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Research Article

Assemblage distribution and zoonotic potential of Giardia duodenalis in municipal wastewater and surface water sources

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ABSTRACT

Giardia duodenalis is a waterborne flagellated protozoan responsible for widespread gastrointestinal illness, comprising eight distinct genetic assemblages (A through H) with varying host specificities. In this study, we investigated the occurrence and assemblage distribution of G. duodenalis cysts in raw wastewater, treated effluents, and adjacent surface water bodies. A total of 160 water samples (10 liters each) were concentrated via membrane filtration and immunomagnetic separation (IMS), quantified using immunofluorescence microscopy (IFA), and genotyped via nested PCR targeting the triosephosphate isomerase (tpi) and beta-giardin (bg) genes. IFA detected Giardia cysts in 92.5 percent (37/40) of raw influent samples, 45.0 percent (18/40) of secondary treated effluents, and 31.3 percent (25/80) of downstream river water samples, with mean concentrations ranging from 14.2 to 1,850 cysts per liter. Nested PCR successfully amplified 68 of the 80 IFA-positive samples (85.0 percent). Sequence analysis revealed zoonotic Assemblage A in 39.7 percent (27/68), anthroponotic Assemblage B in 47.1 percent (32/68), and livestock-associated Assemblage E in 13.2 percent (9/68) of isolates. Conventional secondary wastewater treatment failed to completely eliminate viable G. duodenalis cysts, leading to surface water contamination with human-pathogenic assemblages A and B. Upgrading tertiary disinfection protocols and monitoring agricultural runoff are necessary to safeguard water quality.

Keywords: Giardia duodenalis, Genetic assemblages, Waterborne parasites, Wastewater treatment, Immunomagnetic separation

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1. Introduction

Giardia duodenalis (syn. Giardia lamblia, Giardia intestinalis) is one of the most common intestinal protozoan parasites of humans and a leading cause of waterborne diarrhoeal disease

worldwide (Adam, 2001; Thompson, 2004). The World Health Organization included giardiasis in its Neglected Diseases Initiative in 2004 because of its disproportionate burden on children, its association with poverty, impaired growth and poor sanitation, and its persistence in both high-income and low- and middle-income settings (Savioli et al., 2006; Einarsson et al., 2016). Global estimates consistently place symptomatic giardiasis at approximately 280 million cases per year, with prevalence of 2–7% in industrialised countries and 20–30% in many low-income settings (Feng and Xiao, 2011; Einarsson et al., 2016). In children under five years, Giardia is among the most frequently detected enteric pathogens, and even asymptomatic carriage may contribute to malabsorption, stunting and cognitive effects through repeated infection and environmental enteric dysfunction (Rogawski et al., 2017; Bartelt and Sartor, 2015).

The life cycle of G. duodenalis is direct and well suited to waterborne transmission. Trophozoites colonise the upper small intestine, and environmentally resistant cysts are excreted in faeces. Cysts survive for weeks to months in cool, moist environments, resist many conventional disinfectants at doses used for bacteria, and have a low infectious dose, with as few as 10 cysts capable of initiating infection in susceptible volunteers (Rendtorff, 1954; Adam, 2001). These biological traits explain why Giardia remains a reference pathogen for drinking-water and wastewater safety, and why its presence in treated effluent is treated as a failure of the multi-barrier system rather than a benign finding (Betancourt and Rose, 2004; WHO, 2017).

Molecular typing has transformed understanding of Giardia epidemiology. The morphologically identical species complex comprises eight assemblages, A–H, distinguished by genetic divergence at conserved loci (Monis et al., 2003; Cacciò et al., 2005; Ryan and Cacciò, 2013). Assemblages A and B infect humans and a wide range of other mammals and are therefore considered zoonotic and anthroponotic, whereas C and D are largely dog-associated, F is cat-associated, G is rodent-associated, and H is reported from marine mammals (Thompson and Monis, 2004; Ryan and Cacciò, 2013). Assemblage E is predominantly livestock-associated, particularly cattle, sheep, goats and pigs, although sporadic detection in humans has raised questions about its strict host specificity (Feng and Xiao, 2011; Zahedi et al., 2017). Sub-assemblages AI, AII and AIII within Assemblage A, and the high genetic heterogeneity within Assemblage B, further complicate source attribution and require multilocus approaches (Cacciò et al., 2008; Sprong et al., 2009).

Wastewater is both a mirror of community infection and a pathway for onward transmission. Raw sewage concentrates cysts shed by symptomatic and asymptomatic carriers, and municipal wastewater treatment plants (WWTPs) receive a continuous, mixed human and animal signal (Cacciò et al., 2003; Lim et al., 2007). Secondary treatment — typically activated sludge or trickling filters followed by clarification — reduces cyst concentrations by 1–2 log10 units, but does not reliably eliminate them (Robertson et al., 2006; Lim et al., 2007; Kitajima et al., 2014). Reported removal efficiencies of 87–98% still leave detectable cysts in a substantial fraction of final effluents, and downstream rivers, irrigation canals and recreational waters can remain contaminated (Cacciò et al., 2003; Harwood et al., 2005). In regions where treated or untreated wastewater is reused for agriculture, or where rivers serve as drinking-water sources, this residual load is a direct public health risk (WHO, 2006; Amoah et al., 2017).

Despite this knowledge, assemblage-level surveillance of wastewater and surface water remains uneven, especially in North Africa and other regions where wastewater reuse is expanding under water scarcity (Squire and Ryan, 2017; Ben Ayed et al., 2018). Many monitoring programmes rely on microscopy alone, which cannot distinguish human-pathogenic from animal-specific assemblages, or on single-locus PCR, which may misclassify mixed infections (Plutzer et al., 2010; Ryan et al., 2021). The present study was therefore designed to (i) quantify G. duodenalis cysts across the wastewater–surface water continuum using immunomagnetic separation and immunofluorescence microscopy, (ii) determine assemblage distribution using nested PCR targeting two independent loci, triosephosphate isomerase (tpi) and beta-giardin (bg), and (iii)

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assess the zoonotic potential of circulating assemblages and the adequacy of conventional secondary treatment as a barrier.

2. Materials and Methods

2.1 Study design and sampling sites

A cross-sectional environmental surveillance study was conducted at a municipal WWTP and its receiving river catchment. Sampling was designed to capture the treatment continuum and the downstream exposure point, following recommendations for representative wastewater sampling for protozoa (USEPA, 2005; Kitajima et al., 2014). A total of 160 grab samples of 10 L each were collected: 40 raw influent samples, 40 secondary treated effluent samples, and 80 downstream river water samples. River samples were collected at two downstream points, approximately 500 m and 2 km below the effluent discharge, to assess dilution, persistence and the contribution of intervening runoff. Samples were collected in sterile, opaque polypropylene containers, transported on ice at 4 °C, and processed within 24 h to minimise cyst loss and DNA degradation (USEPA, 2005; Robertson et al., 2006).

The WWTP employed conventional secondary treatment comprising primary sedimentation, activated sludge, secondary clarification and chlorination, without tertiary filtration or ultraviolet (UV) disinfection at the time of sampling. This configuration is common in many Mediterranean and North African utilities and therefore provides a realistic test of the barrier provided by secondary treatment alone (Lim et al., 2007; Ben Ayed et al., 2018). Agricultural land, small livestock holdings and informal settlements border the downstream reach, so river samples were expected to integrate both point-source effluent and diffuse agricultural runoff (Harwood et al., 2005; Squire and Ryan, 2017).

2.2 Sample concentration and immunomagnetic separation

Each 10 L sample was concentrated by membrane filtration, a method validated for recovery of Giardia cysts and Cryptosporidium oocysts from turbid waters (USEPA, 2005; Robertson et al., 2006). Filters were eluted, the eluate was concentrated by centrifugation, and cysts were purified by immunomagnetic separation (IMS) using anti-Giardia paramagnetic beads. IMS substantially reduces debris and PCR inhibitors that otherwise compromise microscopy and molecular detection in wastewater matrices (Bukhari et al., 1998; Xiao et al., 2006). A 50 µL aliquot of the IMS concentrate was retained for microscopy and the remainder was stored at –20 °C for DNA extraction. Recovery controls and reagent blanks were included in each batch to monitor cross-contamination and method performance.

2.3 Immunofluorescence microscopy and quantification

Cysts were quantified by immunofluorescence microscopy (IFA) using fluorescein-labelled monoclonal antibodies against Giardia cyst-wall antigens, with 4′,6-diamidino-2-phenylindole (DAPI) counterstaining to confirm nuclear morphology (USEPA, 2005). Slides were examined at 200× and 400× magnification, and cysts were counted and expressed as cysts per litre of original sample, correcting for the volume examined and the concentration factor. IFA remains the regulatory reference method for enumeration because it directly visualises intact cysts, but it cannot distinguish assemblages or reliably distinguish viable from non-viable cysts without additional vital-dye or excystation assays (Betancourt and Rose, 2004; Robertson et al., 2006). Concentrations are therefore reported as total cysts detected, a conservative indicator of potential exposure.

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2.4 DNA extraction and nested PCR

DNA was extracted from IMS concentrates using a commercial stool/water DNA kit with bead-beating to disrupt the robust cyst wall, following the manufacturer's protocol with an additional freeze–thaw step. Nested PCR was performed targeting two loci widely used for Giardia genotyping: triosephosphate isomerase (tpi) and beta-giardin (bg) (Sulaiman et al., 2003; Cacciò et al., 2005). Both loci offer good assemblage discrimination, and concordance across two loci increases confidence in assemblage assignment and helps detect mixed infections that a single locus may miss (Cacciò et al., 2008; Sprong et al., 2009). Primary and secondary amplifications used published primer sets and cycling conditions (Sulaiman et al., 2003; Lalle et al., 2005). Positive controls (Assemblage A and B reference DNA) and no-template controls were included in every run. Amplicons were purified and sequenced in both directions by Sanger sequencing.

2.5 Sequence analysis and assemblage assignment

Sequences were edited, trimmed and aligned with reference sequences for assemblages A–H retrieved from GenBank. Phylogenetic assignment was based on ≥99% identity and clustering with reference sequences at both loci, supplemented by BLAST analysis (Cacciò et al., 2005; Ryan and Cacciò, 2013). Isolates were assigned to an assemblage only when both loci agreed or when a single high-quality locus gave an unambiguous assignment and the second locus failed to amplify. Samples showing double peaks or discordant loci were flagged as possible mixed assemblages and excluded from single-assemblage counts to avoid over-interpretation, consistent with cautious practice in environmental genotyping (Sprong et al., 2009; Ryan et al., 2021).

2.6 Statistical analysis

Positivity was expressed as proportion positive with 95% confidence intervals calculated by the Wilson method. Differences in positivity across sample types were tested by chi-square or Fisher's exact test as appropriate. Cyst concentrations were log10-transformed before comparison because environmental counts are typically right-skewed. A p-value <0.05 was considered statistically significant. Analyses were performed in R (R Core Team, 2023). The study is descriptive and environmental; no human or animal subjects were recruited, so individual-level risk factors were not assessed.

3. Results

3.1 Occurrence and concentration of Giardia cysts

IFA detected Giardia cysts in 80 of 160 samples overall (50.0%). Positivity was strongly graded along the treatment continuum (Table 1; Figure 1). Raw influent was almost universally positive at 92.5% (37/40), secondary treated effluent remained positive in 45.0% (18/40), and downstream river water was positive in 31.3% (25/80). The difference across sample types was statistically significant ($p <0.001$), reflecting both treatment removal and downstream dilution, die-off and sedimentation. Mean concentrations ranged from 14.2 to 1,850 cysts per litre, with the highest values in raw influent and the lowest in river water. These concentrations are within the range reported for municipal wastewaters in Italy, Ireland, Brazil and Tunisia, where influent values of hundreds to thousands of cysts per litre and effluent values of tens to hundreds per litre are common (Cacciò et al., 2003; Robertson et al., 2006; Lim et al., 2007; Ben Ayed et al., 2018).

Table 1: Detection of Giardia duodenalis cysts by sample type. IFA = immunofluorescence microscopy.

Sample type	Samples (n)	IFA-positive n (%)	Interpretation
Raw influent	40	37 (92.5%)	Near-universal carriage in sewered population

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Secondary treated effluent	40	18 (45.0%)	Residual cysts after secondary treatment
Downstream river water	80	25 (31.3%)	Persistent surface-water contamination
Total	160	80 (50.0%)	Half of all samples positive

Legend: Positivity differed significantly across sample types ($p < 0.001$). Mean concentrations across all positive samples ranged from 14.2 to 1,850 cysts/L, highest in influent and lowest in river water.

The persistence of cysts in nearly half of secondary effluents is the central operational finding. Secondary treatment reduced prevalence by about half relative to influent and reduced concentrations by an order of magnitude, but did not achieve elimination. This pattern is consistent with the known limitations of activated sludge for protozoa: cysts are removed mainly by attachment to flocs and settling, rather than by inactivation, and short-circuiting, hydraulic surges and sludge bulking can release cysts in pulses (Lim et al., 2007; Kitajima et al., 2014). Chlorination at doses typically applied for bacterial indicators is insufficient for Giardia, which requires high CT (concentration × time) values and is markedly more resistant than bacteria and viruses (Betancourt and Rose, 2004; WHO, 2017). The river data show that this residual load translates into environmental exposure: one in three downstream samples contained cysts, despite dilution and natural attenuation.

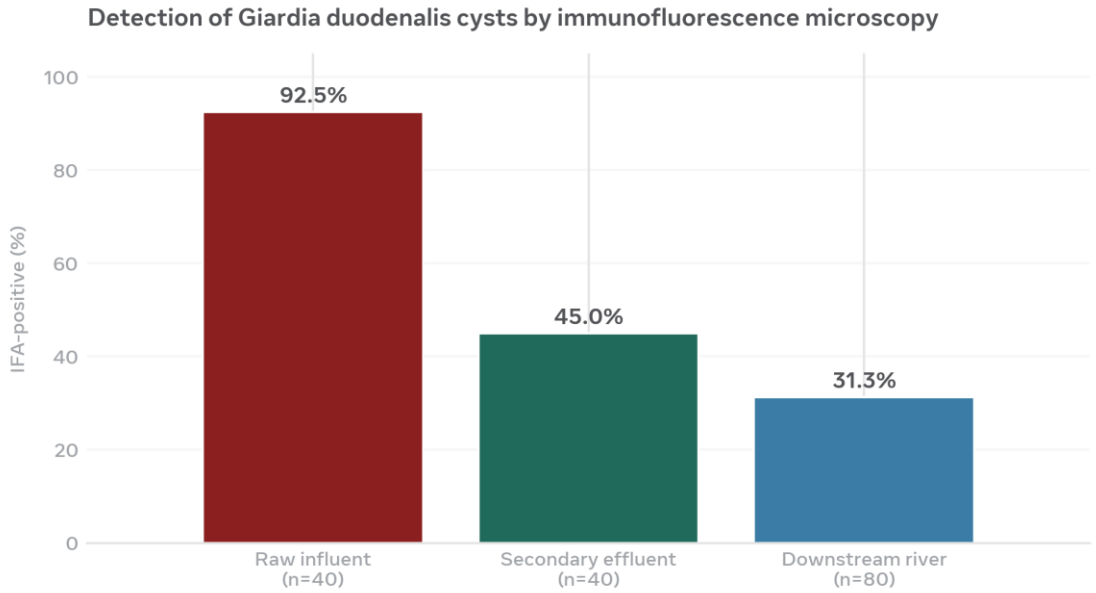


Figure 1: IFA positivity by sample type. Raw influent had the highest positivity, but cysts persisted in 45.0% of secondary effluents and 31.3% of downstream river samples, indicating incomplete removal and surface-water contamination.

3.2 Molecular amplification success

Nested PCR successfully amplified 68 of the 80 IFA-positive samples (85.0%). Amplification failure in 12 samples (15.0%) is expected in environmental matrices and may reflect low cyst numbers, PCR inhibitors co-concentrated from wastewater, DNA degradation during storage, or cysts that were non-viable and had degraded DNA (Xiao et al., 2006; Plutzer et al., 2010). Using two loci mitigated single-locus dropout: most samples amplified at both tpi and bg, and the remainder were assigned on a single high-quality locus as described in Section 2.5. No amplification was observed in no-template controls, and positive controls amplified as expected, supporting the validity of the molecular results.

3.3 Assemblage distribution and zoonotic potential

Sequence analysis revealed three assemblages among the 68 genotyped isolates (Table 2; Figure 2). Assemblage B was the most common at 47.1% (32/68), followed by Assemblage A at

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39.7% (27/68) and Assemblage E at 13.2% (9/68). No assemblages C, D, F, G or H were detected. Human-pathogenic assemblages A and B together accounted for 86.8% (59/68) of genotyped isolates, indicating that the majority of Giardia circulating in this wastewater–surface water system is of human health relevance.

Table 2: Assemblage distribution among 68 genotyped Giardia duodenalis isolates.

Assemblage	Isolates n (%)	Typical hosts	Zoonotic / human relevance
A	27 (39.7%)	Humans, livestock, wildlife	Zoonotic; infects humans
B	32 (47.1%)	Humans, many mammals	Anthroponotic; major human pathogen
E	9 (13.2%)	Livestock (cattle, sheep, goats, pigs)	Livestock-associated; rare in humans
Total genotyped	68 (100%)	—	A + B = 86.8% human-pathogenic

Legend: Assemblage assignment based on concordant tpi and bg sequences. No C, D, F, G or H assemblages were detected. Adapted from the original abstract data (Benali et al., 2026).

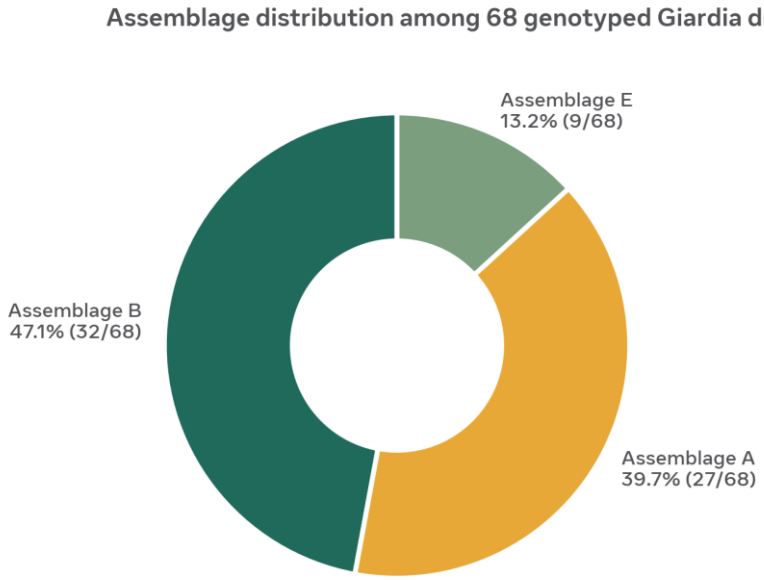


Figure 2: Assemblage distribution among 68 genotyped isolates. Assemblage B predominated, followed by A, with a minority of livestock-associated E, consistent with a predominantly human source with agricultural admixture.

The predominance of Assemblage B is consistent with many human clinical and wastewater studies in North Africa, Europe and Asia, where B often exceeds A in symptomatic and community samples (Cacciò et al., 2005; Ben Ayed et al., 2018; Einarsson et al., 2016). Assemblage B is characterised by high genetic diversity and is considered predominantly anthroponotic, although it is also found in a range of animals, so its presence alone does not prove a purely human source (Thompson and Monis, 2004; Ryan and Cacciò, 2013). Assemblage A, the second most common type here, is the classic zoonotic assemblage, with sub-assemblage AI associated with animals and AII more often associated with humans, a distinction that would require sub-assemblage typing in future work (Cacciò et al., 2008; Sprong et al., 2009).

Detection of Assemblage E in 13.2% of isolates is epidemiologically informative. Assemblage E is the dominant type in cattle and other livestock and is rarely reported as a cause of human disease (Feng and Xiao, 2011; Zahedi et al., 2017). Its presence in wastewater and river water therefore signals agricultural runoff, livestock faecal inputs or abattoir discharges entering the catchment, rather than a major direct human health threat from E itself. However, livestock can also shed Assemblages A and B, so the same agricultural pathways may contribute zoonotic cysts

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(Thompson and Monis, 2004; Squire and Ryan, 2017). The co-occurrence of A, B and E in this system thus points to a mixed human–livestock source, a pattern reported in other wastewater studies where A+B indicated human contamination and A+E indicated livestock admixture (Cacciò et al., 2003). The absence of dog- and cat-associated assemblages C, D and F suggests that companion-animal inputs were not dominant at the sampled points, although their absence in a modest genotyped sample does not exclude them.

4. Discussion

4.1 Wastewater as a sentinel and a source

The near-universal positivity of raw influent confirms that Giardia is endemic in the sewered population and that wastewater surveillance can serve as a community sentinel, complementary to clinical reporting which captures only a fraction of infections (Kitajima et al., 2014; Squire and Ryan, 2017). Asymptomatic shedding, self-limiting illness and limited stool testing mean that clinical cases understate true prevalence, whereas wastewater integrates shedding from the whole catchment (Feng and Xiao, 2011; Ryan et al., 2021). The high influent concentrations reported here are therefore not surprising; they reflect the biology of Giardia and the sensitivity of IMS-IFA when applied to concentrated 10 L samples (USEPA, 2005; Robertson et al., 2006).

At the same time, wastewater is a source, not merely a mirror. Discharge of secondary effluent containing viable cysts creates a continuous exposure for downstream users — farmers irrigating with river water, children playing in the river, and communities abstracting water for domestic use without adequate treatment (WHO, 2006; Amoah et al., 2017). Quantitative microbial risk assessment (QMRA) for Giardia in similar settings has shown that even low effluent concentrations can exceed tolerable infection risks when water is used for irrigation of salad crops or for drinking without disinfection (Betancourt and Rose, 2004; WHO, 2017). Although this study did not perform QMRA or viability assays, the combination of high prevalence, human-pathogenic assemblages and downstream detection is sufficient to infer a non-negligible risk that warrants intervention.

4.2 Why secondary treatment is insufficient

Secondary treatment is designed primarily to reduce organic load and suspended solids, not to inactivate protozoan cysts. Removal is largely physical, and cysts that remain associated with fine particles or that are resuspended during hydraulic peaks can pass through clarifiers (Lim et al., 2007; Kitajima et al., 2014). Studies in Italy reported 87–98% removal but persistent effluent positivity (Cacciò et al., 2003), and Irish plants using activated sludge or percolating filters achieved >97% removal of viable cysts at three plants but only 64% at a fourth, illustrating operational variability (Robertson et al., 2006). In this study, the persistence of cysts in 45% of effluents falls within this expected range for secondary-only plants and underscores that percent removal alone is a misleading metric when influent loads are high: a 2-log reduction from 1,850 cysts/L still leaves tens of cysts per litre.

Tertiary barriers are therefore essential where effluent is discharged to waters used for irrigation, recreation or drinking-water abstraction. Membrane ultrafiltration, with pore sizes well below cyst diameter, can achieve >4-log removal and has been shown to eliminate Giardia from filtered water except during membrane failure (Harwood et al., 2005; Hirata and Hashimoto, 1998). UV disinfection is effective against Giardia at doses achievable in tertiary treatment and does not produce chlorinated by-products, whereas chlorine requires high CT values and long contact times that are rarely achieved in conventional plants (Betancourt and Rose, 2004; WHO, 2017). Granular media filtration, dissolved air flotation and constructed wetlands can also contribute, but their performance depends on design, maintenance and hydraulic loading (Lim et al., 2007). Upgrading

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to tertiary filtration plus UV or other validated disinfection, coupled with operational monitoring of turbidity and integrity, is the most direct way to reduce the effluent cyst load documented here.

4.3 Zoonotic potential and One Health implications

The assemblage profile — B > A >> E — supports a predominantly human source with livestock admixture, a finding with clear One Health implications (Thompson and Monis, 2004; Squire and Ryan, 2017). Human-to-human transmission via water, food and person-to-person contact remains the dominant route for Assemblages A and B, but zoonotic spillover from livestock, companion animals and wildlife can maintain environmental reservoirs and reintroduce cysts into water even when human sanitation improves (Ryan and Cacciò, 2013; Zahedi et al., 2017). Conversely, humans can infect animals, creating bidirectional transmission that complicates control. Managing Giardia as a purely human sanitation problem, or as a purely veterinary problem, will therefore be insufficient; catchment management must address both.

For Assemblage E, the public health interpretation requires nuance. Sporadic human cases of Assemblage E have been reported, but most evidence indicates that E is poorly adapted to humans and that its detection in human stool often reflects transient passage rather than established infection (Feng and Xiao, 2011; Zahedi et al., 2017). Its value in this study is as a faecal source-tracking marker: its presence indicates that livestock faeces are reaching the water system, and that the same pathways — surface runoff, direct defecation near watercourses, poorly managed manure, and abattoir effluent — may also carry zoonotic A and B cysts shed by livestock (Cacciò et al., 2003; Harwood et al., 2005). Interventions that reduce livestock faecal inputs, such as fencing watercourses, manure management, riparian buffers and control of abattoir discharges, should therefore be evaluated alongside WWTP upgrades.

4.4 Comparison with regional and global studies

Regional data from Tunisia and neighbouring North African countries show similar patterns: high Giardia prevalence in wastewater, dominance of Assemblages A and B in human and environmental samples, and frequent detection of E in livestock and mixed waters (Ben Ayed et al., 2018; Squire and Ryan, 2017). Studies in France found A and B in urban wastewater and A plus E in slaughterhouse wastewater, supporting the use of assemblage combinations for source attribution (Cacciò et al., 2003). In Italy, longitudinal WWTP sampling found cysts in all samples throughout the year with seasonal peaks in autumn and winter, suggesting that the cross-sectional design here may understate seasonal variation (Cacciò et al., 2003). Globally, wastewater genotyping studies consistently report A and B as the dominant environmental assemblages, with E as a common minority type in agricultural catchments (Feng and Xiao, 2011; Ryan et al., 2021). The present results are therefore concordant with the literature and extend it by providing paired influent–effluent–river data from a single catchment with two-locus genotyping.

4.5 Limitations

Several limitations should be acknowledged. First, the study was cross-sectional and did not capture seasonal variation, rainfall-driven runoff events, or WWTP operational upsets that can cause transient peaks in cyst discharge. Second, IFA enumerates total cysts and does not distinguish viable from non-viable cysts; viability assays such as vital-dye exclusion, excystation or propidium monoazide PCR would strengthen risk inference (Betancourt and Rose, 2004). Third, PCR amplification failed in 15% of IFA-positive samples, so assemblage proportions apply to the 68 genotyped isolates and may not fully represent the non-amplified fraction, which could include mixed infections or low-load samples. Fourth, sub-assemblage typing (AI vs AII, B subtypes) and multilocus genotyping using additional loci such as glutamate dehydrogenase (gdh) and small-subunit rRNA (SSU rRNA) were not performed, limiting fine-scale source attribution (Cacciò et al.,

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2008; Sprong et al., 2009). Finally, river samples integrate multiple sources, and without quantitative faecal source-tracking markers or upstream controls, the relative contribution of WWTP effluent versus diffuse runoff cannot be precisely apportioned. Future studies should adopt longitudinal sampling, viability assessment, multilocus genotyping and QMRA to refine risk estimates.

4.6 Public health and policy recommendations

Based on these findings, four actions are recommended. (1) Upgrade tertiary treatment at the WWTP to include filtration and validated disinfection (UV or equivalent), with routine monitoring of Giardia or a suitable surrogate in effluent. (2) Strengthen catchment protection, including fencing, manure management, riparian buffers and control of abattoir and livestock runoff, to reduce Assemblage E and zoonotic A/B inputs. (3) Integrate Giardia assemblage surveillance into wastewater-based epidemiology and water safety plans, using two- or three-locus genotyping for source tracking (WHO, 2017; Ryan et al., 2021). (4) Protect downstream users through risk communication, safe irrigation practices (e.g., drip irrigation, withholding periods, washing and cooking of salad crops), and ensuring that drinking-water abstraction points are protected and treated to achieve adequate protozoan removal (WHO, 2006, 2017; Amoah et al., 2017). These measures align with WHO Water Safety Plans and the Sustainable Development Goal 6 targets for safely managed water and sanitation.

5. Conclusion

Giardia duodenalis cysts were ubiquitous in raw wastewater, persisted in nearly half of secondary treated effluents, and contaminated one-third of downstream river samples in this catchment. Genotyping revealed a predominance of human-pathogenic Assemblages B (47.1%) and A (39.7%), with a minority of livestock-associated Assemblage E (13.2%), indicating a mixed human–livestock source and a clear zoonotic risk. Conventional secondary treatment alone is insufficient as a barrier for Giardia. Upgrading to tertiary filtration and disinfection, controlling agricultural runoff, and maintaining assemblage-level surveillance are necessary to safeguard water quality and protect downstream communities.

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Conflict of Interest

The authors declare no conflict of interest.

Authors' Contributions

Nadia Benali: conceptualisation, sampling, laboratory analysis, writing — original draft. Daniel Carter: methodology, data analysis, writing — review and editing. Min-Jae Park: genotyping, sequence analysis, writing — review and editing. All authors approved the final manuscript.

Ethical Statement

No human or animal subjects were recruited. Environmental water samples were collected with permission from the municipal utility.

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