe course, although severe chronic courses are also possible. Deaths are rare, but can occur in infants and the elderly. With regard to toxoplasmas, immunocompromised persons (such as those due to HIV/AIDS, chemotherapy or after transplantation) may develop severe clinical manifestations, which are sometimes fatal. If an initial infection with toxoplasma occurs during pregnancy, this can lead to severe malformations of the unborn child and even miscarriages.

[3] Line D - Validity of available data
Insufficient data and the lack of routine detection methods for protozoa in reclaimed wastewater make a conclusive risk assessment of the use of reclaimed wastewater for the irrigation of fruit and vegetables with regard to human pathogenic protozoa difficult.

[4] Line E - Controllability by the consumer
Because of the low infectious dose, consumers have no means of controlling the risk apart from heating fruit and vegetables completely before consumption. It is therefore all the more important that no human pathogenic protozoa are present in the irrigation water used for plant foods that are consumed raw.

GERMAN FEDERAL INSTITUTE FOR RISK ASSESSMENT (BfR)

1 Subject of the assessment

Regulation (EU) 2020/741 on minimum requirements for water reuse entered into force on 26 June 2020 and will become applicable on 26 June 2023. Very little experience exists in Germany on agricultural irrigation with reclaimed wastewater. Safe use of reclaimed wastewater in agricultural irrigation requires, among other things, that no adverse health effects on consumers are to be expected. Therefore, the BfR has assessed the health risk of using reclaimed wastewater for the purpose of irrigating plants to be used as food or feed. The focus of this statement is on pathogenic protozoa (human pathogenic parasites) that may be associated with reclaimed wastewater in Germany. The following protozoa are considered: *Cryptosporidium* spp, *Giardia duodenalis* and *Toxoplasma gondii*. It is estimated and, where possible, assessed whether these pathogens can get onto the plants to be irrigated via reclaimed wastewater in concentrations that are of concern to consumers. It was further examined whether, in terms of consumer protection, restrictions on the use of reclaimed wastewater, additional validation parameters for wastewater treatment or additional controls of the reclaimed wastewater with regard to hazards from human pathogenic protozoa are necessary.

2 Results

After evaluating the available data, reports and publications on the occurrence of human pathogenic protozoa in concentrations of concern in reclaimed wastewater, the BfR concludes that a risk assessment for the use of this irrigation water for plants is not conclusively possible due to a current lack of data. In general, there is a need for research on the topic. Results and recommendations for action are based on known properties of the pathogens and published studies which, however, do not specifically refer to reclaimed or processed wastewater or whose validity must be checked or at least verified due to small sample sizes.

The pathogens considered here are stable single-celled parasites that are widespread in the environment and can get on food via various pathways. Thus, the safety of the irrigation water used can only contribute in part to minimising the risk. However, the data currently available show that contamination of all known irrigation waters is possible. The probability of occurrence of the protozoa under consideration in wastewater is to be regarded as higher due to the development cycle of cryptosporidia and giardia in particular, and due to the natural infection occurrence of these pathogens in humans and animals. Therefore, the possible burden of protozoa in wastewater is to be assessed as higher compared to drinking water. Furthermore, the described stabilities of the protozoa considered here in relation to common decontamination agents and decontamination procedures, which are also used in the treatment and processing of wastewater, indicate that human pathogenic protozoa can occur in processed wastewater and that the pathogens can get onto plants through irrigation. They are then characterised there again by a high environmental stability. Cryptosporidia show a very high resistance. They are therefore also suitable as reference pathogens for the evaluation of reclaimed wastewater with regard to the presence of human pathogenic protozoa and possibly, because of their stability, also as principal indicators of microbial quality. However, a suitable method would first have to be developed and standardised for this purpose. A standardised method is

ISO 15553 "Water Quality - Isolation and Identification of Cryptosporidium oocysts and Giardia cysts from Water", which, however, is only suitable as a filter method for water with few solid or suspended particles (e.g. drinking water, water from swimming pools).

Furthermore, the protozoa described are characterised by low infectious doses, which have been reported as low as one oocyst or cyst. Furthermore, when comparing the different irrigation systems, the BfR concludes that contamination is possible with each of the irrigation methods considered.

In the interests of consumer health protection, BfR therefore recommends that only irrigation water of a quality comparable to that of drinking water be used for plants intended for raw consumption, because it can be assumed that the concentration of human pathogenic protozoa in it has been reduced as far as possible. The World Health Organization (WHO) Guidance on Potable Reuse recommends a reduction of 8.5 $\log_{10}$ levels of enteric protozoa (*Cryptosporidium)* as a default performance target for safe treatment of wastewater for use as drinking water (WHO, 2017). Even if this is not a direct benchmark for the concrete validation of wastewater treatment plants, this WHO recommendation nevertheless indicates that very high requirements are necessary to reduce human pathogenic protozoa in the reclaimed wastewater as far as possible.

Furthermore, there is a risk of infection not only through raw consumption of such irrigated produce, but also through contact with contaminated reclaimed wastewater via the irrigation systems and with remaining reclaimed wastewater on surfaces, such as the plant parts, and subsequent droplet / smear infection.

The protozoa assessed are also characterised by high host tropism and are also capable of causing mild to severe disease in livestock.

However, the protozoa described are heat sensitive. Safe destruction of the pathogens is guaranteed when food is heated to a product temperature of at least 70 °C for a period of 2 minutes. It follows that in the case of plants that are not consumed raw, adverse health effects are not to be expected from irrigation with reclaimed wastewater as long as it can be ensured that sufficient heating of the food is carried out before consumption.

In the case of irrigation with reclaimed wastewater, adverse health effects with regard to human pathogenic protozoa are not to be expected also if it can be ensured that the edible parts of a plant do not come into contact with the irrigation water. Indirect transmission of the human pathogenic protozoa considered here, e.g. uptake via roots into the fruits, can be ruled out according to the current state of knowledge due to the size and life cycles of the pathogens.

3 Rationale

3.1 Risk assessment

3.1.1 Hazard identification

3.1.1.1 *Cryptosporidium* spp.

Classification (taxonomy)

Cryptosporidia are a genus of unicellular parasites belonging to the Apicomplexa. So far, more than 40 different species have been described, of which at least 20 have been associated with human infections. Of particular importance are the species *Cryptosporidium* (*C.*) *parvum* and *C. hominis*, which are thought to be responsible for the largest proportion of human infections (O'Leary et al., 2021). This assessment will therefore only deal with *C. hominis* and *C. parvum.*

Resilience (tenacity) in the environment

Due to their stable oocyst wall, cryptosporidia are characterised by great resistance to many chemical disinfectants, such as those based on potassium bichromate, glutaraldehydes or peracetic acid. According to the disinfectants listed with the German Veterinary Medical Society, unicellular parasites are reliably killed by agents that use chloro-cresols as a base (DVG, 2021).

Studies demonstrate the infectivity of oocysts in the environment for months. However, the survival of oocysts is dependent on numerous environmental factors that influence both their degradation and infectivity (Hamilton et al., 2018).

Diagnostics

Currently, the best standardised method is DIN EN ISO 18744 for the detection of *Cryptosporidium* and *Giardia* on leafy vegetables and berries. However, this method is expensive, labour-intensive and not suitable for use in high-throughput laboratories, which is why the method is implemented only hesitantly in both official and commercial laboratories.

Furthermore, a large number of different sensitive methods for antigen detection and typing have been described, which, however, are often not sufficiently validated and have not been standardised. What all these methods have in common is that they cannot determine the infectivity of the oocysts found.

Another issue is that concentrations of *Cryptosporidium oocysts* in drinking water, surface water and wastewater samples can be very low. Lengthy procedures must be carried out to process the large volume of water sample (100 l scale) for the concentration of protozoa (Connell et al., 2001).

Indicator for assessing the presence of protozoa in drinking water

The World Health Organisation has classified cryptosporidia as an indicator for assessing drinking water quality (WHO, 2008). This is because 1) the oocysts have a marked resistance to chemical disinfection (including chlorine) and environmental conditions, 2) the pathogens are difficult to remove from drinking water without expensive and lengthy filtration due to their small size (4-6µm), 3) they have a low infectious dose (10-1000 oocysts depending on the species), 4) they are widespread in water bodies due to their widespread distribution in livestock and their high-level excretion, and 5) they are infectious after excretion (excretion), without prior maturation (WHO, 2008).

3.1.1.2 *Giardia duodenalis*

Taxonomy
The pathogens causing the disease Giardiasis belong to the species *Giardia* (*G.*) *duodenalis* (synonym: *G. lamblia, G. intestinalis*). They are flagellated, unicellular intestinal parasites of the genus *Giardia* within the order *Diplomonadina.*

Based on morphological differences, the genus *Giardia* is currently divided into a total of eight species (Ryan et al., 2019). Giardia are distributed worldwide and have a very broad host range. They have been found in humans as well as in numerous domestic and wild animals (Feng and Xiao, 2011a; Bechlars et al., 2014; Heyworth, 2016). However, the only species that can infect humans, in addition to numerous mammalian species, and is thus a zoonotic agent, is *G. duodenalis* (Feng and Xiao, 2011b). This species is divided into assemblages A-H based on genotypic differences, with mainly assemblages A and B considered to be human pathogens (Plutzer et al., 2010; Leung et al., 2019). There are also isolated reports of human diseases caused by assemblages C, D and F (Ryan et al., 2019).

The pathogen is divided into two stages: "cysts" and "trophozoites". Cysts represent the infectious form of the pathogen. They are immobile, environmentally resistant stages that have a solid wall, contain four nuclei and have an oval shape 8-12 µm long. Trophozoites represent the vegetative and at the same time disease-associated form of the parasite. They have four pairs of flagella, two cell nuclei, a so-called adhesive disc and have a pear-like shape.

Tenacity in the environment
Giardia cysts have a very high stability against environmental influences (Utaaker et al., 2017). Studies have shown that cysts can remain infectious for months in cool, moist areas (Feng and Xiao, 2011b).

Cysts are insensitive to disinfectants at concentrations commonly used in sewage treatment plants to reduce bacterial contamination (Caccio et al., 2003). Adeyemo *et al.* (2019) found that *Giardia cysts were* responsive to chlorination and UV treatment, with *Giardia cysts* showing lower resistance than *Cryptosporidium oocysts* (Adeyemo et al., 2019). At a chlorine concentration of 2 ppm, viable cysts were no longer detectable after an exposure time of 30 minutes. Also, according to this study, a 60-minute low-dose treatment with UV-C rays (20.8 mJ/cm^2) killed all the cysts.

Stability of Giardia in water

Within the framework of a project on river hygiene, in which point measurements and analyses were carried out in German flowing waters, only low concentrations of *Giardia cysts could be* detected so far, so that Giardia did not appear as a dominant pathogen here (UBA, 2020). However, depending on the catchment area, higher concentrations of *Giardia cysts* could also be detected. In addition, it was proven that cysts survive in the sediment for a long time and can enter the water phase when stirred up.
The stability of cysts in water is temperature-dependent and increased at cooler temperatures. Studies summarised in a review by Erickson and Ortega (2006) showed that cysts in tap water at a temperature of 0-4 °C can be infectious for a period of 56 days, while at temperatures of 20-28 °C the cysts were infectious for up to 14 days. This temperature dependence is also evident in water from freshwater lakes. Here, infectious cysts were detected at a water temperature of 0-4 °C for 56 days and at a temperature of 17-20 °C for 28 days. In river water, viable cysts were even detected for up to 84 days at 0-4 °C, and up to 28 days at a water temperature of 20-28 °C. In seawater, *Giardia cysts* can survive for more than 65 days at 4 °C (Erickson and Ortega, 2006).

Stability of Giardia in the soil

Studies described in a review by Erickson and Ortega (2006) were able to show that the infectivity of cysts in soil is also temperature-dependent. Thus, after 49 days, the number of infectious cysts was reduced by only 11 % at a temperature of 4 °C, while at a temperature of 25 °C no infectious cysts could be detected after only seven days.

Stability of Giardia on plant foods

Although various studies have investigated the survival of these parasites in water and other environmental matrices, data on the stability of *Giardia cysts* on food is currently very sparse. Whether the cysts found on food actually remain infectious long enough to cause disease in humans has not yet been adequately investigated (Buret et al., 2020).

In a study by Utaaker *et al.* (2017), the parameters of temperature and humidity were considered to be the critical factors for the survival of *Giardia cysts,* especially in relation to fresh foods such as salads and raw vegetables. It has been demonstrated that *Giardia cysts* can survive very well on salads in a moist and cool environment (refrigerator temperature). However, when lettuces were stored in a closed container at room temperature, almost 50 % of the cysts died within the first 24 h. The cysts were found to be very resistant to the treatment. When stored in an open container at room temperature in a closed room, no more infectious *Giardia cysts* were detectable after 20 hours. Therefore, the authors of the study concluded that under the common storage conditions in the production plant, in trade or in private households, *Giardia cysts* probably cannot survive for longer periods on salads. However, this study did not include packaged products such as "ready-to-eat" salads, which are stored in a cool place in the trade in Germany, for example. Hence, if cold storage does occur, *Giardia cysts* may remain viable for longer and pose a risk to consumers if the contaminated food is consumed (Utaaker et al., 2017).

Diagnostics

DIN EN ISO 18744:2016 specifies a method applicable for the detection and enumeration of *Cryptosporidium oocysts* and *Giardia cysts* on or in food, but this method does not allowdetermination of the viability or infectivity of *Cryptosporidium oocysts* and *Giardia cysts that* may be present. Furthermore, this labour-intensive method is associated with high cost and is therefore not widely used to date. Alternatively, other methods are available for detection of *Giardia cysts* on food. Various elution procedures and detection methods based on immunofluorescence (Robertson and Gjerde, 2001; Amoros et al., 2010; Bechlars et al., 2014; Utaaker et al., 2017), rapid staining (e.g. iodine staining (Alemu et al., 2019)) and molecular biology (Utaaker et al., 2017) have been used in numerous studies on the presence of *G. duodenalis in* fresh produce worldwide.

3.1.1.3 *Toxoplasma gondii*

Taxonomy

Toxoplasma (*T.*) *gondii* is a unicellular obligate intracellular parasite classified within the phylum Apicomplexa in the subclass Coccidia and the family Sarcocystidae. *T. gondii* is the only species of the genus *Toxoplasma*[1] .

In Europe and North America, the population structure is clonal and the majority of *T. gondii* isolates can be assigned to three genotypes (types I, II, III), all of which are infectious for humans (Howe and Sibley, 1995). The majority of infections in humans and animals in Europe are attributed to genotype II and to a lesser extent to genotype III (Schlüter et al., 2014; Galal et al., 2019).

T. gondii oocysts have a larger diameter than *Cryptosporidium oocysts.* Unsporulated *T. gondii oocysts* have a spherical to globular shape with a size of 10x12 µm, while sporulated oocysts have a spherical to elliptical shape with a size of 11x13 µm (Dubey et al., 1998).

Tenacity in the environment

Oocysts sporulate in the outside world after a few days and represent a highly resistant permanent form that can survive frost and remain viable and infectious in soil for up to 18 months (Frenkel et al., 1975). Studies on survival at different temperatures showed that sporulated oocysts remained infectious for at least 28 days when stored at -20 °C, for at least 200 days at 10-25 °C, for at least 13 months at 0 °C and even for at least 54 months at 4 °C. However,

[1] http://taxonomicon.taxonomy.nl/TaxonTree.aspx?id=1309&src=0

they became infectious at 60 °C within 18 months but were killed within one minute at 60 °C (Dubey, 1998).

Sporulated oocysts also have a high tolerance to strong acids, detergents, disinfectants, sodium hypochlorite, chlorine, potassium dichromate and chloramine and are not reliably killed by common wastewater treatment and treatment processes, such as chlorination, ozone treatment and treatment with UV-C rays (Dumètre and Dardé, 2003; Wainwright et al., 2007a; Wainwright et al., 2007b; Dumetre et al., 2008; Jones and Dubey, 2010; Bahia-Oliveira et al., 2017; Shapiro et al., 2019).

Sporulated oocysts remained infectious after exposure times of up to 12 min to 6 mg/L ozone (*Ct* 69.96 mg x min/L) and up to 24 h to 100 mg/L chlorine or 5.25 % sodium hypochlorite (Wainwright et al., 2007b).

Treatment with UV-C rays has a killing effect against oocysts, but cannot reliably inactivate large amounts of oocysts. In studies by Wainwright *et al.* (2007), a killing effect of 40 mJ/cm^2 pulsed and 45 mJ/cm^2 continuous treatment with UV-C rays could be demonstrated, but isolated oocysts remained infectious even after high doses of up to 755 mJ/cm^2 (Wainwright et al., 2007a). Similarly, UV-C irradiation at 40 mJ/cm^2 reduced infectivity by 4.5 log steps but did not completely kill large numbers of oocysts (Dumetre et al., 2008). Ware *et al.* (2010) showed in animal experiments a reduction in infectivity by 1, 3 and 3.58 log steps when treated with UV-C rays at 4 mJ/cm^2, 10 mJ/cm^2 and ≥15 mJ/cm^2, respectively; at high initial numbers of >5000 oocysts, individual animals were nevertheless infected (Ware et al., 2010).

Diagnostics
No standardised or official methods are available for the detection of *T. gondii oocysts* in water, soil or plant foods. Various approaches and methods for detection in these matrices are described in the literature, but have not been sufficiently validated. The few studies in which validation experiments were carried out are not comparable due to the diversity of the techniques used in the individual steps. The review articles by Slana *et al.* (2021), Dumètre and Darde (2003), VanWormer *et al.* (2013) and Bahia-Oliveira (2017) provide an overview.

In principle, concentrations of oocysts in soil and water can be very low, so the recovery rate can be increased by examining larger sample volumes. When examining water, the oocysts are usually concentrated in a first step, e.g. by filtration (capsule, ultra, membrane filtration), flocculation, flotation or centrifugation (Shapiro et al., 2019). For detection in soil or plant foods, the oocysts are first detached from the sample using a suitable wash buffer and then concentrated from the wash buffer using one of the methods mentioned above (Slana et al., 2021). No commercially available monoclonal antibodies are available for immunomagnetic separation, similar to the detection methods for cryptosporidia and giardia. The concentrated sample can be examined microscopically for the presence of oocysts, but no differentiation from morphologically similar parasites (such as the genera *Hammondia*, *Besnoitia* and *Neospora*) is possible. Molecular biological methods are available for unequivocal identification (e.g. qPCR, nested PCR). When using a molecular biological detection method, an internal amplification control should be used, as residues of soil or organic material can often lead to PCR inhibition (Slana et al., 2021).

In the literature, LAMP (loop-mediated isothermal amplification) has often been used to detect *T. gondii.* This is a very sensitive method, which, however, entails a high probability of cross-contamination (e.g. of the controls or between the samples). In addition, in the case of positive reactions, no sequencing of the amplified products can be carried out for confirmation, which can lead to a lower specificity compared to PCR or qPCR.

Alternatively, the concentrated sample can be examined in a bioassay (e.g. cat, mouse, pig) for the detection and assessment of infectivity, but this is very time-, labour- and cost-intensive and should be avoided for ethical reasons. As an outlook and alternative to animal testing, techniques such as reverse-transcription qPCR, live staining with propidium iodide or the use of propidium monoazide in combination with qPCR could possibly be used to detect infectious oocysts (Travaillé et al., 2016; Kim et al., 2021).

Within the currently running EJP project TOXOSOURCES[2] (H2020 One Health European Joint Programme), a method for the detection of *T. gondii* in ready-to-eat green leaf lettuce was developed and established in ten European countries. The method, which was additionally validated in an EU-wide ring trial, represents an initial basis for the development of a standardised method.

3.1.2 Hazard characterisation

3.1.2.3 *Cryptosporidium* spp.

Development cycle / transmission to humans
Transmission occurs via ingestion of oocysts, which are the infectious form of cryptosporidia. These contain four sporozoites, which split off in the small intestine due to the influence of pancreatic enzymes, bile salts and temperature and adhere to the surface of the microvilli of the intestinal epithelial cells. After passing through several developmental stages, very strong reproduction occurs, producing both male microgamonts and female macrogamonts. Subsequently, fusion of the two occurs, resulting in the emergence of two different types of oocysts. While the thin-walled ones (approx. 20 %) are only surrounded by one membrane, remain in the intestine and can lead to re-infection, the thick-walled ones (approx. 80 %) are excreted with the faeces. Approximately 5-21 days after oral ingestion, the thick-walled oocysts begin to be excreted in the stool/faeces, and up to 100 million oocysts can be shed per excretion. The oocysts are infectious upon excretion and thus allow direct and immediate faecal-oral transmission. Recent findings show that cryptosporidia are not exclusively obligate intracellular parasites that only reproduce within a host, but also have extracellular stages, whose significance for the life cycle, however, has not yet been clarified (RKI, 2004).

[2] https://onehealthejp.eu/jrp-toxosources/

Cryptosporidia are ubiquitous and, as they are not host-specific, can infect a large number of different animal species. While *C. hominis* occurs almost exclusively in humans, cattle, horses, goats and sheep, but also dogs, cats and birds are considered hosts for *C. parvum* (Bouzid et al., 2013).

Direct and indirect routes of transmission of Cryptosporidium have been described. Direct transmission is via the faecal-oral route from infected hosts, including animal-to-animal, animal-to-human (zoonotic), human-to-animal (anthropozoonotic) and human-to-human (anthroponotic) transmissions (Davies and Chalmers, 2009).

Indirect transmission occurs through contact with faecal contaminated material and objects. Cryptosporidiosis is one of the most common intestinal diseases associated with contaminated water, e.g. from swimming pools or water sources that have not been previously heated or insufficiently filtered, such as river, lake or spring water. Further sources of danger result from irrigation of foodstuffs such as salads, vegetables and fruits with potentially contaminated water. These are often consumed raw, so pathogens are not killed (RKI, 2004; Bouzid et al., 2013).

<u>Description of the disease in humans</u>
The infectious dose for cryptosporidia is low, ranging from 10 to 1000 oocysts, depending on the species (RKI, 2004). Although the lowest infectious dose for *C. hominis has* been calculated to be 10 oocysts, it is considered that one oocyst could be sufficient to cause infection in humans by direct or indirect routes (Hamilton et al., 2018).

The incubation period is between one and 14 days. Infection can be asymptomatic or symptomatic. The latter is characterised by prolonged watery diarrhoea, which can lead to dehydration and severe weight loss. Other symptoms may include severe abdominal pain or cramps, nausea, vomiting and headache (Carter et al., 2020). Symptoms usually last one to two weeks, but can persist for up to four weeks (RKI, 2004).

People at increased risk of infection include, above all, small children between one and two years of age, travellers to developing countries and those who drink untreated water. Particularly at risk are people with severely weakened immune systems, especially those infected with HIV, as well as cancer and transplant patients. In addition, there is an increased risk of infection in people who have more contact with the risk groups described and infected animals. In immunocompetent persons, the infection is self-limiting. However, infectious oocysts are still excreted many weeks after the symptoms have subsided. In the risk groups described, complications and even death can occur after infection. Extra-intestinal manifestations can occur, especially in AIDS patients and possibly in patients with generally severe immunosuppression. Frequently, the bile duct system is involved; more rarely, an infection can also lead to inflammation of the pancreas (pancreatitis), the appendix (appendicitis), the ears (otitis) or infestation of the lungs with respiratory symptoms (RKI, 2004; Bouzid et al., 2013).

Therapy

An effective therapy for cryptosporidiosis is currently not available. Often, only the symptoms are treated, in the form of administration of fluids and electrolytes. In AIDS patients, intensified antiviral therapies are carried out to strengthen the immune system (RKI, 2004).

Geographical and seasonal features

Data from the ECDC (European Centre for Disease Prevention and Control) show that cryptosporidiosis in Europe has a seasonal increase in late spring and late summer to early autumn (Caccio and Chalmers, 2016). In studies, spring cases have more often been attributed to *C. parvum and* are probably the result of increased exposure to oocysts shed by young animals, as this coincides with the calving and lambing seasons (McLauchlin et al., 2000). On the other hand, the late summer-early autumn peak has been attributed mainly to *C. hominis and* is probably associated with increased travel and exposure to recreational waters (Davies and Chalmers, 2009).

Looking at the annual distribution of reported cases of the disease in Germany, pronounced seasonal fluctuations can also be seen here, with a maximum of cases in the second half of the year (end of July to mid-November) (RKI, 2004).

Occurrence in Europe and Germany

In EU/EEA countries, 8000-14000 cases are reported annually, and the incidence in different countries varies between 0.01 and 12 cases/100,000 population (ECDC, 2019).

In Germany, since the introduction of the Infection Protection Act (2001), there has been an obligation to report direct or indirect detection of cryptosporidia as soon as it indicates an acute infection. Current reporting figures are accessible via SurvStat@RKI[3] . According to the Robert Koch Institute (RKI), between approx. 900 and 2,000 cases are reported annually in Germany alone (incidence approx. 2 cases per 100,000 inhabitants). However, the number of undetected cases is probably much higher, as a laboratory diagnostic examination is only initiated if there is a concrete suspicion of cryptosporidiosis and no harmonised methods for detection are available. Caccio and Chalmers (2016) assume a hundredfold higher annual incidence in Germany.

In 2013, a major outbreak was reported in Halle (167 confirmed cases), which was attributed to pollution of a floodplain after a heavy rain event (Gertler et al., 2015).

Outbreaks caused by the consumption of water or plant foods

Cryptosporidia and other protozoa can get into food during preparation and processing, but also in the field through infected animals or flooding events. Disease outbreaks are repeatedly

[3] www.rki.de/survstat

registered; for a detailed description, we refer to Caccio and Chalmers (2016). Table 1 shows selected outbreaks in Europe, exclusively caused by the consumption of plant food or water.

Table 1: Examples of cryptosporidia outbreaks via plant food and drinking water

Country	Year	Breakout cause	Species	Assumed number of cases	Reference
Finland	2012	Frisée salad	*C. parvum*	>250	Åberg et al., 2016
England and Scotland	2012	Ready salad mix	*C. parvum*	N/D	McKerr et al., 2015
Sweden	2011	Drinking water	*C. hominis*	~20.000	Rehn et al., 2015
Sweden	2010	Drinking water	*C. hominis*	~27.000	Rehn et al., 2015
England	2008	Drinking water	*C. cuniculus*	422	Puleston et al., 2014

N/D = no data

3.1.2.2 *Giardia duodenalis*

Development cycle / transmission to humans

Giardia have a direct life cycle. Infection of the host takes place via oral ingestion of cysts through contaminated water, direct faecal-oral contact or, less frequently, with food. After contact with the acidic environment of the stomach, the cysts excyst in the proximal small intestine. During this process, two motile, vegetative growth forms, the trophozoites, are released from each cyst. After release, the trophozoites reproduce asexually by binary longitudinal division and attach themselves to the enterocytes of the small intestine. However, they do not penetrate the intestinal epithelium. In the presence of bile in combination with an alkaline environment in the jejunum (empty intestine), so-called encystation occurs; i.e. some trophozoites are remodelled into cysts again (Adam, 2001). Both cysts and trophozoites are excreted in the stool, the latter not surviving in the environment outside the body (Burnett, 2018). Cysts are excreted in large numbers in the stool/faeces of the host (Feng and Xiao, 2011b; Utaaker et al., 2017). An infected human can excrete 1-10 billion cysts daily (Burnett, 2018). Once the cysts are excreted, they are infectious. Oral ingestion of cysts by the host completes the development and infection cycle.

The species *Giardia duadenalis* is ubiquitously distributed and humans are considered the main reservoir. However, cats, dogs, beavers, cattle and a variety of other animal species can also be a source of infection for humans. Humans become infected through oral ingestion of contaminated tap water or untreated fresh water from lakes or streams. However, giardiasis can also be transmitted through the consumption of contaminated food, close contact with infected people/animals or sexual contact (Burnett, 2018). The risk of infection increases among travellers in developing countries, where the infection is common and inadequate hygiene standards favour it. It is estimated that up to one third of people living in developing countries have been infected with Giardia at least once (Burnett, 2018).

Description of the disease in humans

For Giardia, it is assumed that an amount of less than 10 cysts is sufficient for an infection (Rendtorff, 1954). More recent infection models confirm this and suggest that the oral ingestion of one cyst is sufficient for infection (Caccio, 2004).

Giardiasis is one of the most common intestinal parasitoses in humans worldwide, with an estimated 200 million people infected (Certad et al., 2017). Prevalence rates range between 2 and 7 % in developed countries and between 20 and 30 % in developing countries (Dixon, 2021). Infants and young children, the elderly, travellers and immunocompromised persons are among the high-risk groups, with children generally showing symptomatic infections more frequently than adults (Leung et al., 2019).

The colonisation of the epithelial cells leads to functional disorders and can also cause inflammation. After an incubation period of one to three weeks (Kucik et al., 2004; Dawson, 2005), first symptoms appear, which usually subside after two to four weeks (Lebwohl et al., 2003). Depending on various factors, such as patient age, previous exposure, parasite load and virulence, immune status and nutritional status of the affected person, different symptoms of the disease occur. Typical symptoms include acute, usually mild and self-limiting diarrhoea, with initially often copious and watery stools that become greasy and foul-smelling as the disease progresses (Naz et al., 2018). Other symptoms described include malaise, loss of appetite, nausea, vomiting, flatulence, fatigue, asthenia, abdominal pain or cramps, and even weight loss (Adam, 2001; Pietrzak et al., 2005; Naz et al., 2018). Less common symptoms include mild fever, chills and headache (Leung et al., 2019).

Even if the infection is asymptomatic in about 50-75% of infected persons (Lebwohl et al., 2003), asymptomatic persons can excrete cysts for several months or even longer (Pickering et al., 1984; Lopez-Romero et al., 2015). Re-infections are also possible. In general, repeated infections lead to milder symptoms (Kohli et al., 2008; Halliez and Buret, 2013; Bechlars et al., 2014). In young children and immunocompromised persons, the symptoms are usually longer-lasting and can have a more severe course (LoGalbo et al., 1982; Halliez and Buret, 2013; Bechlars et al., 2014). Severe chronic courses are also possible here. Deaths are rare, but can occur in infants and the elderly (Rumsey and Waseem, 2021).

Only a few studies are available on hospitalisation in cases of the disease. As the disease is usually mild, there is currently little data on hospitalisation, especially in developed countries. According to the European Food Safety Authority (EFSA), the hospitalisation rate in outbreak cases was 0.9 % in 2019 (EFSA, 2021, EU One Health Zoonoses Report 2019). In another study, which recorded children hospitalised with diarrhoeal disease in Greece, Giardia was found to be the cause of illness in only one out of 294 children, corresponding to a hospitalisation rate of 0.5 % (Kafetzis et al., 2001). In contrast, a Polish study for 2017 describes a hospitalisation rate of 24.5 % (Kitowska et al., 2019).

Therapy

As a rule, giardiasis can be treated well. In addition to symptomatic treatment of fluid loss, nitroimidazole preparations such as metronidazole, tinidazole or nitazoxanide are available for treatment, with metronidazole being the drug of choice. However, monotherapy approaches with nitroimidazole preparations can lead to therapy failure due to resistance, so that a combination therapy becomes necessary. Here, the combination of nitroimidazole preparations together with paromomycin or with albendazole or with mepacrine/quinacrine is considered effective. However, well-designed, randomised and controlled trials are still lacking and an optimal combination therapy has not yet been established (Escobedo et al., 2016; Carter et al., 2018).

Occurrence in Europe and Germany

According to the Infection Protection Act (IfSG) § 7, direct or indirect detection of the pathogen is reportable in Germany. In 2018, a total of 4,283 confirmed cases of the disease were reported to the RKI, in 2019 a total of 4,154 cases, in 2020 a total of 2,408 cases, in 2021 a total of 1,854 cases and in 2022 by the beginning of July 1,033 cases so far (SurvStat@RKI 2.0, https://survstat.rki.de, as of 04.07.2022). Nationally, the infection is mostly associated with travel abroad. In 2019, for example, 3,296 travel-associated cases were reported to the RKI. The most frequently mentioned country of infection was India (222 mentions, 18 %), followed by Spain, Colombia, Egypt, Italy and Turkey. The frequency of imported cases showed a clear seasonal pattern with a marked peak in January and two smaller peaks in April and October (RKI, 2020).

Outbreaks caused by the consumption of water or plant foods

Giardiasis is monitored in the whole population in most EU/EEA countries and is one of the most common reported food- and waterborne parasitic diseases. In 2020 alone, 6,249 cases of the disease were reported in the EU, including 1,661 in Germany[4] . However, an estimated number of unreported cases must be expected, especially in Eastern Europe. A quarter of the EU Member States have no surveillance systems for giardiasis and do not report any cases (ECDC, 2019).

Despite its public health significance, epidemiological data on foodborne illness caused by *G. duodenalis* are currently lacking. It is estimated that the pathogen causes 28.2 million cases of diarrhoeal disease per year worldwide due to food contamination (Ryan et al., 2019).

---

[4] http://atlas.ecdc.europa.eu/public/index.aspx?Dataset=27&HealthTopic=20

So far, very few foodborne outbreaks caused by *Giardia have* been documented, as surveillance systems in many countries are inadequate and, in addition, the available detection methods are not standardised (Ryan et al., 2019). Data on foodborne outbreaks in Germany are not yet available.

EFSA has documented 14 foodborne outbreaks caused by *Giardia in* Europe in 2019, five fewer than in 2018. However, the report does not address the specific food causing the outbreak. Three other outbreaks were due to the consumption of water, table water and "well water" respectively. In 2019, a total of 233 people fell ill, with 199 people falling ill in connection with an outbreak in Italy alone (EFSA, 2021, EU One Health Zoonoses Report 2019).

As investigations of food-associated outbreaks between 1971 and 2011 in the USA showed, fresh plant products, especially those consumed raw, are a source of infection for humans (Adam et al., 2016). However, in the few food-associated outbreaks described, it is not clear whether the contamination already occurred in the field, during harvesting or during preparation. Irrigation with wastewater containing cysts has been identified as a possible cause of food contamination in this context (Chalmers et al., 2020). Waterborne transmission is a very significant route of infection and outbreaks with large numbers of ill people have been documented worldwide (Nygard et al., 2006; Guzman-Herrador et al., 2015).

The following table gives examples of some of the documented Giardia outbreaks via plant foods and drinking water:

Table 2: Examples of Giardia outbreaks via plant food and drinking water

Country	Year	Source of infection	Confirmed Cases of illness	Reference
USA	2016	mixed green salad	25	CDC NORS (described in (Ryan et al., 2019) https://www.cdc.gov/foodborneoutbreaks/ Default.aspx)
USA	2005	Fresh fruit and vegetables	50	New York State Dept of Health (Ryan et al., 2019) https://www.health.ny.gov/statistics/diseases/ foodborne/outbreaks/2005/docs/report.pdf)
USA	1986	Fruit salad	9	(Porter et al., 1990)
Norway	2004	contaminated drinking water	1300	(Nygard et al., 2006)
Italy	2018-2019	Presumably contaminated drinking water	238	(Resi et al., 2021)

3.1.2.3 *Toxoplasma gondii*

Development cycle / transmission to humans

Due to its wide distribution and diverse infection routes, *T. gondii is* considered one of the most important parasitic zoonotic pathogens worldwide. The parasite is capable of infecting almost all warm-blooded vertebrates, including birds, with only cats and other felids serving as definitive hosts (Tenter et al., 2000).
The development cycle of *T. gondii is* divided into a sexual and an asexual reproduction phase. Three different developmental stages can be distinguished (oocysts, tachyzoites, bradyzoites), all of which are infectious for both the intermediate and the final host. Transmission of the parasite is not only possible between intermediate and final hosts, but can also occur between two different intermediate or final hosts (Tenter et al., 2000).

Sexual reproduction of the parasite occurs exclusively in the intestinal epithelium of the final host, during which several million oocysts are produced and excreted in the faeces (Dubey and Frenkel, 1972). In the environment, depending on the prevailing conditions, sporulation of the oocysts occurs within 1-21 days (11-25 °C), which only attain their infectivity through this maturation process (Dubey et al., 1970). Through oral ingestion of sporulated oocysts from the environment, new final or intermediate hosts can become infected. In this process, the oocyst wall is lysed during the gastrointestinal passage, releasing contained sporozoites. After infection of the intestinal epithelium, there is a strong asexual multiplication and spread of the pathogen in the body in the form of rapidly dividing tachyzoites. With onset of the immune response, this acute stage changes into a chronic phase of infection. During this phase, the division rate of the tachyzoites slows down and they differentiate into bradyzoites, hundreds of which persist in the host, protected within tissue cysts in various organs and the musculature, for years or even for life (Robert-Gangneux and Darde, 2012). Consumption of raw meat containing tissue cysts eventually leads to infection of the final host or other intermediate hosts. The bradyzoites are released during the gastrointestinal passage, infect the intestinal epithelium and a new asexual (intermediate host) or sexual (final host) reproduction phase is initiated.

Due to the wide distribution and host range, all infected warm-blooded farm, slaughter and wild animals can generally be considered as reservoirs. The most important horizontal transmission routes for humans are: 1) oral ingestion of infectious tissue cysts through consumption of raw or inadequately heated meat or inadequately heated meat products made from such meat, 2) oral ingestion of sporulated oocysts from the environment via smear infections (e.g. through direct contact with cat faeces or soil contaminated with them) or through consumption of contaminated plant foods and water. In the case of an initial infection during pregnancy, the pathogen can also be transmitted vertically (tachyzoites) to the unborn child (Tenter et al., 2000).

Description of the disease in humans

With an estimated infection rate of 30 %, toxoplasmosis is one of the most common parasitic zoonotic diseases in humans worldwide.

In immunocompetent individuals, 80-90 % of postnatally acquired infections are asymptomatic and therefore go undetected. In 10-20 % of cases, mild non-specific flu-like symptoms with fever, headache, muscle pain, sore throat, fatigue and lymph node swelling (predominantly in the head and neck area, less frequently generalised) may occur in the acute phase of infection (Hill and Dubey, 2002; Robert-Gangneux and Darde, 2012; RKI, 2018). First clinical symptoms occur 10-14 days after infection (EFSA, 2007). Inflammation of the retina and choroid of the eye (ocular toxoplasmosis) with visual disturbances and even blindness can also occur in immunocompetent children and adults. Severe diseases, such as inflammation of the heart muscle, lungs, brain and liver, have already been described in immunocompetent individuals, but occur very rarely (EFSA, 2007; Schlüter et al., 2014). There are indications that particularly severe diseases are associated with highly virulent atypical genotypes (EFSA, 2007; Schlüter et al., 2014).

Immunocompromised individuals (e.g. due to HIV/AIDS, chemotherapy or after transplantation) can often develop severe clinical manifestations, usually due to reactivation of latent infections, which can sometimes be fatal. The most frequent manifestation is inflammation of the brain, but pneumonia can also occur. In addition, ocular toxoplasmosis and the disseminated form of toxoplasmosis can occur with the involvement of various organs (EFSA, 2007; RKI, 2018). Without prophylactic therapy, 47 % of AIDS patients chronically infected with *T. gondii* may reactivate and develop encephalitis (Zangerle et al., 1991).

If an initial (first time) infection occurs during pregnancy, the pathogen can be transmitted transplacentally to the unborn child (prenatal or congenital infection) and lead to severe physical and neurological malformations, such as inflammation of the retina and choroid of the eye, hydrocephalus and cerebral calcifications, and even miscarriage (Mylonas et al., 2013). In up to 20 % of cases, the pathogen is transmitted to the foetus (Li et al., 2014). However, only 27 % of infected newborns show specific symptoms (Dunn et al., 1999). However, late damage may not manifest itself until several years after childbirth, mainly affecting the eyes, the central nervous system and the hearing (Tenter and Fehlhaber, 2002).

In animal studies, infection with *Toxoplasma oocysts* resulted in more severe disease compared to infection with tissue cysts (Dubey, 2021). Although discussed more frequently in the literature (Hill and Dubey, 2002; VanWormer et al., 2013), this has not yet been clearly demonstrated in human infections (Meireles et al., 2015; Dubey, 2021).

Nothing is known about the minimum infective dose in humans (EFSA, 2007). In experimentally infected pigs, an infective dose of 1-10 oocysts was determined. After administration of one oocyst, tachyzoites could already be detected in 60 % (17/28) of the experimentally infected pigs; after administration of 10 oocysts, this was possible in 92 % (13/14) of the animals (Dubey et al., 1996). A large drinking water-associated outbreak with 176 cases in Brazil, in which presumably relatively few oocysts introduced by cats contaminated millions of litres of drinking water, also suggests a low infectious dose in humans (de Moura et al., 2006; Dubey, 2021).

Therapy

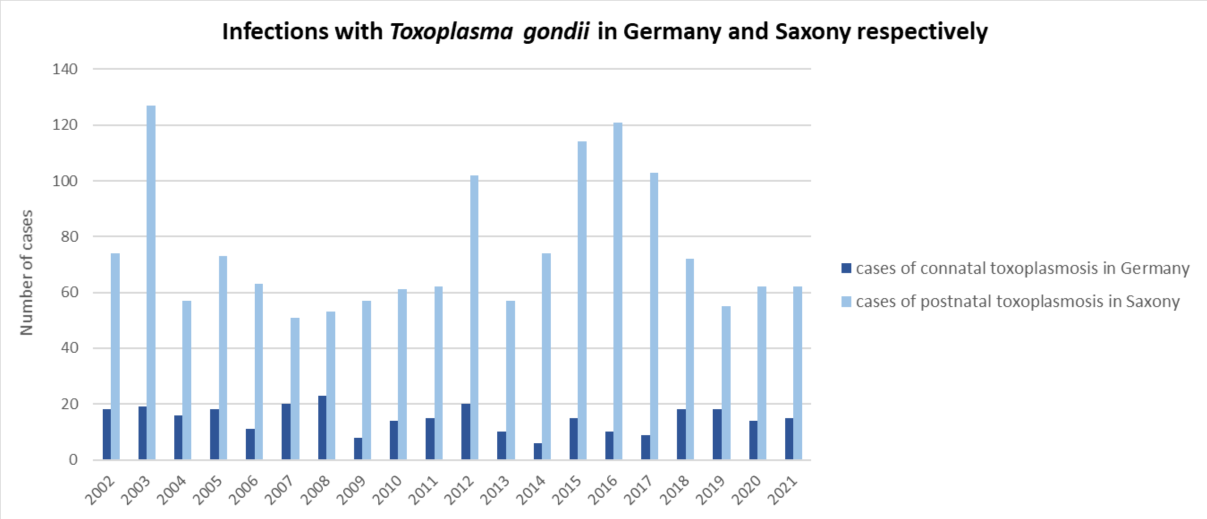
The treatment of toxoplasmosis differs depending on the type of disease. For example, active *toxoplasma infections in* immunosuppressed patients or ocular toxoplasmosis are treated. Treatment is also recommended in the case of an innitial infection during pregnancy, and infected newborns are also treated. Various drugs are available for treatment, such as spiramycin, pyrimethamine, sulphadiazine, clindamycin and, if necessary, atovaquone. The aim of treatment is to interrupt protein biosynthesis and folic acid synthesis of toxoplasma. It should be noted, that the drug effect is limited to *Toxoplasma tachyzoites,* whereas the drugs are almost ineffective against *Toxoplasma bradyzoites* (RKI, 2018).

Occurrence in Europe and Germany

Direct or indirect detection of *T. gondii* in connatal infections of humans is notifyable according to the Infection Protection Act (RKI, 2018). Between 2002 and 2021, 6-23 cases of connatal toxoplasmosis were reported directly to the RKI each year (Fig. 1). However, this is assumed to be a severe under-reporting, as there is no routine screening of pregnant women in Germany and usually only those cases clinically or serologically conspicuous at the time of birth are reported (RKI, 2018). In Saxony, there is also an obligation to report postnatally acquired toxoplasmosis. Here, 51-127 cases of postnatal toxoplasmosis were reported directly to the RKI every year between 2002 and 2021 (Fig. 1).

In 2016, a nationwide representative study conducted for the first time by the RKI determined a seroprevalence of 55 % in the entire adult German population (Wilking et al., 2016). The seroprevalence rose from 20 % in the age group of young adults (18-29 years) to 76.8 % among senior citizens (70-79 years). Higher seroprevalence rates were detected in the population in eastern Germany than in western Germany, which could be due to the increased consumption of raw minced pork in the east (Wilking et al., 2016). From the seroprevalences determined, an incidence of postnatally acquired infections of 1,099 per 100,000 inhabitants was estimated. Furthermore, it was calculated on the basis of these data that 74.1 % of all pregnancies in Germany are at risk and that a first infection occurs in approx. 6,400 pregnancies per year. This could lead to approx. 1,300 prenatal infections and 345 newborns with apparitional clinical symptoms every year (Wilking et al., 2016).

Figure 1: Number of cases of connatal toxoplasmosis in Germany not reported by name to the RKI since 2002 and reported cases of postnatal toxoplasmosis in Saxony (source: Robert Koch Institute: SurvStat@RKI 2.0, https://survstat.rki.de, query date: 09.11.2021).



Oocysts-associated toxoplasmosis outbreaks:

In Germany, no outbreaks caused by *T. gondii have been* described so far. In Europe, only one oocyst-associated outbreak has been reported so far among 171 schoolchildren in Turkey, where transmission via soil/dust and its contamination by stray cats is suspected (Doganci et al., 2006).

An overview of *T. gondii-associated* outbreaks worldwide is provided by two systematic reviews from 2015 (n=38) and 2019 (n=34) (Meireles et al., 2015; Pinto-Ferreira et al., 2019) and a recent review from 2021 (Dubey, 2021). In this review, 44-52% of the *T. gondii* associated outbreaks analysed and occurring worldwide between the years 1965 and 2013 were attributed to oocysts, water contaminated with oocysts in 20-21%, contaminated soil/sand in 18-26% (10/38) and contaminated raw vegetables in 3-6% of outbreaks identified as sources of infection (Meireles et al., 2015; Pinto-Ferreira et al., 2019). In most outbreaks, the source of infection (matrix as well as parasite stage) was determined by epidemiological correlations and has rarely been established by direct detection of *T. gondii (*de Moura et al., 2006). Compared to tissue cyst-associated outbreaks, in which usually only smaller groups/clusters are affected, higher numbers of cases occur in oocyst-associated outbreaks, especially in contaminated drinking water (Meireles et al., 2015; Pinto-Ferreira et al., 2019).

In the first water-associated outbreak in Panama (1979), 31 soldiers contracted toxoplasmosis from drinking river water treated with iodine tablets, a type of treatment generally thought to be effective in killing bacteria and *G. lamblia* (Benenson et al., 1982; Dubey, 2021). Several drinking water-associated outbreaks, e.g. in Canada and Brazil with 100 and 178 cases respectively, have demonstrated access of *T. gondii* positive cats to drinking water supplies or the immediate environment (Bowie et al., 1997; de Moura et al., 2006). In the largest outbreak described so far, with 248 cases of ocular toxoplasmosis in India, no source of infection could be identified. However, due to the geographical clustering of cases in the city of Coimbatore and its suburbs, contamination of municipal drinking water is also assumed (Balasundaram et al., 2010).

Table 3: Selected oocyst-associated toxoplasmosis outbreaks

Country	Year	Breakout cause	Assumed number of cases	Symptomatic cases	Reference
Panama	1979	Iodised river water	31	91 %	(Benenson et al., 1982)
Canada	1995	municipal drinking water	100	82 %	(Bowie et al., 1997)
Brazil	2001	municipal drinking water	176	88 %	(de Moura et al., 2006)
Turkey	2002	Earth/Dust (School)	171	100 %	(Doganci et al., 2006)
India	2004	unclear; possibly municipal drinking water	248	100 %	(Balasundaram et al., 2010)
Brazil	2009	Lettuce (Escarole)	11	72 %	(Ekman et al., 2012)
Brazil	2013	Juice (Açai berries)	73	100 %	(Morais et al., 2016)

The BfR identified only two publications on oocyst-associated outbreaks via plant-based food, but these lack precise information on the origin of the oocyst contamination. In Brazil, there was an outbreak among 11 employees of an industrial plant in 1993, in which lettuce (escarole) was identified as the probable source of infection (Ekman et al., 2012). Another outbreak in Brazil with 73 confirmed cases was caused by juice from açai berries (Morais et al., 2016).

3.1.3 Exposure estimation and assessment

3.1.3.3 *Cryptosporidium* spp.

Raw and waste water

Overall, *Cryptosporidium* spp. have been widely detected in raw water and wastewater samples; for an extensive list of worldwide studies, please refer to Kerry *et al*. (Hamilton et al., 2018). Only studies from Germany are discussed here.

The study by Karanis *et al.* (1998) was conducted to assess the prevalence of *Cryptosporidium* spp. in German water supplies and the efficiency of surface water treatment plants to remove *Cryptosporidium* spp. by conventional treatment. Water samples from six surface water treatment plants in different parts of Germany were analysed for the presence of the pathogens. *Giardia spp.* or *Cryptosporidium* spp. or both were found in 76.2 % of the raw water sources examined. The average number of *Cryptosporidium oocysts* detected was 116 per 100 litres (max. 1081/100 L). In the intermediate clarification steps (including flocculation and several filtration steps), *Giardia* spp. or *Cryptosporidium* spp. or both were found in 33.3 % (50/150) of the samples. 29.8 % (14/47) of the drinking water samples were positive for *Cryptosporidium* spp. (max. 20.8/100 L).

Another German study evaluated the occurrence of *Cryptosporidium* spp. in sewage samples and removal efficiency (Ajonina et al., 2012). Treated wastewater and untreated influent sewage samples were collected seasonally over a two-year period. Oocysts were repeatedly detected in influent and effluent samples from the sewage treatment plant with a mean concentration of 782 oocysts per litre (50-1280).

In another study in the Rhine basin, 64 (31.1 %) of 206 sewage samples were positive for *Cryptosporidium oocysts*, with up to 1745 oocysts found per litre (Gallas-Lindemann et al., 2013b).

Vegetable food
Few studies are available on the occurrence of cryptosporidia on plant foods.

In the study by Rzezutka *et al.* (2010), various samples of fresh vegetables and soft fruits were taken at farmers' markets in the Lublin region of Poland between 2006 and 2007; *C. parvum oocysts were* detected in six of 128 vegetable samples (range 1-47 oocysts), but in none of the 35 fruit samples.

In a similar study in Norway between August 1999 and January 2001, 19 out of 475 fruit and vegetable samples were positive for *Cryptosporidium* spp. Detections were found in five lettuce samples (26 %) and 14 (74 %) mung bean sprout samples, with detected oocyst concentrations averaging about three oocysts per 100 g of produce (Robertson and Gjerde, 2001).

In Greece, a total of 72 fruit and vegetable samples from supermarkets and open markets were also examined. *Cryptosporidium oocysts were* detectable in two out of 72 (2.8%) samples (Sakkas et al., 2020).

In Italy, *Cryptosporidium* spp. were detected in 0.9 % of 648 samples when testing ready-to-eat salads, which are intended for immediate consumption without prior washing (Caradonna et al., 2017).

In a small study, *Cryptosporidium* spp. or *Giardia* spp. were detected in 12 (10) of 19 Spanish salad products tested (Amoros et al., 2010).

3.1.3.2 *Giardia duodenalis*

Raw and waste water
Overall, there are several studies worldwide looking at Giardia in surface water and wastewater (for details of these studies, see review by Hamilton et al., 2018).

Here, two European studies (Germany, Italy) and one study from Brazil will be discussed as examples, which deal with reduction of pathogens through treatment of wastewater. Karanis *et al.* (1998) sampled six surface water treatment plants in Germany over a period of slightly more than two years. One aim of the study was to record the spread of Giardia in the water supply and the efficiency of pathogen elimination by conventional treatment. *Giardia* spp. or *Cryptosporidium* spp. or both pathogen species were found in 76.2 % of the raw water sources investigated. On average, 88.2 *Giardia cysts* were detected per 100 litres of water (max. 1314/100 L). Although a reduction of the pathogens was achieved in the subsequent treatment steps, complete elimination could not be achieved. Thus, after the intermediate clarification steps (including flocculation and several filtration steps), *Giardia* spp. or *Cryptosporidium* spp. or both pathogen species were found in 33.3 % (50/150) of the samples. *Giardia cysts were* detected in 14.9 % (7/47) of the drinking water samples (max. 16.8/100 L). A combination of slow sand filtration, infiltration, disinfection, sand and activated carbon filtration was found to be the most effective water treatment technology to achieve effective pathogen reduction (Karanis et al., 1998).

In a study conducted in Italy in four wastewater treatment plants at each stage of the treatment process over a period of one year, *Giardia cysts* could be detected in the influents of all wastewater treatment plants throughout the year. On average, the number of cysts ranged from 2.1 to 4.2 per litre. In all wastewater treatment plants, the highest number of cysts was detected in autumn and winter. After all wastewater treatment steps, an elimination of cysts between 87 % and 98.4 % was achieved, depending on the type of treatment (Caccio et al., 2003).

In another study by a Brazilian working group, *Giardia cysts were* detected in 35.8 % of the samples tested (n=53) in the examination of reclaimed wastewater from two wastewater treatment plants. The number of cysts ranged from < 0.03 to 16 cysts per litre (Razzolini et al., 2020).

<u>Vegetable food</u>

In a survey of the global prevalence of human pathogens on fresh produce in production/packaging facilities, Giardia had the highest mean prevalence among the parasites studied (Van Pelt et al., 2018). Contamination of vegetables and fruits with *Giardia cysts* has been reported in many countries, and the mean prevalence is estimated at 4.8% (276/5739; 95% CI: 4.2-5.4%) (Li et al., 2020).

In different studies conducted in several European countries, *G. duodenalis cysts were* detected on fresh plant foods with varying prevalence: Norway: 2.1 % (Robertson and Gjerde, 2001), Turkey: 20 % (Erdogˇrul and Şener, 2005), Spain: 52.6 % (Amoros et al., 2010), Italy: 0.6 % (Caradonna et al., 2017).

According to the Food and Agriculture Organisation of the United Nations (FAO, 2011), approximately 70 % of water consumption worldwide is attributed to the agricultural sector. Water used for irrigation or food washing can be contaminated with *Giardia cysts* through contamination with animal or human faeces, thus contaminating food with the pathogen (Budu-Amoako et al., 2011).

For example, in investigations carried out as part of a Norwegian study by Robertson and Gjerde (2001) on parasite infestation of various fruits and vegetables, *Giardia cysts were* detected in 10 of 475 samples. The affected foods were dill and lettuce (2 positive samples each), mung bean sprouts (3 positive samples), radish sprouts (one positive sample) and strawberries (2 positive samples). The pathogen concentrations detected here were low and averaged 3.3 cysts per 100 g of fruit and vegetables. *Giardia cysts* were also detected in water used for irrigation of fields and production of bean sprouts in Norway in this study (Robertson and Gjerde, 2001).

Amoros *et al.* (2010) were also able to detect cysts in both the food and water samples in a study in Spain on lettuce and the irrigation water used. Here, *Giardia cysts* were detected in 52.6 % of the food samples and in 100 % of the water samples. In the water samples, an average of 40 cysts per litre of water was detected (Amoros et al., 2010).

3.1.3.3 *Toxoplasma gondii*

Occurrence of *Toxoplasma gondii* oocysts in water:
T. gondii oocysts originate exclusively in the intestinal epithelium of cats and other felids (Dubey and Frenkel, 1972) and only enter the environment via their faeces, where they are further spread via soil, air and water and can thus also get on plant foods. They are released from soil and faeces by rainwater and thus also enter irrigation systems, rivers, lakes, seawater and sewage systems. However, since they are not excreted via human faeces, unlike *Cryptosporidium oocysts* and Giardia cysts, comparatively lower concentrations of *T gondii oocysts* are to be expected in wastewater. Nevertheless, they can enter urban and industrial wastewater, e.g. through the disposal of cat faeces via the toilet or through cleaning of objects and plant foods (Bahia-Oliveira et al., 2017).

The BfR identified only two studies from Germany on the occurrence of *T. gondii oocysts* in water. In a smaller study by Ajonina *et al.* (2018), wastewater from a wastewater treatment plant in Hamburg was analysed for the presence of *T. gondii DNA* before (n=16) and after treatment (n=9) over a period of eight months. *T. gondii* could not be detected in any of the 25 samples, but the number of samples is too small to make a reliable statement (Ajonina et al., 2018). In a somewhat more extensive study by Gallas-Lindemann *et al.* (2013a), wastewater was sampled over a period of nine months within eight wastewater treatment plants along the Rhine in North Rhine-Westphalia. *T. gondii* was detectable in 8.9 % (4/45) of the samples before and in 10.5 % (4/38) of the samples after wastewater treatment, whereby an entry of oocysts after wastewater treatment by access of cats could not be excluded. In contrast, no *T.*

gondii could be detected in the tap water (n=1), raw water (n=1), groundwater (n=4) and surface water (n=6) samples taken, but here, too, the low sample numbers do not allow a valid statement (Gallas-Lindemann et al., 2013a).

In other European studies, *T. gondii* was detected in 43 % of wastewater samples in Bulgaria (Sotiriadou and Karanis, 2008), but not in wastewater studies from France and Italy (Moulin et al., 2010; Marangi et al., 2015). In addition, *T. gondii* has also been detected in surface, ground and tap water as well as in rivers and lakes in Europe and worldwide (Sotiriadou and Karanis, 2008; Bahia-Oliveira et al., 2017).

Occurrence of *Toxoplasma gondii* oocysts on plant foods:
In Germany, no studies have yet been carried out on the occurrence of *T. gondii oocysts* in samples of plant foods. Therefore there is a considerable need for research. In Europe, only five studies have been published so far, the results of which can be seen in Table 4. The majority of the studies show prevalences between 5 and 20 %. By far the highest prevalences were observed in Spain and Portugal on berries and various vegetables, although in this study only a few samples with above-average sample sizes of several hundred grams were examined (Marques et al., 2020). This implies that pathogen transmission through consumption of plant foods must also be considered. Especially vegetables or fruits grown close to the ground could be more contaminated with oocysts through soil contact, such as root vegetables, salads or strawberries. However, oocysts can also get onto plant foods by use of contaminated water for irrigation and further treatment.

Table 4: Occurrence of *Toxoplasma gondii* on plant foods in Europe

Country	Year	Matrix	Prevalence	Reference
Czech Republic	N/D	Cucumbers	11,9 % (13/109)	(Slany et al., 2019)
Czech Republic	N/D	Carrots	7,5 % (7/93)	(Slany et al., 2019)
Czech Republic	N/D	Lettuce (lettuce, iceberg lettuce, little gem, lollo lettuce)	8,9 % (8/90)	(Slany et al., 2019)
Poland	2006-2008	Strawberries	0 % (0/60)	(Lass et al., 2012)
Poland	2006-2008	Radish	5 % (3/60)	(Lass et al., 2012)
Poland	2006-2008	Carrots	19,5 % (9/46)	(Lass et al., 2012)
Poland	2006-2008	Salad	18 % (9/50)	(Lass et al., 2012)
Italy	2015-2016	RTE mixed salads (curly and escarole lettuce, radishes, carrots, rocket)	0.8 % or 6.9 % (5/72 pool samples from a total of 648 individual samples)	(Caradonna et al., 2017)
Spain, Portugal	2018-2019	Vegetables (bulk, RTE, packaged products: Lettuce, carrots, parsley, water cress, coriander, RTE mixed salads, rocket)	42,9 % (12/28)	(Marques et al., 2020)

Country	Year	Matrix	Prevalence	Reference
Spain, Portugal	2018-2019	Berries (strawberries, raspberries, blueberries)	42,9 % (3/7)	(Marques et al., 2020)
Switzerland	2019	Salad	6 % (6/100)	(Guggisberg et al., 2020)

N/D.: no data

A comprehensive review article on the occurrence and diagnostics of *T. gondii oocysts* in soil, water, plant foods and bivalve molluscs was published within the EJP project TOXOSOURCES (López-Ureña et al., 2022) In addition, a multicentre study on the occurrence of *T. gondii oocysts* in ready-to-eat green lettuce was conducted in ten European countries.

3.1.3.4 Consumption of fresh fruit and vegetables in Germany

Fresh fruit and vegetables are an important component of a healthy diet. These foods are therefore regularly consumed in significant quantities by almost all consumer groups in Germany. An estimate of the consumption of fresh fruit and vegetables in Germany can be found in a statement published by the BfR in 2020 (BfR statement no. 021/2020 of 21 April 2020: "Reclaimed waste water: Avoid bacterial pathogens on fresh fruit and vegetables").

3.1.4 Risk characterisation

3.1.4.2 Possibilities and limits of risk characterisation

Due to the currently very limited data available on documented disease outbreaks in which the infections were demonstrably caused by the consumption of plant foods, a conclusive risk characterisation on the use of reclaimed wastewater for the irrigation of plants intended for consumption is only possible to a limited extent with regard to the protozoa *Cryptosporidium* spp., *G. duodenalis* and *T. gondii.* The data on the prevalence and stability of cryptosporidia, giardia and toxoplasma on fresh fruit and vegetables listed in the present statement also do not allow for a valid statement on the matter. Nevertheless, they show that these protozoa are present on such foods in Europe. Concrete data for Germany are currently not available in this regard. The different detection rates on plant foods determined in the European studies may be due to the use of different detection methods.

All protozoa assessed here show high stability in the environment. It is true that the data on stability on fresh fruit and vegetables is currently insufficient. However, it can be assumed that the protozoa described here are infectious on plant foods over a longer period of time and can cause diseases after their raw consumption. The ingestion of a very small number of oocysts/cysts is sufficient for this. In contrast, however, infected individuals excrete very large quantities of *Cryptosporidium oocysts* and Giardia cysts in their stool/faeces, which implies the possibility of sewage contamination.

Plant foodstuffs can be demonstrably contaminated with *Cryptosporidium oocysts* or Giardia cysts through irrigation water. The occurrence in municipal wastewater depends on the infection status of the population or livestock in the catchment area of wastewater production. As already described in chapter 3.1.1, all protozoa discussed here show moderate to good resistance to disinfection measures and disinfectants in the doses or concentrations commonly used for wastewater treatment. Although a reduction can be achieved through treatment of wastewater during the various processing steps according to current knowledge, complete elimination is not possible.

T. gondii oocysts originate exclusively in the intestinal epithelium of cats and other felids (Dubey and Frenkel, 1972) and only enter the environment via their faeces, where they are further spread via soil, air and water and can thus also get on plant foods. They are dissolved from soil and faeces by rainwater and thus also enter water ditches, rivers, lakes, seawater and sewage systems. However, since they do not occur in human faeces, unlike *Cryptosporidium oocysts* and Giardia cysts, comparatively lower concentrations of *T. gondii oocysts* are expected in wastewater. Nevertheless, they can enter municipal wastewater, e.g. by disposing of cat faeces in the toilet or by cleaning objects and plant foods.

In view of the evidence provided so far of the protozoa considered here in wastewater and their high stability and environmental resistance, there is a high probability of contamination of plant foodstuffs after they have been irrigated with reclaimed wastewater, depending on the treatment and irrigation methods used. It is possible that there will be an increase in cases of cryptosporidiosis, giardiasis or toxoplasmosis in Germany if the reclaimed wastewater gets directly onto plant parts to be eaten raw or if protozoa are transferred to these plant parts with contaminated soil particles. However, by heating the food sufficiently (at least two minutes at 70 °C on all parts of the product), the protozoa considered here can be killed.

A complete removal of these pathogens is unlikely even in drinking water, as the disease outbreaks described here due to contaminated drinking water show. It can be assumed, however, that due to the described life and infection cycles of these pathogens alone, there are higher concentrations in wastewater than in drinking water. It must be examined to what extent further treatment can effectively reduce these pathogens.

3.1.4.2 Risk characterisation based on conceivable scenarios

For the protozoa mentioned in this assessment opinion, the data situation in different areas is currently insufficient for a conclusive risk characterisation. Therefore, further risk characterisation for different irrigation systems is based on a scenario in which it is assumed that toxoplasma, giardia and cryptosporidia are not completely eliminated or inactivated by wastewater treatment.

3.1.4.2.1 Subsurface drip irrigation

It is possible that infectious human pathogenic protozoa such as toxoplasma, giardia and cryptosporidia get into the ground itself as well as plant parts growing in the ground with the reclaimed wastewater and remain infectious there for longer periods of time. Contamination of above-ground growing plant parts is possible, especially by transfer from contaminated areas to the edible plant parts. This can happen, for example, during harvesting. Uptake of the pathogens via the root system of the plant however is unlikely.

In this scenario, the probability of contamination of plant parts intended for consumption growing in the ground is to be assessed as high. The probability of contamination of plant parts growing above the ground, on the other hand, is to be assessed as lower.

3.1.4.2.2 Drip irrigation

It is possible that infectious human pathogenic protozoa such as toxoplasma, giardia and cryptosporidia get into the ground itself as well as plant parts growing in the ground with the reclaimed wastewater and remain infectious there for longer periods of time. Furthermore, contamination of plant parts growing above the ground is possible if plant parts close to the ground come into direct contact with reclaimed wastewater or pathogen-contaminated soil particles get onto the plant.

In this scenario, the probability of contamination of plant parts intended for consumption growing in the ground is to be assessed as high. The probability of contamination of plant parts growing above the ground is to be assessed as moderate due to the possibility of contaminated soil particles being transferred, for example during harvesting, to edible plant parts.

3.1.4.2.3 Furrow irrigation

It is possible that infectious human pathogenic protozoa such as toxoplasma, giardia and cryptosporidia get into the ground itself as well as plant parts growing in the ground with the reclaimed wastewater and remain infectious there for longer periods of time. Contamination of plant parts growing in the ground must be assumed. Contamination of plant parts growing above the ground with protozoa is also possible if plant parts come into direct contact with the reclaimed wastewater (e.g. in the case of overflowing water ditches, water splashes from the water ditches or the movement of pathogen-containing soil particles through water).

In this scenario, the probability of contamination of plant parts intended for consumption growing in the ground is to be assessed as high. The probability of contamination of above-ground growing plant parts intended for consumption is to be assessed as moderate to high.

3.1.4.2.4 Sprinkler systems

It is possible that infectious human pathogenic protozoa such as toxoplasma, giardia and cryptosporidia get into the ground itself as well as plant parts growing in the ground with the reclaimed wastewater and remain infectious there for longer periods of time. For this reason, contamination of both plant parts growing in and above the ground must be assumed with this type of irrigation.

In this scenario, the probability of contamination of plant parts intended for consumption growing in or above the ground is to be assessed as high.

3.1.4.2.5 Hydroponic culture

It is possible that infectious human pathogenic protozoa, such as toxoplasma, giardia and cryptosporidia, are present in the reclaimed wastewater and remain infectious over longer periods of time. Contamination of plant parts growing in the water must be assumed. Contamination of other plant parts is possible if they come into direct contact with the irrigation water.

In this scenario, the probability of contamination of plant parts growing in the water and intended for consumption must be considered high. The probability of contamination of other plant parts intended for consumption is considered low, although such contamination cannot be ruled out.

3.1.4.3 Assessment of the quality of the data

The basis of the present assessment opinion on the possible infection of humans through the consumption of fruit and vegetables irrigated with reclaimed wastewater during cultivation was available data from the public health system in Germany, the EFSA and the scientific literature. The quality of the available data and information related to the characteristics of the protozoa, their transmission to humans and the diseases caused by these pathogens can be considered satisfactory.

Cryptosporidia
Data on the occurrence of cryptosporidia on fresh plant food, in soils and reclaimed wastewater are available, but there are hardly any statements on the infectivity of the oocysts found due to the lack of infectivity assays.

Giardia
The quality of data on the occurrence of Giardia in water and on fresh plant foods is also considered satisfactory. The quality of the data regarding the stability of Giardia in soil and water as well as the data quality regarding the reduction of Giardia in the context of the treatment or processing of wastewater is also considered satisfactory. Data on the stability of Giardia on fresh fruit and vegetables are currently only available to a very limited extent. However, in combination with the data on stability in soil and water, they allow a tendency assessment with regard to stability on these foods. However, there are currently insufficient data on foodborne outbreaks caused by Giardia.

Toxoplasma
The data on the occurrence of *T. gondii* in wastewater in Germany and Europe is currently very limited. In Germany, there are only a few small, but no representative studies on the occurrence of *T. gondii in wastewater in* general or in different sections of wastewater treatment plants that would allow for reliable data on the occurrence and effectiveness of different treatment or processing techniques. Furthermore, standardised methods for sensitive detection in the matrix water - which is susceptible to PCR inhibition - are needed to make reliable statements.

3.1.4.4 Need for further research

In order to generate data to further assess the risks posed by protozoa in the use of reclaimed wastewater, research would be needed in the following areas:

- Development of standardised methods for the detection of infectious protozoa on food (so far only partially available and not ubiquitously applicable)

- Further studies to improve the data situation with regard to stability of protozoa on fresh plant foods, taking into account the respective cultivation and storage conditions.

- Development of further methods for chemical/physical inactivation/elimination of protozoa in the treatment of wastewater. For example, *Cryptosporidium oocysts* could be used as a reference pathogen, as they have a higher resistance than *Giardia cysts* or *T. gondii oocysts.*

- Testing of effective inactivation methods of cryptosporidia, giardia and toxoplasma in water treatment with regard to plant and environmental compatibility, the residue problem and possible negative effects on human health.

- Comparison of available and commonly used inactivation methods for drinking water and wastewater and their effects on the microbiological quality of the reclaimed water, especially with regard to resistant human pathogenic protozoa - "best practices".

4 Risk management options, recommended measures

4.1 Limiting the use of reclaimed wastewater for irrigation

Based on the result of the risk characterisation (chapter 3.1.4), it is generally recommended to use only irrigation water with a quality comparable to drinking water for plants intended for raw consumption. It is assumed that irrigation water of drinking water quality has the lowest contamination with regard to the protozoa described here. The WHO Guidance on Potable Reuse recommends a reduction of 8.5 $\log_{10}$ levels of enteric protozoa (*Cryptosporidium)* as a default performance target for safe treatment of wastewater for use as drinking water (WHO, 2017). Even if this is not a direct benchmark for the concrete validation of wastewater treatment plants, this WHO recommendation nevertheless indicates that very high requirements are necessary to reduce human pathogenic protozoa in the reclaimed wastewater as far as possible.

In the case of plants that are not consumed raw, adverse health effects are not to be expected from irrigation with reclaimed wastewater, as long as it can be ensured that sufficient heating of the food takes place before consumption.

Plants where the edible part is not exposed to direct irrigation sometimes occupy a special position. An example of this is the irrigation of fruit trees. When these plants are irrigated with reclaimed wastewater, health hazards are not to be expected if it can be ensured that, for example, bearing fruit as the edible part does not come into contact with the irrigation water. In this case, however, fallen fruit would then no longer be suitable for raw consumption. In addition, it must be mentioned that the pathogens can also enter the foodstuffs to be irrigated from the environment. An additional entry via the irrigation water thus increases the risk for consumers to become infected with these pathogens and should therefore be avoided.

4.2 Testing for protozoa in the validation of wastewater treatment processes

Overall, the regulations and parameters specified in Regulation (EU) 2020/741 for the validation of new and existing reclamation facilities are not considered sufficient to ensure the safe removal of human pathogenic protozoa from wastewater in every case. This is because the parameters listed in Annex 1, Section 2, Table 4 of Regulation (EU) 2020/741 (*Clostridium perfringens* spores/spore-forming sulphate-reducing bacteria) for the validation of a new reclamation facility must be viewed critically with regard to their suitability as indicator germs.

The indicators described here are not protozoa, but bacterial spores. Although a high stability of the spores can be assumed, a congruence of the reduction evaluations with the specified log reductions is questionable, especially because very low infectious doses for the protozoa considered here can lead to illness, as already shown in Chapter 3.1.1. In addition, the absence of this indicator germ, as described in the regulation, does not automatically mean an actual absence of protozoa, according to the BfR. In the opinion of the BfR, it would therefore be advisable to pursue the alternative formulated in the Regulation (EU) 2020/741 of also using cryptosporidia as a reference pathogen for protozoa and, if possible, to develop a suitable detection method for this.

Further information on the BfR website on the topic:

BfR Opinion No 021/2020 "Reclaimed waste water: preventing bacterial pathogens on fresh fruit and vegetables":

https://www.bfr.bund.de/cm/349/reclaimed-waste-water-preventing-bacterial-pathogens-on-fresh-fruit-and-vegetables.pdf

BfR Opinion No 019/2022 "Reclaimed wastewater: preventing viral pathogens on plant foods":

https://www.bfr.bund.de/cm/349/reclaimed-wastewater-preventing-viral-pathogens-on-plant-foods.pdf

5 References

Aberg, R., Sjoman, M., Hemminki, K., Pirnes, A., Rasanen, S., Kalanti, A., Pohjanvirta, T., Caccio, S.M., Pihlajasaari, A., Toikkanen, S., Huusko, S., and Rimhanen-Finne, R.

(2015). Cryptosporidium parvum Caused a Large Outbreak Linked to Frisee Salad in Finland, 2012. *Zoonoses Public Health* 62**,** 618-624.

Adam, E.A., Yoder, J.S., Gould, L.H., Hlavsa, M.C., and Gargano, J.W. (2016). Giardiasis outbreaks in the United States, 1971-2011. *Epidemiol Infect* 144**,** 2790-2801.

Adam, R.D. (2001). Biology of Giardia lamblia. *Clin Microbiol Rev* 14**,** 447-475.

Adeyemo, F.E., Singh, G., Reddy, P., Bux, F., and Stenstrom, T.A. (2019). Efficiency of chlorine and UV in the inactivation of Cryptosporidium and Giardia in wastewater. *PLoS One* 14**,** e0216040.

Ajonina, C., Buzie, C., Ajonina, I.U., Basner, A., Reinhardt, H., Gulyas, H., Liebau, E., and Otterpohl, R. (2012). Occurrence of Cryptosporidium in a wastewater treatment plant in North Germany. *J Toxicol Environ Health A* 75**,** 1351-1358.

Ajonina, C., Buzie, C., Möller, J., and Otterpohl, R. (2018). The detection of Entamoeba histolytica and Toxoplasma gondii in wastewater. *Journal of Toxicology and Environmental Health, Part A* 81**,** 1-5.

Alemu, G., Mama, M., Misker, D., and Haftu, D. (2019). Parasitic contamination of vegetables marketed in Arba Minch town, southern Ethiopia. *BMC Infect Dis* 19**,** 410.

Amoros, I., Alonso, J.L., and Cuesta, G. (2010). Cryptosporidium oocysts and giardia cysts on salad products irrigated with contaminated water. *J Food Prot* 73**,** 1138-1140.

Bahia-Oliveira, L., Gomez-Marin, J., and Shapiro, K. (2017). "Toxoplasma gondii," in *Water and Sanitation for the 21st Century: Health and Microbiological Aspects of Excreta and Wastewater Management (Global Water Pathogen Project). (R. Fayer and W. Jakubowski (eds), Part 3: Specific Excreted Pathogens: Environmental and Epidemiology Aspects - Section 3: Protists),* eds. J.B. Rose & B. Jimenez-Cisneros. (Michigan State University: E. Lansing, MI, UNESCO).

Balasundaram, M.B., Andavar, R., Palaniswamy, M., and Venkatapathy, N. (2010). Outbreak of acquired ocular toxoplasmosis involving 248 patients. *Arch Ophthalmol* 128**,** 28-32.

Bechlars, S., Bier, N., Strauch, E., and Dieckmann, R. (2014). "Analysis of potential virulence factors of Vibrio parahaemolyticus isolated from German coastal waters", in: *Conference VIBRIO 2014.* (Edinburgh, UK Abstract book).

Benenson, M.W., Takafuji, E.T., Lemon, S.M., Greenup, R.L., and Sulzer, A.J. (1982). Oocyst-transmitted toxoplasmosis associated with ingestion of contaminated water. *N Engl J Med* 307**,** 666-669.

BfR (2018). "Wildfleisch: Gesundheitliche Bewertung von humanpathogenen Parasiten".).

BfR (2020). "Aufbereitete Abwässer: Bakterielle Krankheitserreger auf frischem Obst und Gemüse vermeiden".).

Bouzid, M., Hunter, P.R., Chalmers, R.M., and Tyler, K.M. (2013). Cryptosporidium pathogenicity and virulence. *Clin Microbiol Rev* 26**,** 115-134.

Bowie, W.R., King, A.S., Werker, D.H., Isaac-Renton, J.L., Bell, A., Eng, S.B., and Marion, S.A. (1997). Outbreak of toxoplasmosis associated with municipal drinking water. The BC Toxoplasma Investigation Team. *Lancet* 350, 173-177.

Budu-Amoako, E., Greenwood, S.J., Dixon, B.R., Barkema, H.W., and Mcclure, J.T. (2011). Foodborne illness associated with Cryptosporidium and Giardia from livestock. *J Food Prot* 74, 1944-1955.

Buret, A.G., Caccio, S.M., Favennec, L., and Svard, S. (2020). Update on Giardia: Highlights from the seventh International Giardia and Cryptosporidium Conference. *Parasite* 27, 49.

Burnett, M.W. (2018). Giardiasis. *J Spec Oper Med* 18, 106-107.

Caccio, S.M. (2004). [New methods for the diagnosis of Cryptosporidium and Giardia]. *Parassitologia* 46, 151-155.

Caccio, S.M., and Chalmers, R.M. (2016). Human cryptosporidiosis in Europe. *Clin Microbiol Infect* 22, 471-480.

Caccio, S.M., De Giacomo, M., Aulicino, F.A., and Pozio, E. (2003). Giardia cysts in wastewater treatment plants in Italy. *Appl Environ Microbiol* 69, 3393-3398.

Caradonna, T., Marangi, M., Del Chierico, F., Ferrari, N., Reddel, S., Bracaglia, G., Normanno, G., Putignani, L., and Giangaspero, A. (2017). Detection and prevalence of protozoan parasites in ready-to-eat packaged salads on sale in Italy. *Food Microbiol* 67, 67-75.

Carter, B.L., Chalmers, R.M., and Davies, A.P. (2020). Health sequelae of human cryptosporidiosis in industrialised countries: a systematic review. *Parasit Vectors* 13, 443.

Carter, E.R., Nabarro, L.E., Hedley, L., and Chiodini, P.L. (2018). Nitroimidazole-refractory giardiasis: a growing problem requiring rational solutions. *Clin Microbiol Infect* 24, 37-42.

Certad, G., Viscogliosi, E., Chabe, M., and Caccio, S.M. (2017). Pathogenic Mechanisms of Cryptosporidium and Giardia. *Trends Parasitol* 33, 561-576.

Chalmers, R.M., Robertson, L.J., Dorny, P., Jordan, S., Kärssin, A., Katzer, F., La Carbona, S., Lalle, M., Lassen, B., Mladineo, I., Rozycki, M., Bilska-Zajac, E., Schares, G., Mayer-Scholl, A., Trevisan, C., Tysnes, K., Vasilev, S., and Klotz, C. (2020). Parasite detection in food: Current status and future needs for validation. *Trends in Food Science & Technology* 99, 337-350.

Connell, K., Clancy, J., Reglo, S., Messner, M., Rodgers, C., Fricker, C., and Telliard, W.A. (2001). Discussion of: ‘Evaluation of USEPA Method 1622 for Detection of Cryptosporidium Oocysts in Stream Waters’. *American Water Works Association* 93, 106–109.

Davies, A.P., and Chalmers, R.M. (2009). Cryptosporidiosis. *BMJ* 339, b4168.

Dawson, D. (2005). Foodborne protozoan parasites. *Int J Food Microbiol* 103, 207-227.

De Moura, L., Bahia-Oliveira, L.M., Wada, M.Y., Jones, J.L., Tuboi, S.H., Carmo, E.H., Ramalho, W.M., Camargo, N.J., Trevisan, R., Graça, R.M., Da Silva, A.J., Moura, I., Dubey, J.P., and Garrett, D.O. (2006). Waterborne toxoplasmosis, Brazil, from field to gene. *Emerg Infect Dis* 12**,** 326-329.

Dixon, B.R. (2021). Giardia duodenalis in humans and animals - Transmission and disease. *Res Vet Sci* 135**,** 283-289.

Doganci, L., Tanyuksel, M., Araz, E.R., Besirbellioglu, B.A., Erdem, U., Ozoguz, C.A., Yucel, N., and Ciftcioglu, A. (2006). A probable outbreak of toxoplasmosis among boarding school students in Turkey. *Clin Microbiol Infect* 12**,** 672-674.

Dubey, J.P. (1998). Toxoplasma gondii oocyst survival under defined temperatures. *J Parasitol* 84**,** 862-865.

Dubey, J.P. (2021). Outbreaks of clinical toxoplasmosis in humans: five decades of personal experience, perspectives and lessons learned. *Parasites & Vectors* 14**,** 263.

Dubey, J.P., and Frenkel, J.K. (1972). Cyst-induced toxoplasmosis in cats. *J Protozool* 19**,** 155-177.

Dubey, J.P., Lindsay, D.S., and Speer, C.A. (1998). Structures of Toxoplasma gondii Tachyzoites, Bradyzoites, and Sporozoites and Biology and Development of Tissue Cysts. *Clinical Microbiology Reviews* 11**,** 267-299.

Dubey, J.P., Lunney, J.K., Shen, S.K., Kwok, O.C., Ashford, D.A., and Thulliez, P. (1996). Infectivity of low numbers of *Toxoplasma gondii* oocysts to pigs. *J. Parasitol.* 82**,** 438-443.

Dubey, J.P., Miller, N.L., and Frenkel, J.K. (1970). The Toxoplasma gondii oocyst from cat feces. *The Journal of experimental medicine* 132**,** 636-662.

Dumètre, A., and Dardé, M.-L. (2003). How to detect Toxoplasma gondii oocysts in environmental samples? *FEMS Microbiology Reviews* 27**,** 651-661.

Dumetre, A., Le Bras, C., Baffet, M., Meneceur, P., Dubey, J.P., Derouin, F., Duguet, J.P., Joyeux, M., and Moulin, L. (2008). Effects of ozone and ultraviolet radiation treatments on the infectivity of Toxoplasma gondii oocysts. *Vet Parasitol* 153**,** 209-213.

Dunn, D., Wallon, M., Peyron, F., Petersen, E., Peckham, C., and Gilbert, R. (1999). Mother-to-child transmission of toxoplasmosis: risk estimates for clinical counselling. *The Lancet* 353**,** 1829-1833.

DVG (2021). *Deutsche Veterinärmedizinische Gesellschaft : DVG gelistete Desinfektionsmittel* [Online]. Available: https://www.desinfektion-dvg.de/index.php?id=2150 [Accessed November 2021].

ECDC (2019). "European Centre for Disease Prevention and Control: Cryptosporidiosis - Annual Epidemiological Report for 2017", in: *Annual Epidemiological Report on Communicable Diseases in Europe.* (Stockholm: ECDC).

EFSA (2007). Scientific Opinion of the Panel on Biological Hazards on a request from EFSA on Surveillance and monitoring of Toxoplasma in humans, foods and animals. *The EFSA Journal* 583**,** 1-64.

Ekman, C.C., Chiossi, M.F., Meireles, L.R., Andrade Junior, H.F., Figueiredo, W.M., Marciano, M.A., and Luna, E.J. (2012). Case-control study of an outbreak of acute toxoplasmosis in an industrial plant in the state of Sao Paulo, Brazil. *Rev Inst Med Trop Sao Paulo* 54**,** 239-244.

ErdogˇRul, Ö., and Şener, H. (2005). The contamination of various fruit and vegetable with Enterobius vermicularis, Ascaris eggs, Entamoeba histolyca cysts and Giardia cysts. *Food Control* 16**,** 557-560.

Erickson, M.C., and Ortega, Y.R. (2006). Inactivation of protozoan parasites in food, water, and environmental systems. *J Food Prot* 69**,** 2786-2808.

Escobedo, A.A., Lalle, M., Hrastnik, N.I., Rodríguez-Morales, A.J., Castro-Sánchez, E., Cimerman, S., Almirall, P., and Jones, J. (2016). Combination therapy in the management of giardiasis: What laboratory and clinical studies tell us, so far. *Acta Trop* 162**,** 196-205.

Feng, Y., and Xiao, L. (2011a). Zoonotic potential and molecular epidemiology of Giardia species and giardiasis. *Clin Microbiol Rev* 24**,** 110-140.

Feng, Y., and Xiao, L. (2011b). Zoonotic Potential and Molecular Epidemiology of Giardia Species and Giardiasis. *Clinical Microbiology Reviews* 24**,** 110-140.

Frenkel, J.K., Ruiz, A., and Chinchilla, M. (1975). Soil survival of toxoplasma oocysts in Kansas and Costa Rica. *Am J Trop Med Hyg* 24**,** 439-443.

Galal, L., Hamidović, A., Dardé, M.L., and Mercier, M. (2019). Diversity of *Toxoplasma gondii* strains at the global level and its determinants. *Food and Waterborne Parasitology* 15**,** e00052.

Gallas-Lindemann, C., Sotiriadou, I., Mahmoodi, M.R., and Karanis, P. (2013a). Detection of Toxoplasma gondii oocysts in different water resources by Loop Mediated Isothermal Amplification (LAMP). *Acta Trop* 125**,** 231-236.

Gallas-Lindemann, C., Sotiriadou, I., Plutzer, J., and Karanis, P. (2013b). Prevalence and distribution of Cryptosporidium and Giardia in wastewater and the surface, drinking and ground waters in the Lower Rhine, Germany. *Epidemiol Infect* 141**,** 9-21.

Gertler, M., Durr, M., Renner, P., Poppert, S., Askar, M., Breidenbach, J., Frank, C., Preussel, K., Schielke, A., Werber, D., Chalmers, R., Robinson, G., Feuerpfeil, I., Tannich, E., Groger, C., Stark, K., and Wilking, H. (2015). Outbreak of Cryptosporidium hominis following river flooding in the city of Halle (Saale), Germany, August 2013. *BMC Infect Dis* 15**,** 88.

Guggisberg, A.R., Alvarez Rojas, C.A., Kronenberg, P.A., Miranda, N., and Deplazes, P. (2020). A Sensitive, One-Way Sequential Sieving Method to Isolate Helminths' Eggs and Protozoal Oocysts from Lettuce for Genetic Identification. *Pathogens* 9.

Guzman-Herrador, B., Carlander, A., Ethelberg, S., Freiesleben De Blasio, B., Kuusi, M., Lund, V., Löfdahl, M., Macdonald, E., Nichols, G., Schönning, C., Sudre, B., Trönnberg, L., Vold, L., Semenza, J.C., and Nygård, K. (2015). Waterborne outbreaks in the Nordic countries, 1998 to 2012. *Euro Surveill* 20.

Halliez, M.C., and Buret, A.G. (2013). Extra-intestinal and long term consequences of Giardia duodenalis infections. *World J Gastroenterol* 19**,** 8974-8985.

Hamilton, K.A., Waso, M., Reyneke, B., Saeidi, N., Levine, A., Lalancette, C., Besner, M.C., Khan, W., and Ahmed, W. (2018). Cryptosporidium and Giardia in Wastewater and Surface Water Environments. *J Environ Qual* 47**,** 1006-1023.

Heyworth, M.F. (2016). Giardia duodenalis genetic assemblages and hosts. *Parasite* 23**,** 13.

Hill, D., and Dubey, J.P. (2002). *Toxoplasma gondii*: transmission, diagnosis and prevention. *Clinical Microbiology and Infection* 8**,** 634-640.

Howe, D.K., and Sibley, L.D. (1995). Toxoplasma gondii comprises three clonal lineages: correlation of parasite genotype with human disease. *J Infect Dis* 172**,** 1561-1566.

Jones, J.L., and Dubey, J.P. (2010). Waterborne toxoplasmosis – Recent developments. *Experimental Parasitology* 124**,** 10-25.

Kafetzis, D.A., Maltezou, H.C., Zafeiropoulou, A., Attilakos, A., Stavrinadis, C., and Foustoukou, M. (2001). Epidemiology, clinical course and impact on hospitalization costs of acute diarrhea among hospitalized children in Athens, Greece. *Scand J Infect Dis* 33**,** 681-685.

Karanis, P., Schoenen, D., and Seitz, H.M. (1998). Distribution and removal of Giardia and Cryptosporidium in water supplies in Germany. *Water Treatment-Microorganisms and Particles* 37**,** 9-18.

Kim, M., Shapiro, K., Rajal, V.B., Packham, A., Aguilar, B., Rueda, L., and Wuertz, S. (2021). Quantification of viable protozoan parasites on leafy greens using molecular methods. *Food Microbiol* 99**,** 103816.

Kitowska, W., Milczarek, M., and Sadkowska-Todys, M. (2019). Giardiasis (lambliasis) in Poland in 2017. *Przegl Epidemiol* 73**,** 499-509.

Kohli, A., Bushen, O.Y., Pinkerton, R.C., Houpt, E., Newman, R.D., Sears, C.L., Lima, A.A., and Guerrant, R.L. (2008). Giardia duodenalis assemblage, clinical presentation and markers of intestinal inflammation in Brazilian children. *Trans R Soc Trop Med Hyg* 102**,** 718-725.

Kucik, C.J., Martin, G.L., and Sortor, B.V. (2004). Common intestinal parasites. *Am Fam Physician* 69**,** 1161-1168.

Lass, A., Pietkiewicz, H., Szostakowska, B., and Myjak, P. (2012). The first detection of Toxoplasma gondii DNA in environmental fruits and vegetables samples. *Eur J Clin Microbiol Infect Dis* 31**,** 1101-1108.

Lebwohl, B., Deckelbaum, R.J., and Green, P.H. (2003). Giardiasis. *Gastrointest Endosc* 57, 906-913.

Leung, A.K.C., Leung, A.a.M., Wong, A.H.C., Sergi, C.M., and Kam, J.K.M. (2019). Giardiasis: An Overview. *Recent Pat Inflamm Allergy Drug Discov* 13, 134-143.

Li, J., Wang, Z., Karim, M.R., and Zhang, L. (2020). Detection of human intestinal protozoan parasites in vegetables and fruits: a review. *Parasit Vectors* 13, 380.

Li, X.L., Wei, H.X., Zhang, H., Peng, H.J., and Lindsay, D.S. (2014). A meta analysis on risks of adverse pregnancy outcomes in Toxoplasma gondii infection. *PLoS One* 9, e97775.

Logalbo, P.R., Sampson, H.A., and Buckley, R.H. (1982). Symptomatic giardiasis in three patients with X-linked agammaglobulinemia. *J Pediatr* 101, 78-80.

Lopez-Romero, G., Quintero, J., Astiazarán-García, H., and Velazquez, C. (2015). Host defences against Giardia lamblia. *Parasite Immunol* 37, 394-406.

López Ureña, N. M., Chaudhry, U., Calero Bernal, R., Cano Alsua, S., Messina, D., Evangelista, F., Betson, M., Lalle, M., Jokelainen, P., Ortega Mora, L. M., Álvarez García, G. (2022). Contamination of Soil, Water, Fresh Produce, and Bivalve Mollusks with Toxoplasma gondii Oocysts: A Systematic Review. Microorganisms 10, 1-38.

Marangi, M., Giangaspero, A., Lacasella, V., Lonigro, A., and Gasser, R.B. (2015). Multiplex PCR for the detection and quantification of zoonotic taxa of Giardia, Cryptosporidium and Toxoplasma in wastewater and mussels. *Mol Cell Probes* 29, 122-125.

Marques, C.S., Sousa, S., Castro, A., and Da Costa, J.M.C. (2020). Detection of Toxoplasma gondii oocysts in fresh vegetables and berry fruits. *Parasites & Vectors* 13, 180.

Mclauchlin, J., Amar, C., Pedraza-Díaz, S., and Nichols, G.L. (2000). Molecular epidemiological analysis of Cryptosporidium spp. in the United Kingdom: results of genotyping Cryptosporidium spp. in 1,705 fecal samples from humans and 105 fecal samples from livestock animals. *J Clin Microbiol* 38, 3984-3990.

Meireles, L.R., Ekman, C.C.J., De Andrade, H.F., and Luna, E.J.D.A. (2015). Human toxoplasmosis outbreaks and the agent infecting form. Findings from a systematic review. *Revista do Instituto de Medicina Tropical de São Paulo* 57, 369-376.

Morais, R.D.a.P.B., Freire, A.B.C., Barbosa, D.R.L., Silva, L.D.C.T.D., Pinheiro, A.F., Costa, S.S.D., Ramos, F.L.D.P., Bichara, C.N.C., Lima, L.J.B., Silva, A.V.D., Souza, S.R.P.D., Piqueira Neto, L.P., Gonçalves, N.V., Póvoa, M.M., and Carmo, E.L.D. (2016). Surto de toxoplasmose aguda no Município de Ponta de Pedras, Arquipélago do Marajó, Estado do Pará, Brasil: características clínicas, laboratoriais e epidemiológicas. *Revista Pan-Amazônica de Saúde* 7, 143-152.

Moulin, L., Richard, F., Stefania, S., Goulet, M., Gosselin, S., Gonçalves, A., Rocher, V., Paffoni, C., and Dumètre, A. (2010). Contribution of treated wastewater to the microbiological quality of Seine River in Paris. *Water Res* 44, 5222-5231.

Mylonas, I., Groß, U., Hlobil, H., Friese, K., and Wintergerst, U. (2013). "Toxoplasmose," in *Infektionskrankheiten der Schwangeren und des Neugeborenen,* eds. K. Friese, I. Mylonas & A. Schulze. Springer Verlag).

Naz, A., Nawaz, Z., Rasool, M.H., and Zahoor, M.A. (2018). Cross-sectional epidemiological investigations of Giardia lamblia in children in Pakistan. *Sao Paulo Med J* 136**,** 449-453.

Nygard, K., Schimmer, B., Sobstad, O., Walde, A., Tveit, I., Langeland, N., Hausken, T., and Aavitsland, P. (2006). A large community outbreak of waterborne giardiasis-delayed detection in a non-endemic urban area. *BMC Public Health* 6**,** 141.

O'leary, J.K., Blake, L., Corcoran, G.D., Sleator, R.D., and Lucey, B. (2021). Development of a novel, high resolution melting analysis based genotyping method for Cryptosporidium parvum. *Eur J Protistol* 79**,** 125799.

Pickering, L.K., Woodward, W.E., Dupont, H.L., and Sullivan, P. (1984). Occurrence of Giardia lamblia in children in day care centers. *J Pediatr* 104**,** 522-526.

Pietrzak, A., Chodorowska, G., Urban, J., Bogucka, V., and Dybiec, E. (2005). Cutaneous manifestation of giardiasis - case report. *Ann Agric Environ Med* 12**,** 299-303.

Pinto-Ferreira, F., Caldart, E.T., Pasquali, A.K.S., Mitsuka-Breganó, R., Freire, R.L., and Navarro, I.T. (2019). Patterns of Transmission and Sources of Infection in Outbreaks of Human Toxoplasmosis. *Emerg Infect Dis* 25**,** 2177-2182.

Plutzer, J., Ongerth, J., and Karanis, P. (2010). Giardia taxonomy, phylogeny and epidemiology: Facts and open questions. *International Journal of Hygiene and Environmental Health* 213**,** 321-333.

Porter, J.D., Gaffney, C., Heymann, D., and Parkin, W. (1990). Food-borne outbreak of Giardia lamblia. *Am J Public Health* 80**,** 1259-1260.

Razzolini, M.T.P., Breternitz, B.S., Kuchkarian, B., and Bastos, V.K. (2020). Cryptosporidium and Giardia in urban wastewater: A challenge to overcome. *Environ Pollut* 257**,** 113545.

Rendtorff, R.C. (1954). The experimental transmission of human intestinal protozoan parasites. II. Giardia lamblia cysts given in capsules. *Am J Hyg* 59**,** 209-220.

Resi, D., Varani, S., Sannella, A.R., De Pascali, A.M., Ortalli, M., Liguori, G., Benvenuti, M., Re, M.C., Pirani, R., Prete, L., Mazzetti, C., Musti, M., Pizzi, L., Sanna, T., and Caccio, S.M. (2021). A large outbreak of giardiasis in a municipality of the Bologna province, north-eastern Italy, November 2018 to April 2019. *Euro Surveill* 26.

RKI (2004). Robert Koch Institut: Kryptosporidiose - RKI-Ratgeber *Epidemiologisches Bulletin* 34**,** 279-283.

RKI (2018). Robert Koch-Institut: RKI-Ratgeber Toxoplasmose. *Epidemiologischen Bulletin* 42**,** 451-457.

RKI (2020). Robert Koch Institut: Aktuelle Daten und Informationen zu Infektionskrankheiten und Public Health - Epidemiologisches Bulletin. *Epidemiologisches Bulletin* 50**,** 13-14.

Robert-Gangneux, F., and Darde, M.L. (2012). Epidemiology of and diagnostic strategies for toxoplasmosis. *Clinical Microbiology Reviews* 25**,** 264-296.

Robertson, L.J., and Gjerde, B. (2001). Occurrence of parasites on fruits and vegetables in Norway. *J Food Prot* 64**,** 1793-1798.

Rumsey, P., and Waseem, M. (2021). "Giardia Lamblia Enteritis," in *StatPearls*. (Treasure Island (FL): StatPearls Publishing

Copyright © 2021, StatPearls Publishing LLC.).

Ryan, U., Hijjawi, N., Feng, Y., and Xiao, L. (2019). Giardia: an under-reported foodborne parasite. *Int J Parasitol* 49**,** 1-11.

Rzezutka, A., Nichols, R.A., Connelly, L., Kaupke, A., Kozyra, I., Cook, N., Birrell, S., and Smith, H.V. (2010). Cryptosporidium oocysts on fresh produce from areas of high livestock production in Poland. *Int J Food Microbiol* 139**,** 96-101.

Sakkas, H., Economou, V., Bozidis, P., Gousia, P., Papadopoulou, C., and Karanis, P. (2020). Detection of Cryptosporidium and Giardia in foods of plant origin in North-Western Greece. *J Water Health* 18**,** 574-578.

Schlüter, D., Daubener, W., Schares, G., Gross, U., Pleyer, U., and Luder, C. (2014). Animals are key to human toxoplasmosis. *Int J Med Microbiol* 304**,** 917-929.

Shapiro, K., Bahia-Oliveira, L., Dixon, B., Dumètre, A., De Wit, L.A., Vanwormer, E., and Villena, I. (2019). Environmental transmission of Toxoplasma gondii: Oocysts in water, soil and food. *Food and Waterborne Parasitology* 15**,** e00049.

Slana, I., Bier, N., Bartosova, B., Marucci, G., Possenti, A., Mayer-Scholl, A., Jokelainen, P., and Lalle, M. (2021). Molecular Methods for the Detection of Toxoplasma gondii Oocysts in Fresh Produce: An Extensive Review. *Microorganisms* 9**,** 167.

Slany, M., Dziedzinska, R., Babak, V., Kralik, P., Moravkova, M., and Slana, I. (2019). Toxoplasma gondii in vegetables from fields and farm storage facilities in the Czech Republic. *FEMS Microbiol Lett* 366.

Sotiriadou, I., and Karanis, P. (2008). Evaluation of loop-mediated isothermal amplification for detection of Toxoplasma gondii in water samples and comparative findings by polymerase chain reaction and immunofluorescence test (IFT). *Diagn Microbiol Infect Dis* 62**,** 357-365.

Tenter, A.M., and Fehlhaber, K. (2002). Toxoplasmose: Eine lebensmittelübertragene Parasitose. *Bundesgesundheitsblatt - Gesundheitsforschung - Gesundheitsschutz* 45**,** 549-555.

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

Travaillé, E., La Carbona, S., Gargala, G., Aubert, D., Guyot, K., Dumètre, A., Villena, I., and Houssin, M. (2016). Development of a qRT-PCR method to assess the viability of Giardia intestinalis cysts, Cryptosporidium spp. and Toxoplasma gondii oocysts. *Food Control* 59, 359-365.

UBA (2020). "Viren und Parasiten in Abwasser und Flüssen sowie Handlungsempfehlungen für Flüsse mit kurzzeitigen Verschmutzungen; Abschlussbericht Umweltbundesamt (FKZ 02WRM1364B)".).

Utaaker, K.S., Skjerve, E., and Robertson, L.J. (2017). Keeping it cool: Survival of Giardia cysts and Cryptosporidium oocysts on lettuce leaves. *Int J Food Microbiol* 255, 51-57.

Van Pelt, A.E., Quinones, B., Lofgren, H.L., Bartz, F.E., Newman, K.L., and Leon, J.S. (2018). Low Prevalence of Human Pathogens on Fresh Produce on Farms and in Packing Facilities: A Systematic Review. *Front Public Health* 6, 40.

Vanwormer, E., Fritz, H., Shapiro, K., Mazet, J.A., and Conrad, P.A. (2013). Molecules to modeling: Toxoplasma gondii oocysts at the human-animal-environment interface. *Comp Immunol Microbiol Infect Dis* 36, 217-231.

Wainwright, K.E., Lagunas-Solar, M., Miller, M.A., Barr, B.C., Gardner, I.A., Pina, C., Melli, A.C., Packham, A.E., Zeng, N., Truong, T., and Conrad, P.A. (2007a). Physical inactivation of Toxoplasma gondii oocysts in water. *Applied and environmental microbiology* 73, 5663-5666.

Wainwright, K.E., Miller, M.A., Barr, B.C., Gardner, I.A., Melli, A.C., Essert, T., Packham, A.E., Truong, T., Lagunas-Solar, M., and Conrad, P.A. (2007b). Chemical inactivation of Toxoplasma gondii oocysts in water. *Journal of Parasitology* 93, 925-931.

Ware, M.W., Augustine, S.a.J., Erisman, D.O., See, M.J., Wymer, L., Hayes, S.L., Dubey, J.P., and Villegas, E.N. (2010). Determining UV inactivation of Toxoplasma gondii oocysts by using cell culture and a mouse bioassay. *Applied and environmental microbiology* 76, 5140-5147.

WHO (2017). Potable reuse: guidance for producing safe drinking-water. ISBN 9789241512770.

WHO (2008). World Health Organization: Guidelines for Drinking-water Quality- Third Edition Incorporating the first and second addenda- Volume 1 Recommendations.

Wilking, H., Thamm, M., Stark, K., Aebischer, T., and Seeber, F. (2016). Prevalence, incidence estimations, and risk factors of *Toxoplasma gondii* infection in Germany: a representative, cross-sectional, serological study. *Scientific Reports* 6, 22551.

Zangerle, R., Allerberger, F., Pohl, P., Fritsch, P., and Dierich, M.P. (1991). High risk of developing toxoplasmic encephalitis in AIDS patients seropositive to Toxoplasma gondii. *Med Microbiol Immunol* 180, 59-66.

About the BfR

The German Federal Institute for Risk Assessment (BfR) is a scientifically independent institution in the portfolio of the Federal Ministry of Food and Agriculture (BMEL). It advises the Federal Government and the Federal States on issues of food, chemical and product safety. The BfR conducts its own research on topics closely related to its assessment tasks.