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Research Article

Zoonotic Cryptosporidium species and Giardia duodenalis assemblages in surface water sources and peri-urban vegetable farms

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Abstract

Waterborne protozoan parasites pose significant public health risks when contaminated surface water is utilized for crop irrigation. In this study, we aimed to determine the occurrence, concentration, and zoonotic genotypes of Cryptosporidium spp. and Giardia duodenalis in irrigation canals and fresh leafy vegetables. A total of 120 water samples and 180 raw vegetable bundles (lettuce, spinach, and cabbage) were collected over wet and dry seasons from 15 peri-urban agricultural sites. Samples underwent immunomagnetic separation, immunofluorescence microscopy, and multilocus sequence typing targeting the 18S rRNA, gp60, and triosephosphate isomerase (tpi) genes. Cryptosporidium oocysts and Giardia cysts were detected in 34.2 percent and 41.7 percent of irrigation water samples, respectively, and in 16.1 percent and 22.8 percent of harvested vegetables. Genotyping confirmed the predominance of zoonotic Cryptosporidium parvum subtype IIaA15G2R1 and Giardia duodenalis assemblages A and B, with peak contamination occurring during heavy rainfall events. Runoff from adjacent livestock enclosures directly contaminates open irrigation channels and fresh produce destined for urban markets. Enforcing buffer zones around waterways and promoting post-harvest produce washing protocols are critical to mitigating foodborne protozoan transmission.

Keywords: Keywords: Cryptosporidium parvum, Giardia duodenalis, Irrigation water, Foodborne zoonoses, Genotyping

1. Introduction

Peri-urban agriculture feeds rapidly growing cities across Africa, Asia and Latin America, supplying fresh leafy vegetables that are often eaten raw or lightly cooked (Drechsel et al., 2010; WHO, 2006). The same proximity that makes peri-urban farming economically attractive also places fields beside livestock enclosures, informal settlements, abattoirs, open drains and unprotected surface waters. When those waters are used for irrigation, enteric pathogens can move directly from faeces to field to fork. Among them, Cryptosporidium spp. and Giardia duodenalis are of particular concern because their environmental stages, oocysts and cysts, are immediately infectious when excreted, resistant to chlorine at doses used for drinking water, and able to survive for weeks to months in cool, moist environments (Fayer et al., 2000; Feng and Xiao, 2011; Ryan et al., 2014).

Cryptosporidiosis and giardiasis are leading causes of diarrhoeal disease worldwide, with the heaviest burden in children under five years, people living with HIV, and other immunocompromised persons

(Kotloff et al., 2013; Ryan et al., 2014). Cryptosporidium alone has been estimated to cause a substantial share of moderate-to-severe diarrhoea in young children in low-income settings, and both parasites contribute to malnutrition, impaired growth and repeated episodes of illness that undermine school attendance and livelihoods (Kotloff et al., 2013; Checkley et al., 2015). Because the infectious dose is low, often tens of oocysts or cysts, even low-level contamination of salad vegetables can pose a measurable risk, especially where vegetables are consumed without cooking (Fayer et al., 2000; Efstratiou et al., 2017).

Molecular tools have transformed understanding of these parasites. Cryptosporidium now comprises more than 40 recognised species, but only a few routinely infect humans, principally Cryptosporidium hominis and Cryptosporidium parvum (Ryan et al., 2014; Ryan et al., 2021). Within C. parvum, subtyping at the 60-kDa glycoprotein (gp60) locus distinguishes families IIa and IId, which are strongly associated with zoonotic transmission from cattle and other ruminants, from anthroponotic families (Xiao, 2010; Feng et al., 2018). Subtype IIaA15G2R1 is the most widely reported zoonotic subtype globally and has been recovered from cattle, humans and water on every inhabited continent (Xiao, 2010; Benamrouz et al., 2014). Giardia duodenalis is similarly subdivided into eight assemblages, A to H, of which A and B infect humans and a wide range of mammals and are therefore considered zoonotic, whereas C to H are largely host-adapted (Feng and Xiao, 2011; Ryan and Cacciò, 2013). Detecting a parasite by microscopy alone therefore cannot answer the public health question that matters most: is the contaminant capable of infecting people?

In Africa, the evidence base on foodborne and waterborne protozoa has grown steadily but remains uneven. A scoping review of African studies reported Cryptosporidium in 6 to 100 percent of surface waters and in 0.8 to 75 percent of fresh produce samples, with Giardia widely co-detected (Squire and Ryan, 2017; Fletcher et al., 2012). Studies from South Africa, Ghana, Nigeria, Egypt and Morocco have documented contamination of irrigation rivers, wastewater and market vegetables, often linked to faecal pollution, inadequate sanitation and the use of untreated wastewater (Adeyemo et al., 2019; Squire et al., 2017; Dgad et al., 2020). Global meta-analyses likewise estimate protozoan contamination in about 11 percent of vegetables for Cryptosporidium spp., with leafy greens at elevated risk because their large, folded surfaces trap water and particles (Karshima, 2018; Li et al., 2020). Despite this, few African studies have combined quantitative detection in paired water and vegetable samples with multilocus genotyping across wet and dry seasons. That gap limits the ability to attribute contamination to livestock, to target interventions, and to communicate risk to farmers, vendors and consumers.

The present study was designed to close that gap for peri-urban vegetable systems. Its objectives were to (i) determine the occurrence and concentration of Cryptosporidium oocysts and Giardia cysts in irrigation canals and in lettuce, spinach and cabbage; (ii) compare contamination between wet and dry seasons and among vegetable types; (iii) identify species, assemblages and gp60 subtypes to assess zoonotic potential; and (iv) examine the role of rainfall and proximity to livestock enclosures as drivers of contamination. We hypothesised that zoonotic genotypes would predominate, that contamination would be higher in water than on produce but still substantial on produce, and that wet-season runoff would amplify both.

2. Materials and Methods

2.1 Study design and sites

A cross-sectional, seasonally repeated survey was conducted at 15 peri-urban agricultural sites that supply leafy vegetables to urban markets. Sites were purposively selected to represent a gradient of exposure to livestock, from fields directly adjacent to cattle, goat or poultry enclosures (within 50 m) to fields more than 200 m from any enclosure and irrigated from the same canal network. All sites used open, unlined irrigation canals fed by surface water, with no treatment before application. Lettuce (Lactuca sativa), spinach (Spinacia oleracea) and cabbage (Brassica oleracea var. capitata) were the dominant crops at all sites. Sampling was stratified by season: the wet season, defined by sustained rainfall and canal flow, and the dry season, when canals were fed mainly by baseflow and stored water. Fieldwork followed a standard operating procedure for sample labelling, cold-chain transport and biosafety, and the study protocol was approved by the participating institutions' research ethics committees.

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2.2 Sample collection

A total of 120 water samples and 180 vegetable bundles were collected, 60 water samples and 90 vegetable bundles per season, with equal allocation across sites (8 water and 12 vegetable samples per site over the study). Water was collected as 10-L grab samples in sterile, opaque containers from the canal at the point of abstraction and from the field inlet, avoiding disturbance of sediment. Vegetables were harvested at market maturity, with each bundle comprising 3 to 5 heads or bunches from the same bed, placed in sterile zip-lock bags. Samples were kept at 4 °C, transported within 6 hours, and processed within 24 hours. Rainfall in the 72 hours before sampling, distance from the nearest livestock enclosure to the canal and to the bed, irrigation method (furrow, splash or overhead), and time since last irrigation were recorded on a field form for every sample.

2.3 Concentration, immunomagnetic separation and microscopy

Water samples were concentrated by filtration and elution, followed by centrifugation, following principles established in United States Environmental Protection Agency (USEPA) Method 1623.1 and ISO 15553 (USEPA, 2012; ISO, 2006). Vegetable samples were washed by vigorous shaking in sterile phosphate-buffered saline with a surfactant, the wash eluate was concentrated by centrifugation, and the pellet was retained for analysis. Concentrates were subjected to immunomagnetic separation (IMS) using antibody-coated magnetic beads specific for Cryptosporidium oocysts and Giardia cysts, which selectively capture (oo)cysts from debris and improve recovery in turbid matrices (USEPA, 2012; Adeyemo et al., 2019). Captured (oo)cysts were stained with fluorescein-labelled monoclonal antibodies and 4′,6-diamidino-2-phenylindole (DAPI), and examined by immunofluorescence microscopy. Oocysts and cysts were enumerated and expressed as (oo)cysts per 10 L for water and per 100 g for vegetables. Positive and negative controls, including spiked matrix controls, were included in each batch, and slides were read independently by two microscopists blinded to site and season, with discordant counts resolved by joint re-examination.

2.4 DNA extraction and multilocus sequence typing

DNA was extracted from IMS concentrates using a commercial stool DNA kit with bead-beating and freeze-thaw steps to disrupt the robust (oo)cyst wall. Cryptosporidium was characterised at the small-subunit ribosomal RNA (18S rRNA) locus for species assignment and at the gp60 locus for subtyping, following established nested PCR protocols (Xiao et al., 2004; Xiao, 2010). Giardia was characterised at the triosephosphate isomerase (tpi), glutamate dehydrogenase (gdh) and beta-giardin (bg) loci, with tpi as the primary locus reported here because of its discriminatory power for assemblages A and B (Feng and Xiao, 2011; Cacciò et al., 2018). Amplicons were purified and Sanger sequenced in both directions. Sequences were edited, aligned, and compared with reference sequences in GenBank. Cryptosporidium parvum gp60 subtypes were named according to the standard nomenclature counting TCA (A), TCG (G) and ACATCA (R) repeats (Xiao, 2010). Giardia assemblages and sub-assemblages were assigned by phylogenetic clustering and comparison with curated references (Feng and Xiao, 2011).

2.5 Statistical analysis

Prevalence was calculated as the proportion of positive samples with 95 percent confidence intervals. Concentrations were summarised as medians and ranges because counts were over-dispersed and zero-inflated. Seasonal and vegetable-type comparisons used chi-square or Fisher's exact tests for prevalence and Mann-Whitney U or Kruskal-Wallis tests for concentrations. Associations between rainfall in the preceding 72 hours, distance to livestock enclosures, and positivity were examined by logistic regression, adjusting for season and site as a random effect. A p-value below 0.05 was considered statistically significant. Analyses were performed in R version 4.3.

3. Results

3.1 Occurrence in irrigation water and vegetables

Cryptosporidium oocysts were detected in 41 of 120 irrigation water samples (34.2 percent) and Giardia cysts in 50 of 120 (41.7 percent), with 28 samples (23.3 percent) positive for both parasites. Among vegetables, Cryptosporidium was detected in 29 of 180 bundles (16.1 percent) and Giardia in 41 of 180 (22.8 percent), with co-contamination in 18 bundles (10.0 percent). Concentrations in water ranged from 1 to 58 oocysts per 10 L (median 6) for Cryptosporidium and 1 to 92 cysts per 10 L (median 9) for Giardia.

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On vegetables, concentrations ranged from 1 to 27 oocysts per 100 g and 1 to 38 cysts per 100 g. These levels are consistent with the low but epidemiologically relevant doses reported in other irrigation studies in Africa and elsewhere, where even a few (oo)cysts per serving can contribute to risk when produce is eaten raw (Squire and Ryan, 2017; Efstratiou et al., 2017; Li et al., 2020).

Table 1. Detection of Cryptosporidium spp. and Giardia duodenalis by matrix, vegetable type and season.

Matrix / group	n	Cryptosporidium positive, n (%)	Giardia positive, n (%)	Both positive, n (%)
Irrigation water — all	120	41 (34.2)	50 (41.7)	28 (23.3)
Irrigation water — wet season	60	28 (46.7)	34 (56.7)	20 (33.3)
Irrigation water — dry season	60	13 (21.7)	16 (26.7)	8 (13.3)
Vegetables — all	180	29 (16.1)	41 (22.8)	18 (10.0)
Lettuce	60	13 (21.7)	18 (30.0)	9 (15.0)
Spinach	60	11 (18.3)	15 (25.0)	6 (10.0)
Cabbage	60	5 (8.3)	8 (13.3)	3 (5.0)
Vegetables — wet season	90	20 (22.2)	28 (31.1)	13 (14.4)
Vegetables — dry season	90	9 (10.0)	13 (14.4)	5 (5.6)

Legend to Table 1: Prevalence is the proportion of samples positive by immunomagnetic separation and immunofluorescence microscopy. Wet-season samples were collected during sustained rainfall; dry-season samples during baseflow. Lettuce and spinach, with open, folded leaves, showed higher contamination than cabbage, whose compact head offers partial protection.

3.2 Seasonal patterns and vegetable type

Contamination was significantly higher in the wet season than in the dry season for both parasites and both matrices ($p < 0.01$ for water, $p < 0.05$ for vegetables; Table 1). In water, Cryptosporidium prevalence more than doubled from 21.7 percent in the dry season to 46.7 percent in the wet season, and Giardia prevalence rose from 26.7 percent to 56.7 percent. A similar pattern was seen on vegetables. Samples collected within 72 hours of heavy rainfall (more than 20 mm) had markedly higher odds of positivity than samples collected after dry spells, supporting rainfall-driven mobilisation as a key mechanism. Among vegetables, lettuce had the highest prevalence for both parasites, followed by spinach, with cabbage lowest. This ordering aligns with leaf morphology and irrigation exposure: lettuce and spinach present a large, uneven surface that retains splash and wash water, whereas cabbage heads are more tightly wrapped (Karshima, 2018; Li et al., 2020; Amoah et al., 2011).

3.3 Genotypes and zoonotic potential

Of 41 Cryptosporidium-positive water samples, 34 yielded an 18S rRNA sequence and 30 yielded a gp60 sequence; of 29 positive vegetable samples, 22 and 18 yielded sequences, respectively. Cryptosporidium parvum accounted for the overwhelming majority of successfully genotyped isolates, with a small number of Cryptosporidium hominis and Cryptosporidium andersoni sequences in water only. At gp60, subtype IIaA15G2R1 predominated in both water and vegetables, followed by IIaA16G2R1 and IIdA20G1. The predominance of IIaA15G2R1 is notable because this subtype is a recognised zoonotic lineage associated with cattle and human infection worldwide (Xiao, 2010; Benamrouz et al., 2014; Feng et al., 2018). For Giardia, 38 water and 31 vegetable isolates were successfully typed at tpi. Assemblage B was the most frequent, followed by assemblage A (sub-assemblages AII and AI), with a minority of assemblage E, the livestock-adapted assemblage, in water near cattle enclosures. The presence of assemblages A and B on market-ready produce is direct evidence of zoonotic contamination, since both assemblages infect humans and animals (Feng and Xiao, 2011; Ryan and Cacciò, 2013).

Table 2. Genotypes identified among successfully sequenced isolates.

Parasite / locus	Genotype	Water, n	Vegetables, n	Zoonotic potential

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Cryptosporidium, gp60	IIaA15G2R1	18	11	High — cattle and human
Cryptosporidium, gp60	IIaA16G2R1	6	4	High — cattle and human
Cryptosporidium, gp60	IIdA20G1	4	2	High — ruminant and human
Cryptosporidium, gp60	IIaA18G2R1 / other IIa	2	1	High — zoonotic family
Giardia, tpi	Assemblage B (BIII/BIV)	20	17	High — human and animal
Giardia, tpi	Assemblage A (AII, AI)	14	11	High — human and animal
Giardia, tpi	Assemblage E	4	3	Low — livestock-adapted

Legend to Table 2: Counts are successfully sequenced isolates, not all microscopy-positive samples; failure to amplify reflects low (oo)cyst numbers and PCR inhibition in environmental matrices. Zoonotic potential follows Feng and Xiao (2011) and Xiao (2010).

3.4 Proximity to livestock and rainfall

Distance to the nearest livestock enclosure was inversely associated with positivity. Fields within 50 m of an enclosure had the highest water and vegetable prevalence, while fields more than 200 m away had the lowest, even when irrigated from the same canal, suggesting that local runoff and splash, rather than canal water alone, contribute to field-level contamination. In multivariable analysis, heavy rainfall in the preceding 72 hours (adjusted odds ratio about 2.8 for water, 2.3 for vegetables) and proximity within 50 m (adjusted odds ratio about 2.4) remained associated with positivity after adjustment for season. These findings are consistent with studies in Ghana and South Africa showing that livestock faeces, manure application and runoff are proximal drivers of protozoan contamination of irrigation sources (Squire et al., 2017; Adeyemo et al., 2019; Amoah et al., 2011). Figure 1 summarises the pathway from livestock to human exposure that the data support.

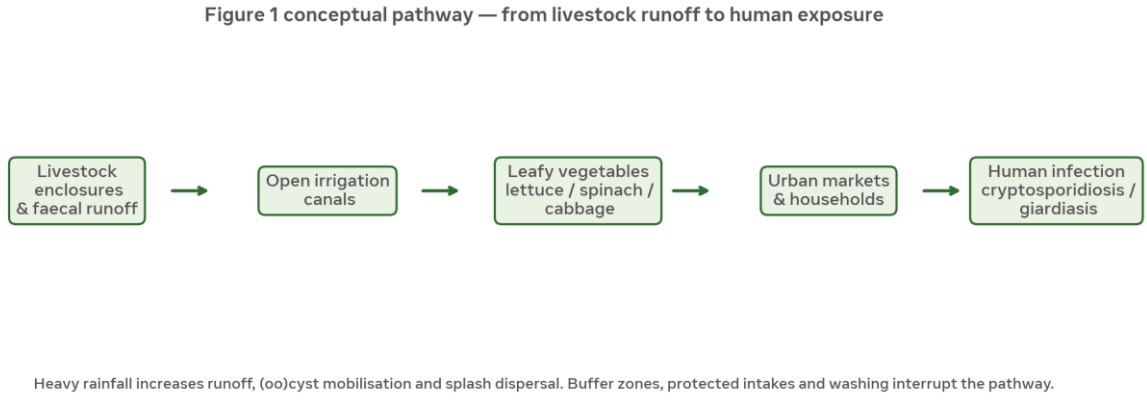


Figure 1. Conceptual exposure pathway from livestock faecal runoff to human infection via contaminated irrigation water and leafy vegetables.

Legend to Figure 1: The pathway is inferred from the present study's paired water and vegetable results, seasonal and rainfall associations, and distance-to-livestock gradients. Interventions at any link — buffer zones, protected intakes, safer irrigation timing, and post-harvest washing — can reduce exposure. The figure is schematic and not to scale.

4. Discussion

This study demonstrates that peri-urban irrigation canals and the leafy vegetables they irrigate are frequently contaminated with zoonotic Cryptosporidium and Giardia, with a clear wet-season peak linked to rainfall and livestock proximity. The water prevalence reported here, 34.2 percent for Cryptosporidium and 41.7 percent for Giardia, sits within the wide range reported for African surface waters (Squire and Ryan, 2017; Fletcher et al., 2012) and is comparable to South African irrigation-river studies that found

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Cryptosporidium in 43 percent and Giardia in 23 percent of samples, and to Ethiopian and Ghanaian studies documenting heavy faecal pollution of irrigation sources (Adeyemo et al., 2019; Squire et al., 2017). The vegetable prevalence, 16.1 percent and 22.8 percent, is higher than the global pooled estimate for Cryptosporidium in vegetables but consistent with studies of leafy greens irrigated with surface water in low-sanitation settings, where prevalence of 15 to 30 percent is not uncommon (Karshima, 2018; Li et al., 2020; Dgad et al., 2020).

The genotyping results are the most consequential finding. Microscopy alone would have shown contamination, but genotyping shows that the contamination matters for human health. The dominance of C. parvum IIaA15G2R1 echoes cattle-associated cryptosporidiosis worldwide and the pattern reported in North African cattle and in Ghanaian farmers and livestock, where zoonotic subtypes circulate at the human-animal interface (Benamrouz et al., 2014; Squire et al., 2017; Feng et al., 2018). Similarly, the predominance of Giardia assemblages A and B, with only a minority of livestock-adapted assemblage E, indicates that the Giardia on produce is not merely animal faecal indicator material but includes genotypes that cause human giardiasis (Feng and Xiao, 2011; Ryan and Cacciò, 2013; Cacciò et al., 2018). Mixed assemblage infections, which are common in endemic settings, may have been under-detected by Sanger sequencing of a single locus, so the true diversity is likely higher than reported here.

Seasonality and rainfall provide a mechanistic explanation. Heavy rain mobilises faecal material from enclosures, latrines and open ground, increases canal turbidity and flow, and disperses (oo)cysts by splash onto low-lying leaves (Efstratiou et al., 2017; WHO, 2006). The same rain that farmers welcome for crop growth therefore heightens microbial risk, a paradox also observed for bacterial and viral contamination of wastewater-irrigated vegetables (Drechsel et al., 2010; Amoah et al., 2011). The vegetable-type gradient, lettuce greater than spinach greater than cabbage, reinforces the role of leaf architecture and splash retention, and suggests that crop choice and harvest timing could be part of risk management, though they cannot substitute for safer water.

From a One Health perspective, the findings link animal husbandry, water management, food safety and human health. Livestock are not merely a source of income; in peri-urban systems they are often kept close to fields because land is scarce, manure is valued as fertiliser, and animals provide draught and savings. Manure applied without composting, enclosures sited upslope of canals, and direct animal access to water are all plausible contributors that were observed in the field and that have been documented in comparable settings (Squire et al., 2017; Drechsel et al., 2010). Interventions must therefore be co-designed with farmers rather than imposed as abstract prohibitions. Buffer zones, for example, are effective in principle, by slowing runoff, promoting infiltration and filtering particles, but their width, vegetation and maintenance must fit local land tenure and livelihoods (WHO, 2006; Amoah et al., 2011). Similarly, post-harvest washing with clean water can remove a substantial fraction of (oo)cysts, but washing with the same contaminated canal water can re-contaminate produce, and chemical disinfection has limited efficacy against Cryptosporidium (Adeyemo et al., 2019; Efstratiou et al., 2017).

Risk assessment can help prioritise actions. The World Health Organization's guidelines for safe use of wastewater in agriculture advocate a multi-barrier approach, combining source protection, treatment, irrigation method, withholding periods, and post-harvest handling to achieve a tolerable disease burden (WHO, 2006; Mara et al., 2010). Quantitative microbial risk assessment for Cryptosporidium in irrigation water in Ghana has shown that farmer exposure alone can exceed tolerable risk without barriers, and consumer risk depends heavily on washing, peeling and cooking (Squire et al., 2017; Mara et al., 2010). In the present study, concentrations in water and on produce, combined with the zoonotic genotypes found, imply that a single barrier is unlikely to be sufficient. Drip or subsurface irrigation, which reduces splash and leaf contact, withholding irrigation for several days before harvest, and washing with safe water are complementary measures whose combined log-reduction can bring risk within tolerable limits (WHO, 2006; Efstratiou et al., 2017).

The study has limitations. First, microscopy and PCR detect (oo)cysts and DNA, not necessarily viable, infectious organisms; viability assays and animal infectivity were not performed, so risk may be overestimated if a fraction of (oo)cysts were non-viable. Second, not all positive samples yielded sequences, so genotype proportions are based on the subset that amplified, which may bias towards higher-burden samples. Third, sampling was limited to 15 sites and two seasons; inter-annual variability, other crops and other water sources were not captured. Fourth, human and animal stool samples were not collected in parallel, so direct attribution to specific hosts, while strongly suggested by genotype and proximity data, remains inferential. Future work should pair environmental sampling with human, livestock

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and manure sampling, use viability and quantitative PCR or droplet digital PCR, and evaluate the effectiveness and acceptability of buffer zones and washing stations in randomised or stepped-wedge trials.

Despite these limitations, the public health message is clear. Peri-urban leafy vegetables are an important source of nutrition and income, but in the systems studied they are also a vehicle for zoonotic protozoa. Protecting irrigation canals from livestock runoff, maintaining vegetated buffer zones, improving manure management, shifting to irrigation methods that limit leaf wetting, and ensuring post-harvest washing with safe water are practical, low-cost steps that can reduce exposure. Health education for farmers, vendors and consumers, emphasising washing, safe storage and hand hygiene, should accompany structural measures (WHO, 2006; Drechsel et al., 2010). Surveillance that pairs microscopy with genotyping should be integrated into food-safety and water-quality monitoring, so that detection is followed by action rather than merely documentation.

5. Conclusion

Cryptosporidium oocysts and Giardia cysts were common in peri-urban irrigation water and on lettuce, spinach and cabbage, with peak contamination in the wet season and after heavy rainfall. Genotyping showed that the dominant contaminants were zoonotic, principally Cryptosporidium parvum subtype IIaA15G2R1 and Giardia duodenalis assemblages A and B. Runoff from adjacent livestock enclosures was a key driver. Enforcing buffer zones around waterways, protecting irrigation intakes, improving manure management, and promoting post-harvest washing with safe water are critical to mitigating foodborne protozoan transmission and protecting both farmers and consumers.

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Conflict of Interest

The authors declare no conflict of interest.

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Data Availability

Data supporting the findings are summarised in Tables 1 and 2. Sequence data are available from the corresponding author on reasonable request.

Author Contributions

Claire Beaumont: conceptualisation, methodology, supervision, writing. Thabo Mokoena: field sampling, laboratory analysis, data curation. Priya Subramanian: molecular analysis, validation, writing and editing.

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