The Study of the Prevalence of Giardia in Various Water Bodies and its Impact on Wildlife Health

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Abstract

The flagellated protozoan *Giardia* is responsible for causing giardiasis. Numerous zoonotic and non-zoonotic *Giardia* species have been known to infect humans, mammals and animals, including non-human primates, rodents, horses, rabbits, bovines, marine life and wildlife. Water bodies are the primary means of *Giardia* transmission. Giardiasis has stages like vesicles and sac. It has been found to be widespread in many parts of the world, demonstrating its terrible effect on mutually human being and mammal health. Sequence analysis of many genes has directed to the description of eight genotypes in *G. duodenalis*. They are A to H assemblages. A phylogenetic tree constructed using sequences from individual loci shows a big cluster of several related subtypes that make up each assemblage. Giardia's ability to survive and spread is influenced by environmental conditions such as pH, humidity, and temperature. Chlorination and UV radiation use are two examples of control factors. Cysts of *Giardia* species are removed from waterbodies with the aid of oxidation factors etc.

Keywords: Giardia spp., Prevalence, Environmental factors, Control measures, Wildlife.

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Introduction

Common parasitic infection, giardiasis spread by the enteric flagellated diplomonad protozoan parasite *Giardia* (*Metamonada, Giardiidae*), which affects a variety of mammals, involving creatures and wildlife (Ayed et al., 2024). In humans and other animals, *Giardia* spp. are frequent intestinal protozoan parasites (Ayed et al., 2024). In *Giardia duodenalis* is shown in Figure 1. In developed regions, it is a main cause of foodborne, also waterborne enteric illness epidemics (Berrilli et al., 2023). Since 1954, there have been more than 300 documented giardiasis outbreaks worldwide, the majority of which have been linked to tainted water (Egan et al., 2024).

Trophozoites of *Giardia*

The pear-shaped trophozoites of *Giardia duodenalis* are 10–20 micrometers long. Two sizable nuclei are typically evident in permanent, pigmented specimens. Moreover, the middle masses, scourges and sucking discs (used for attaching to the host's mucosal epithelium) may be observed (Berrilli et al., 2023).

Cysts of *Giardia*

Cyst is the resistant form of giardiasis that causes disease to occur. Both trophozoites and cysts can be observed in the diagnosis period of feces. It is so persistent that they may live for various months inside icy water. Contaminated food and water, or the fecal-oral pathway can all result in cyst infection. In the tiny intestine, excystation emits trophozoites. Through longitudinal binary fission, trophozoites multiply and remain in the proximal small intestinal lumen, somewhere they may either stay released or adhere to the mucous membrane through a adaxial carrying disc. Ent encystation occurs as the organisms go towards the colon. Cysts is the stage that is extremely seen in non-diarrheal feces (Leonel et al., 2024).

Giardia Species

Giardiasis in humans is primarily caused by *Giardia duodenalis*, which is also known in literature as *G. intestinalis* or *G. lamblia* (Figure 2). This species has also been identified in numerous animal hosts. In mammals, for example, *G. muris* infects rodents, *G. microti* is present in

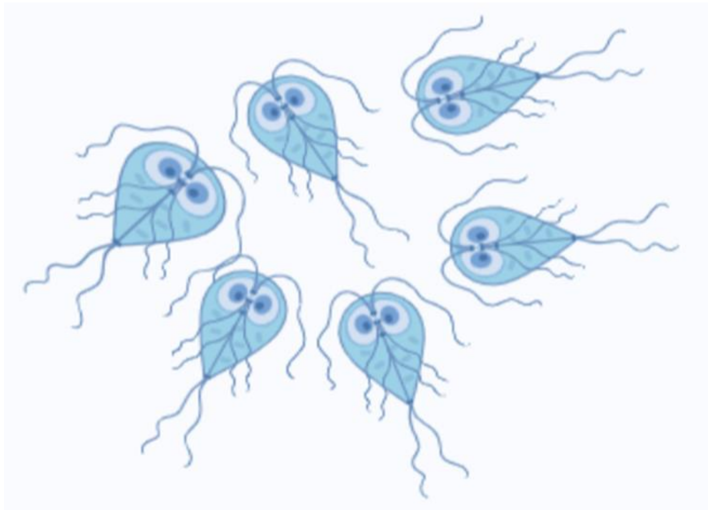

Figure 1: Multiple *Giardia* exhibiting their characteristics pear shape and flagella, representing the active motile stage in the host small intestine. (Generated by biorender)

muskrats, voles, deer and mice, *G. circetidarum* affects hamsters. Beyond mammals, *G. agilis* has been reported in frogs, *G. ardeae* in herons and various bird species and *G. psittaci* in birds like parakeets and budgerigars (Leonel et al., 2024). Among the *Giardia* species, *G. duodenalis* has served as the focus in the most genetic and biological research on this genus (Leonel et al., 2024).

Giardia duodenalis

According to the sequence analysis of various genes, eight genotypes of *G. duodenalis* have been identified. They are assemblages A to H (Elmahallawy et al., 2023). Each assembling consists numerous related subtypes that create a sizable cluster.

Due to their broadest host ranges between the 8 groups of *Giardia*, both 1 and 2 group are thought pathogenic (Zuo et al., 2023). The host ranges of assemblies C through H, which belong to different genotypes, are constrained. However, most reports for F, G and H group present in gnawers, seals, fields, individually (Wang et al., 2023). While ensemble E mainly infects footed animals such as livestock, sheep, pigs and goats, groups C and D are mainly seen in canine species (Šmit et al., 2023). However, infections caused by these host-adapted genotypes of *G. duodenalis* are an exception. Humans contain assemblages C, D, E and F, according to reports (Rahdar & Daylami, 2016). Apart from assemblage E, which has been documented in at least 57 individuals across multiple nations, in this lesser number of cases involved (Rahdar & Daylami, 2016). More research is required to determine these genotypes zoonotic potential (Rahdar & Daylami, 2016).

Mainly group E is usually a genotype of host-specific in footed animals, recent research must documented human cases in different regions of world South America (15 cases), Northern Africa (25 cases), Southeast Asia (7 cases), Oceania (7 cases) (Al-Rashidi & El-Wakil, 2024). Additionally, it was shown that one Egyptian isolate belonged to Group C (Soliman et al., 2011). Group D and F, which were previously discovered in a limited human test sample, in recent studies they have not been present. (Al- Rashidi & El-Wakil, 2024).

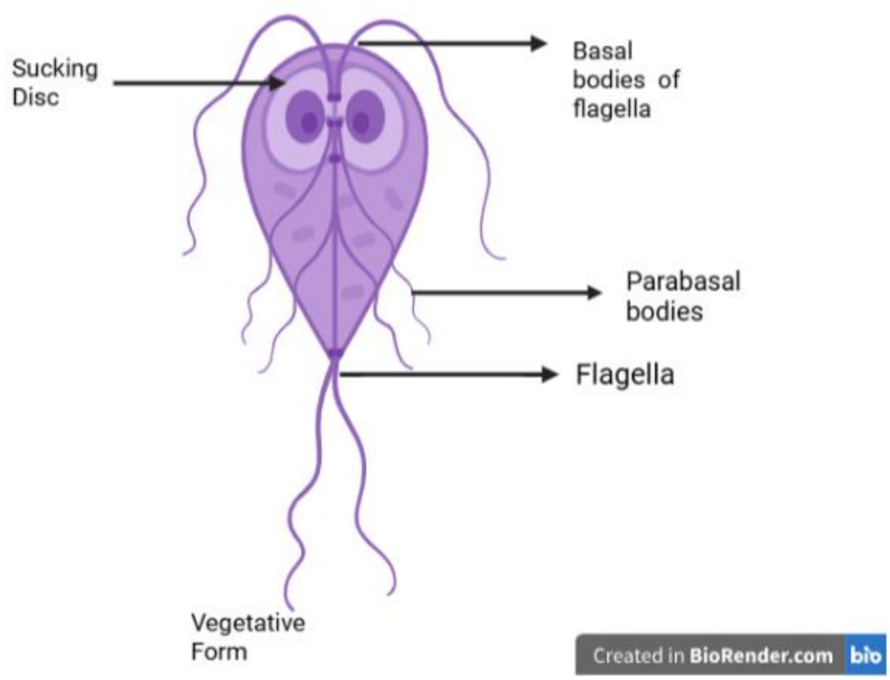

Fig. 2: Structure of *Giardia lamblia* (trophozoite form). The vegetative form of giardia, showing key structures of including sucking disc for intestinal attachment, basal bodies, flagella for motility and parabasal bodies.

In cows' prevalence of *Giardia*

G. duodenalis frequently hosts cattle. According to previous papers, the occurrence of it in cattle ranged from 1.1% to 74.2% (Santin et al. 2012).

Prevalence of goats and sheep

In recent research the infection rate ranges from 0.7 to 33.8%, which means that the occurrence of *Giardia* in lamb and goats is lesser in livestock. Compared to older animals, lambs and goat offspring tended to have greater rates of infection (Chhetri et al., 2019). According to another research, the rate of *Giardia* infection was higher in lambs with diarrhea than in those without (Chhetri et al., 2019).

Prevalence of *Giardia* in Pig

Pig infections have been reported frequently that are caused by *G. duodenalis*; infection range from 0.6 to 26.9%. According to most of the research, weaners and fattening pigs have a greater prevalence of *G. duodenalis* than piglets (Wang et al., 2023). The level of Fecal consistency and cyst excretion did not corelate in recent study (Xiao et al., 2017).

In Cats and Dogs *Giardia* Prevalence

Dogs frequently contract *G. duodenalis*; recent investigations have shown infection rate ranges from 1.9 to 57.9 %. Dogs with symptoms exhibited greater disease rates in recent two researches (Adam et al., 2021; Klotz et al., 2023). Hunting, breeding and sheltering dogs had considerably different rates of its infection, according to one research conducted in Spain (Šmit et al., 2023). Higher infection rates in dogs kept in shelter were noted in certain research (Jagai et al., 2009). The frequent usage of kennels, increased animal intensity and constant beginning of new animals may be the cause of this significant environmental contamination with cysts (Jerlström-Hultqvist et al., 2010).

***G. duodenalis* prevalence in Equines**

Equine *G. duodenalis* infection rates varied from 1.5% to 17.4%. According to reports, donkeys were more likely than horses to be infected (de Paula Baptista et al., 2024). Additionally, this analysis showed that infection rates were higher in donkeys aged 6-12 months and horses older than in other age groups. However, in one research of Turkey horses found no apparent changes in *G. duodenalis* infection rates by age or sex. Most horse samples that tested positive for *G. duodenalis* in recent research had assemblages A and B (de Paula Baptista et al., 2024).

***G. duodenalis* Prevalence in Rabbits**

According to published investigations, the occurrence of *Giardia* in bunnies ranged widely from 1.9 to 72.3%. Even though all rabbits in Nigeria were asymptomatic, the prevalence was high in those under 25 weeks, at 72.3% (Klotz et al., 2023). Infections with *G. duodenalis* in rabbits may be associated with age. According to a specific study, *G. duodenalis* infection was most likely present in juvenile rabbits. Breeds and living conditions had a greater impact on *G. duodenalis* infection rates in rabbits. According to two research studies, rabbits grown outdoors had infection rates that were noticeably greater than those raised indoors (Adam et al., 2001).

According to recent isolate characterization results, rabbits are dominated by zoonotic assemblage B. In rabbits, assemblage E was shown to be highly prevalent in just one study. One rabbit was found to have assemblage A in the same research (Adam et al., 2001).

***G. duodenalis* Prevalence in Rodents**

It is very common for rodents to have *G. duodenalis*. Infection rates ranged from 6.0% to 66.3% in recent research. The fact that Group B is the predominant genotype in rodents, these animals could serve as possible reservoirs for zoonotic *G. duodenalis*. All 52 bamboo rat samples positive test of G. duodenalis were found to have assemblage B in one Chinese investigation (Wang et al., 2014). In Chinchillas, Group B was identified as the predominant *G. duodenalis* in six recent studies. Beavers in Canada and China have been reported to contain Assemblage B (Wang et al., 2014). One Patagonian cavy and one Prevost's squirrel were found to have assemblage B in Croatia. However, some chipmunks were found to have Assemblage A (Franzen et al., 2009).

G. duodenalis and chinchillas appear in China. In a Belgian study, assemblage-specific PCR was used to detect assemblages A, C, and E in chinchillas, but Group B only detected bg gene by direct PCR analysis (Wang et al., 2014).

***G. duodenalis* Prevalence in Wildlife**

G. duodenalis infection can affect various wild animals, including predators, mammals and primates (NHPs). In a few recent research reported that wild animals in different regions have information about the occurrence and genetic identification of Giardia (Nader, 2017). *G. duodenalis* is prevalent in non-human primates, there infection ranges from 4.8 to 57.8%. It was found to be highly prevalent in both captive and farmed NHPs, according to several investigations (Adam et al., 2001). *G. duodenalis* may spread more easily among NHPs if a lot of vulnerable animals are housed in small areas (Nader, 2017). According to chinses study, *G. duodenalis* was most likely spread by water in public park among rhesus macaques (Thompson et al., 2012). It's unclear how *G. duodenalis* infection and NHP age relate to one another. Most *G. duodenalis* NHPs isolates were classified as belonging to Group B, while there were some assemblages A. Some NHPs in China, Thailand, Malaysia, Norway, Brazil and the Central African Republic contained latter. Assemblage A was predominant genotype in 62 orangutan cases in one Malaysian report. Furthermore, NHPs were the site of five assemblages E infection cases (Gonçalves et al., 2024).

Several additional subtypes of assemblages A at the subtype level have been discovered in NHPs, moreover the presence of A1, A2 and other prevalent subtypes at loci. Assemblage B's frequent occurrence indicates that the zoonotic potential of G. duodenalis from NHPs is high (Gonçalves et al., 2024).

Disease rates with Giardia in deer have been reported to range from 0.7% to 23.0%. The hazards and trends of *G. duodenalis* disease in deer are poorly understood. Musk deer less than one year were responsible for 60% of Giardia disease, study (Brynildsrud et al., 2018). Although some deer had assemblages B, C and E, Group A was the predominant *G. duodenalis* genotype in the published investigations. Sub-Groups AIII and AI comprise Group A isolates that were subtyped. The existence of compatible host subtypes suggests that have limited zoonotic potential of isolates from deer, even though assemblage A was commonly identified in deer (Brynildsrud et al., 2018).

Several isolates from wild predators have been genotyped in recent reports. In wild canids, group D seems to be the most prevalent assemblage. In total, one racoon dog and wolves and fox have been found to have it. Seven wolves have been observed to have Assemblage A. On the other hand, three wolves and one jackal have been observed to exhibit assemblage B. Only group A and B were found in a single rummage every in study conducted in Europe (Li et al., 2023). Although two group E infections were discovered in wild dogs, the SSU rRNA locus analysis was used to make the diagnosis. Only ten isolates of lions, servals, lynx, jaguars, cheetahs, leopards, Siberian tigers, and servals were genotyped among wild felids. Five genotypes of *G. duodenalis* have been detected in several isolates from wild mammals in recent investigations (Li et al., 2023).

In the initial report of *G. Duodenalis* the most infected animals have masked palm in group B (Li et al., 2023). In recent research, 18 cases of *Giardia* infection in wild raccoons of group B. The high frequency of assemblage B raises the possibility that raccoons and civets could serve as zoonotic *G. duodenalis* reservoirs. Epidemiological research is required to determine the occurrence and genetic identification of *G. duodenalis* in these different species of animal (Li et al., 2023).

Giardia **Prevalence in Aquatic Animals**

At least recent studies have documented the existence of *Giardia* in aquatic animals (Ligda, 2020). According to one study, zoo-dwelling captive sea lions had greater infection rate than wildlife (Ligda, 2020). The transmission of *G. duodenalis* in these captive animals may have increased due to the exposure to humans and unnatural environments. Limited genotype studies have shown that group A, B, C and F can infect marine creatures. Assessment of Small subunit ribosomal RNA position revealed that group B was prevalent genotypes in Australia marine mammal. In Spain, however, assemblages A was discovered in one whale and six dolphins (Nader, 2017). More molecular information based on widely utilized genetic loci (bg, tpi and gdh) is required to comprehend the division of *G. duodenalis* genetic constitution in oceanic genus. (Ogbuigwe et al., 2022).

Research on *Giardia duodenalis* infections in wild animals remains relatively scarce in Australia, there have been reports of eight instances involving assemblage B and one involving assemblage D in species such as quenda, Tasmanian devils and brush-tailed rock-wallabies (Thompson & Monis, 2012). A separate Australian study utilizing sequencing of the small subunit ribosomal RNA gene found assemblages A, B, C and D in kangaroos, with eight, two and four cases respectively (Thompson & Ash, 2019). Additionally, assemblages A, B and E have been identified in wild pigs in several countries. To gain a deeper understanding of giardia zoonotic ability and host specificity in wildlife, further detailed molecular investigations are required (Thompson & Ash, 2019).

G. duodenalis **Prevalence in birds**

Some characterization were based on information from the SSU rRNA or its locus, Group A and B were generally detected in a variety of birds across multiple nations (Egan et al., 2024b). Group A,B and the combination of A, B were detected in 18 samples of chickens in a study carried out in Cote d'Ivoire (Wielinga et al., 2023). However, assemblage E was present in all *Giardia*- positive pet birds in one Chinese research. In contrast, group Band E were found in 19 and 14 wild birds 19, individually, in a different investigation carried out in China. Geography and living conditions may have an impact on the spread of genotype of G. duodenalis in birds (Wielinga et al., 2023).

G. duodenalis **prevalence in Shellfish**

In shellfish, 4 groups have been found. Three Brazilian oysters and nineteen Italian mussels were found to contain Assemblage A. However, In New Zealand one green-lipped mussel and one blue mussel in Argentina were found to have assemblage B. Assemblies B, C and D were discovered within California two mollusks. Through the filter feeding system giardia syst can concentrate in shellfish, there is no evidence of infection occur in shellfish due to G. duodenalis (Theresa et al., 2021). Successful genotyping or the discovery of cysts in shellfish could only be the result of environmental contamination (Theresa et al., 2021).

According to these findings, if these filter-feeding mussels are consumed raw or undercooked, they may spread the disease to people. Two giardiasis outbreaks have been linked to eating shellfish thus far (Theresa et al., 2021).

Chain of Life of *Giardia*

The chain of life of *Giardia* consists of two primary stages including trophozoites and cysts as described in Figure 3. The host large intestine that has infection cyst particularly resistant to environmental deterioration and disinfectants. The infection continues to spread because many cysts are expelled with the feces of affected people. Most infections are self-limiting, and the primary clinical signs of giardiasis include diarrhea, bloating, malnourishment and weight loss. The great majority of infected people in endemic locations remain asymptomatic, even though symptomatic giardiasis sometimes necessitates treatment (Theresa et al., 2021).

G. duodenalis **Prevalence in Various water Bodies**

Because of its adverse impacts on infants and expecting females, while its associations with poverty, giardiasis has been a part of the WHO Neglected Disease Initiatives since September 2004. Due to inadequate water sources, inadequate sanitation and poor hygiene, the incidence of *Giardia* in nations with limited resources is thought to be between 20 and 30 percent. The prevalences in West Africa and sub-Saharan Africa are estimated to be 8.97% and 7.36 percent, respectively (Egan et al., 2024b).

Giardia **spp. Infection in water bodies of Africa**

Before the year 2000, researchers on the continent were not particularly interested in investigating the distribution and frequency of *Giardia spp.* in Africa. This may be related to the parasite's increased resistance to water treatment chemicals. Interest has been growing over the past 20 years. For most surrounding water bodies, the occurrence of *Giardia* infection as determined by molecular methods was higher than that determined by microscopy procedures. The prevalence varied by country, with Nigeria having the lowest prevalence at 15.4% and Tunisia having the highest at 37.27% (Egan et al., 2024b).

Study in India and Iran

Similar prevalence was found in rivers in India using both molecular (32.0%) and microscopic (31.3%) approaches. But there have also been observations regarding a high occurrence of this pathogen in Iranian municipal and residential wastewater via microscopy methods (100.0%) as opposed to molecular procedures (96.2%) (Nader, 2017). This was ascribed to the lack of proficiency in using microscopes to identify the parasite and the incapacity of molecular techniques to distinguish between the DNA of viable and non-viable Giardia parasites (Nader, 2017).

Animal Giardiasis Prevalence (Malaysia)

Recent investigations have revealed the simultaneous presence of Giardia across human populations, animal hosts and environmental sources. In rural areas of Selangor, Pahang and Perak, Giardia cyst was identified in river water samples at concentrations ranging from 0.1 to 5.97 cysts per liter. Parasites were also detected in 6.7% of the local human population and 4.7% of pet animals (Li et al., 2023). Data from animal studies reported cyst loads exceeding 504 cysts per gram in sheep, 6061 cysts/g in dogs, 7143 cysts/g in domestic cats and more than 16,667 cysts/g in pigs. Surveillance from cattle farms located around the Sungai Basin showed Giardia cysts in 14.6% of bovine fecal samples (n=96), with concentrations ranging from 75 to 1.3x 10^4cysts/g and 6.7% (n=45) of cow wastewater samples contained cysts at levels between 4 and 75 cysts/ml (Li et al., 2023).

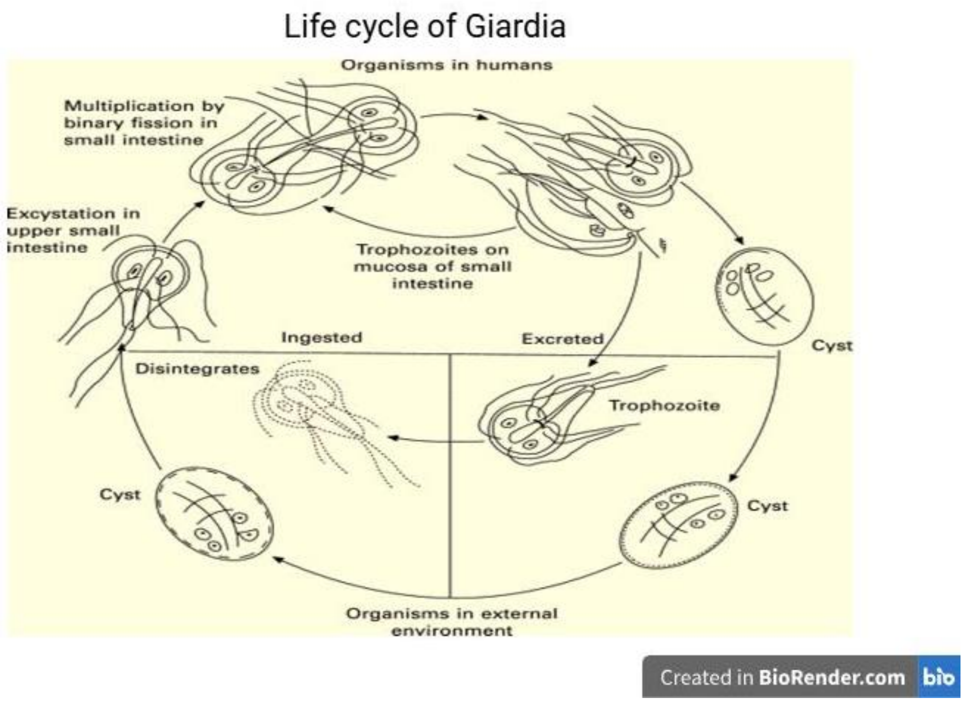

Fig. 3: This diagram illustrates the life cycle of Giardia lamblia showing cyst ingestion, excystation, trophozoite multiplication and excretion of cyst. Cysts survive in the environment and continue the infection cycle.

Furthermore, in Serdang, 14.6% of wild rats tested positive for *Giardia*, while 3% of urban rodents (n=134) were also carriers. In addition, a prevalence of 28.6% was recorded among domestic cats and wild rats in Orang communities, with cyst concentrations ranging from 10 to 59,640 cysts/g (Li et al., 2023). A regional review emphasized the widespread detection of waterborne protozoa, particularly *Giardia* and cryptosporidium, in diverse samples across several Asian nations, especially Malaysia (Li et al., 2023).

Connection between Climatic Factors and *Cryptosporidium*/*Giardia*

Temperature and *Giardia*: In New Zealand a slight positive correlation between average annual temperatures and giardia. Giardiasis cysts were adversely correlated with temperature (expect from winter) in Canada. *Giardia* cyst die-off was expedited by higher temperatures. *Giardia* and temperature are interrelated.

Humidity: while some researchers thought that the spread of cysts contributed to only dry or low humid conditions, the quantity of cysts grew in warm others discovered that humid environments. The findings of Wang et al. complemented the findings of Jagai et al., who showed that the association between humidity and cryptosporidium/Giardia was identical to that rainfall and that cysts could live more readily in humid environments. A weak negative correlation between Giardia and relative humidity (Jagai et al., 2009).

Connection between water Features and *Cryptosporidium/Giardia*

Though limited, the research supporting the impact of water features is instructive. Water discharge generally has a positive correlation with the density of (oo)cysts; however, if the flow is little, the concentration cannot be adequately dispersed (Franzen et al., 2009).

The concentration of *Giardia* cysts associated with dissolved oxygen, whereas the absorption of *Cryptosporidium* oocysts was associated with river flow, water hardness, and pH, and turbidity was involved with both. *Giardia* is significantly impacted by salinity; cysts in canal water survived longer (121.3 h, T99) than those in dark seawater (97.1 h, T99) (Franzen et al., 2009).

Control Measure

Cysts, the infectious form of the protozoan, escape from the stool of the host. Utilizing partially or completely treated wastewater in agriculture has been connected to Giardiasis. They are frequently detected in high conc. (up to 106 cysts L−1) in tests obtained from manure systems worldwide. Regulations and recommendations require a certain quality of wastewater for agricultural flooding and a disinfection step toward the conclusion of the process of wastewater treatment is advised as a preventative measure (Ligda, 2020).

The most widely used disinfection technique for sewage water treatment and the production of retrieved water is chlorination because it is inexpensive and easy to use. However, at the concentrations typically used, it is not very effective at inactivating *Giardia* cyst. For cyst-forming protozoa like Giardia to be successfully chemically inactivated, the cyst wall barrier must be removed (Li et al., 2023). To cause enough damage to shatter the wall, oxidant dosages and exposure durations must be used. After the wall is destroyed, the disinfectant can enter the interior of the cyst and reach the genetic material, which inactivates the cyst. These authors suggest that high chlorine dosages more effectively harm the cyst cytoplasm and destroy the wall. The cysts were injected into a buffer solution without any requirement for chlorine during the study. Depending on the wastewater's chlorine demand, the chlorine concentration needed for good outcomes is typically substantially greater and can surpass 20 mg L−1, USEPA 2011.

In the past ten years, advanced oxidation processes (AOPs) and disinfectants like UV radiation and peracetic acid have emerged as common alternatives for chlorination. It has been widely observed that UV light is effective at inactivating cysts.

Medium-pressure mercury lamps, which are commonly used in wastewater treatment plants (WWTPs). This affects cellular functions like transcription and DNA replication, which in turn causes microorganisms to become inactive. Because light emitting diodes (LEDs) are stronger and eco-friendly than mercury lamps, some researchers have recommended changing them. Additionally, LEDs have the benefit of using a variety of wavelengths and wider spectrum of action, which allows them to reach different cellular components in addition to nucleic acids. This enhances the disinfection process' efficacy, particularly when it comes to resistant organisms like *Giardia* cysts. Using advanced oxidation processes (AOPs) in additional choice. These procedures have the potential to produce high levels of hydroxyl radicals (OH). Most organic molecules can be totally oxidized by OH because of its highly oxidative and non-selective character. Additionally, germs like *Escherichia coli* can be rendered inactive by AOPs. Photolysis, the breakdown of hydrogen peroxide (H_2O_2) using ultraviolet (UV) light, is the most direct way to produce OH (Nader, 2017). In 2014 and 2015, Guimaraes et al. evaluated the effect of advanced oxidation process (AOPs), particularly the association of hydrogen peroxide and ultra-violet light, on cyst walls. AOPs impact on cysts in sewage water samples hasn't been assessed yet, though (Ligda, 2020).

Disinfecting agent's Correlations with Environmental Factors

The properties of wastewater also affect how effective this disinfectant is. The rate at which peracetic acid breaks down is affected by several factors, including pH, temperature, organic matter, solids and salinity, all of which reduce the disinfecting effect of peracetic acid. At pH values lower than 8.2, when its biocidal form, non-dissociated acid (CH_3COH_3H), predominates, peracetic acid has higher action. The ionized form (CH_3CO_3-), which has low capacity for disinfection, is predominant in alkaline circumstances (Franzen et al., 2009).

Peracetic acid performs well as a disinfectant due to the properties of secondary treatment wastewater, like the sewage water in this study, such as pH near neutrality and low levels of organic matter (Franzen et al., 2009).

The Efficiency of AOPs (UV-LED/H2O2)

The combination of 15 mg L−1 H_2O_2 and UV-LED (255/280/400nm) caused destruction to 78% of all cysts on average.

Conclusion

In accordance with the One Health concept, to learn more about how giardiasis spreads at the interaction between humans, animals, and the environment, further epidemiological research is necessary. We may conclude that the existence of agriculture/livestock, settlements and human activities like defecation are directly associated in all types of water samples the presence of *Giardia*. Furthermore, often in crude water and infrequently in treated water. Even though, according to environmental health statistics for rural areas in 2018, 95.89% of people living in rural areas have sanitary latrines and safe water supply, the occurrence of underweight, stunting and wasting in children from rural areas has increased significantly through years. The guidelines for the prevention of giardiasis must be followed to limit its spread in the communities.

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