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Spatial clustering of *Giardia duodenalis* assemblages and sub-assemblages with environmental and anthropozoonotic factors in Busia, Western Kenya

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Abstract

Background Giardiasis is a common intestinal disease caused by the protozoan parasite *Giardia duodenalis*. The transmission of giardiasis is influenced by a complex interplay of environmental hazards such as contaminated water sources, markets, and/or roads or pathways including anthropozoonotic domestic animal-related factors and accessibility to healthcare. However, the risk factors of acquiring *G. duodenalis* including its assemblages and sub-assemblages have not been previously mapped in high burden populations in rural Africa. To mitigate transmission in rural African settings with special reference to Kenya, it is important to map out the clustering of the disease occurrence in relation to risk factors. Accordingly, this study evaluated geospatial clustering of *G. duodenalis* assemblages and sub-assemblages, in relation with environmental and domestic animal-related factors in Busia County, a rural setting of Western Kenya.

Methods In this cross-sectional study, 147 human stools were microscopically and molecularly analyzed by polymerase chain reaction (PCR) for *G. duodenalis* infection. A total of 88 human stool specimens positive for *G. duodenalis* deoxyribonucleic acid were genotyped at the glutamate dehydrogenase (*gdh*) and triose-phosphate isomerase (*tpi*) loci using PCR-restriction fragment length polymorphism (PCR-RFLP). Data on human and livestock population densities were obtained from the Kenya National Bureau of Statistics and incorporated into the geospatial analyses to explore their relationship with infection patterns. Distances from households to the nearest market, stream and/or river, road and/or pathway, and health facility were calculated and linked to geospatial clustering with the disease.

Results Overall, 59.9% of the human stool samples were positive for giardiasis in the sampled population. Geospatial clustering analyses indicated that giardiasis clustered with residing near a market (adjusted; aOR, 1.73), stream and/or river water source (aOR, 1.16), road (aOR, 1.74), an area with larger cattle (aOR, 1.83) or poultry (aOR, 1.36) densities (all $P < 0.05$). Likewise, assemblage A (aOR, 1.14 and 1.06) and sub-assemblage AII (aOR, 1.51 and 1.62) clustered with

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residing near stream and/or river water sources, and in localities with larger cattle density, respectively (all $P < 0.05$). Conversely, assemblage B (aOR, 1.87; 1.46; and 2.98) clustered with distance to the road, including cattle and poultry densities ($P < 0.05$ to $P < 0.01$). Furthermore, sub-assemblages BIII (aOR, 2.04 and 1.22) clustered with distance to the road and cattle densities, while BIV (aOR, 1.62) clustered with poultry densities ($P < 0.05$ to $P < 0.01$).

Conclusion *Giardia duodenalis* assemblage A and B including sub-assemblages AII, BIII, and BIV infections cluster differentially with residence at the nearest stream and/or river water source, market or road or within areas with larger cattle or poultry densities. In contrast, the absence of such environmental and domestic animal-related risk factors may represent areas that are relatively less affected by giardiasis. While we did not explicitly designate "low-risk" zones, the spatial clustering patterns suggest that environments with limited human-animal-environmental interface pressures are less conducive to giardiasis transmission.

Keywords Giardiasis, *Giardia duodenalis* assemblages, Sub-assemblages, Spatial clustering

Introduction

Giardiasis is a common intestinal disease caused by the protozoan parasite *Giardia duodenalis. Giardia duodenalis* is an enteric protozoa that mainly causes water and food-borne giardiasis in humans and animals following ingestion of cyst-contaminated water and/or food [1]. Giardiasis commonly presents with diarrhea, abdominal pain, bloating, nausea, and weight loss, though many infections remain asymptomatic [2]. Pathologically, Giardia disrupts intestinal epithelial integrity, leading to malabsorption, increased permeability, and villus atrophy [3]. Chronic infection can result in growth impairment, cognitive deficits, and post-infectious irritable bowel syndrome [3].

Giardiasis occurs globally, affecting an estimated 243.6 million people and is widespread in low-and-middle-income countries, including sub-Saharan Africa [4]. The large burden of giardiasis is attributable to contamination of the environment and water sources such as boreholes, shallow wells, rivers, streams, and springs by *G. duodenalis* cysts released from domestic animals and humans [5, 6]. In many rural African settings, contamination of domestic water sources by agricultural practices, livestock, and wildlife remains a major driver of giardiasis transmission [7, 8]. In addition, *G. duodenalis* possesses diverse assemblages and sub-assemblages which exhibit a predilection for infecting humans and/or domestic as well as wild animals, contributing to varying degrees of disease severity [9]. However, to the best of our knowledge, this is the first study to investigate whether the disease and the parasite genotypes cluster with risk factors in rural African settings of Kenya.

Geospatial clustering is a technique for assembling geographical points or features into clusters by their spatial proximity and/or shared non-spatial characteristics [10]. This techniques have been applied in mapping risk factors for several infectious diseases [10]. For instance, previous studies in the USA indicated that giardiasis incidence clustered in counties with ubiquitous water consumption from private wells [11]. Although no studies have investigated geo-spatial clustering in the current study area, previous studies in the area indicated that malaria cases clustered with vegetation cover, proximity to water bodies, and human population density [12]. Elsewhere in Africa, spatio-temporal studies in Ghana showed high spatial autocorrelation with districts with a higher aggregation of risk factors for intestinal worm morbidity [13]. In the United States, spatiotemporal mapping of giardiasis at the county level has shown that incidence is higher where private well water sources are common. These associations were further shaped by the presence of septic system sanitation, highlighting the role of water infrastructure in influencing transmission risk [11]. Equally, mapping studies in Ontario City, Canada showed a geospatial correlation of giardiasis with cattle population density and the use of manure for crop production [14], but failed to identify spatial clustering of the disease with specific risk determinants in rural communities [15, 16]. Additionally, recent studies have indicated that the sub-assemblages AII, BIII, and BIV, respectively, clustered in the Western, South-Eastern, and Mid regions of Norway [17]. Altogether, these studies support the notion of localised transmission dynamics largely due to variability in exposures to potential human-animal and environmental sources of the parasite. Therefore, in order to further identify giardiasis clustering with risk factors of transmission, this study investigated the geospatial clustering of *G. duodenalis* positive infections, assemblages and sub-assemblages with environmental and domestic animal-related factors in rural Busia County, Western Kenya.

Materials and methods

Study setting and methods

The description of the study area and methods are presented in our previous publication [18]. Briefly, this cross-sectional study was conducted in Busia county, Western Kenya. Patients presenting with abdominal complaints (nausea, emesis, anorexia, abdominal pain/cramps, bloating, flatulence, diarrhoea, and/or constipation) and

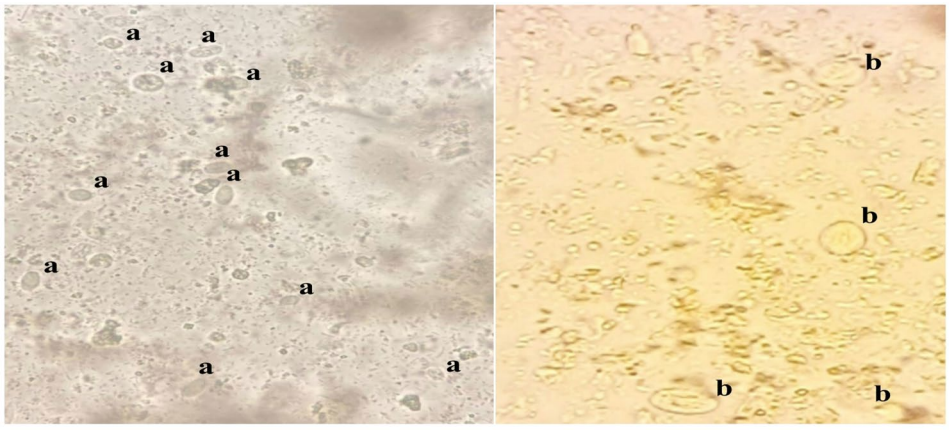

Fig. 1 *Giardia duodenalis* trophozoites (**a**) and cysts (**b**) on 0.9% normal saline wet mount and 5% Lugol's iodine wet preparation at X40 objectives (magnification 400)

Table 1 Primer, PCR conditions and restriction enzyme used in PCR-RFLPs

Genes	Size (bp)	Primers	Conditions	Restriction enzymes
gdh	432	5′-TCAACGTYAAYCGYGGYTTCCGT-3′ 5′-GTTRTCCTTGCACATCTCC-3′ 5′-CAGTACAACTCYGCTCTCGG-3′	1 cycle of 94 °C for 2 min., 56 °C for 1 min. and 72 °C for 2 min., 55 cycles of 94 °C for 30 s, 56 °C for 20 s and 72 °C for 45 s, and a final extension of 72 °C for 7 min.	NIa IV RSaI
tpi	605	R5′-AAATIATGCCTGCTCGTCG-3′ 5′-CAAACCTTITCCGCAAACC-3′		BbvI MnlI
	532	5′-CCCTTCATCGGIGGTAACTT-3′ 5′GTGGCCACCACICCCGTGCC-3′		

Glutamate dehydrogenase (*gdh*) and triose-phosphate isomerase (*tpi*) gene, template size of base pair, primer, PCR conditions and restriction enzymes used in digestion

referred by a clinician to the clinical laboratory for stool examination were analysed for the presence of *G. duodenalis* parasites. The sample size was calculated based on the Cochran sampling approach [19], as per previously reported 10.0% prevalence of giardiasis in a larger study in neighbouring rural settings of Bungoma, Kakamega, and Vihiga Counties in Western Kenya [20]. A total of 147 stool specimens from the patients were enrolled in the study calculated from a power of 80.0%, a critical alpha-value of 0.05, and confidence interval of 95%. Besides, those with other intestinal protozoa such as *Entamoeba histolytica*, helminths and bacterial infections or who had completed medication for the respective infection a fortnight prior to visiting the hospital were excluded from the study.

Microscopic examination was performed as per the CDC guidelines for the presence of parasites in the stools [21]. Briefly, the stool specimens were macroscopically recorded as formed, semi-formed, loose, and presence of pus cells, mucus, foul smelling or greasy; and microscopic for presence of trophozoites and/or cysts of *G. duodenalis*. Parasite density per 1.0 g stool specimen were recorded as (trophozoite and/or cyst/µL of stool). The microscopic examination was performed 3 times per specimen for confirmation, and the criteria for positive *G. duodenalis* were presence of active motile flagellated trophozoites and thick hyaline wall of cysts (Fig. 1). To ensure consistency and accuracy of the microscopic examinations, the stool smears were independently examined and the number of trophozoites and cysts recorded by two microscopists. A third microscopists examined and recorded results of all smears discrepant at ≥ 5% in the first reading (Table 1).

Deoxyribonucleic acid (DNA) was extracted from 88 microscopy confirmed *G. duodenalis*-positive stool specimens using the QiAmp® DNA stool Mini kit (Qiagen, Crawley, West Sussex, UK) as per the manufacturer's protocols. Genotyping of *G. duodenalis* assemblages and sub-assemblages using a multi-loci polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) approach at *glutamate dehydrogenase* (*gdh*) and *triose-phosphate isomerase* (*tpi*) gene loci (Table 1) was performed as previously described [22, 23],].

The Figure (2.1) shows PCR-RFLP analysis of the NIa IV digest of the *gdh*-PCR amplicons separated on ethidium bromide stained 4% agarose gels. Lane M: 50 bp ladder marker; lane 1,3,4,6,7,9,10: sub-assemblages BIII/BIV at 290 and 120 bp; lane 5,8: sub-assemblage AII at 90 and 120 bp; and lane 2: sub-assemblage AI at 90, 120, and 150 bp. Figure 2.2 shows *gdh*-PCR amplicon RSaI digest on ethidium bromide-stained 4% agarose gels. Lane M: 50 bp ladder marker and lane 1–6: sub-assemblage BIII at 130 and 300 bp. Figure 2.3 shows *tpi*-RFLP analysis of the BbvI digest of the TPI-PCR products separated

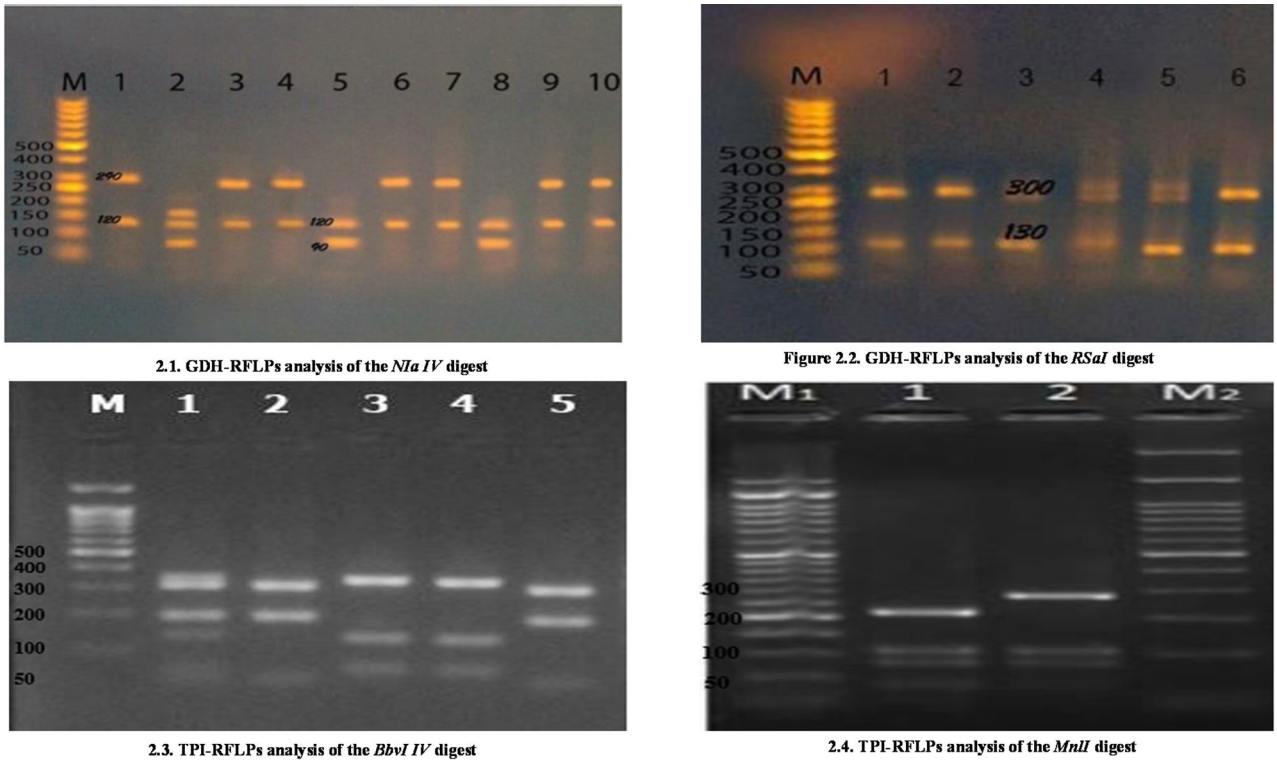

Fig. 2 Agarose gel bands of *gdh* and *tpi*-polymerase chain reaction-restriction fragment length polymorphism analysis

on ethidium bromide stained 4% agarose gels. Lane M: 100 bp ladder marker; lanes 2 and 5: assemblage A; lanes 3 and 4: assemblage B; and lane 1 mixed A and B assemblages. Figure 2.4 shows, *tpi*-RFLPs amplicon MnlI digestion on ethidium bromide stained 4% agarose gels. Lane M1 and M2: 50 bp and 100 bp ladder markers; lane 1: sub-assemblage BIII and lane 2: sub-assemblage BIV.

Environmental information on water sources (boreholes, shallow wells, streams, rivers, springs, rainwater and piped water) was collected using structured questionnaires while data on living alongside domestic animals including cattle, goats, sheep, and/or poultry (chickens, turkeys, ducks, geese, pigeons, and doves) were captured from all the 147 patients using an observational checklist. Data for human and crop farming population densities were obtained from the Kenya Bureau of Statistics (KBS) [24], whereas cattle, goats, and poultry population densities were obtained from the Kenya 2021 Animal Census Report [25]. Information on latitudes and longitudes was obtained using the Geographic Information System (GIS) of landmarks including church, school, health facility, market, and cattle dip tanks nearest the patient's residence. Information on the distance in metres (m) for each patient's residence to the nearest market, streams/rivers, public transport roads, and health facilities was estimated via spatial-autocorrelation calculations using the ArcGIS® 10.3.1 software (ArcGIS® 10.3.1, Version 6.0., California, Esri: GIS Corp., USA). The information on the distance from each patient's residence to the nearest market, streams/rivers, public transport roads, and health facilities was relevant because it provided insight into potential risk factors and exposure pathways for giardiasis transmission. For instance, proximity to markets and transport routes can influence human interactions, sanitation practices, and the spread of infection. Whereas, residing near to streams or rivers reflects possible exposure to contaminated water sources. Distance to health facilities is equally important, as it determines healthcare-seeking behavior, early diagnosis, and timely treatment, which directly affect disease burden and control. Human and animal populations were classified into two groups based on population density in the county as per the 2019 census as follows: A population greater than 527 for humans, 125 for cattle, 500 for goats, and 5,000 for poultry were used as cut-off points with a population density below the cut-off used as the reference for geospatial regression analysis [24,26,]. Distance-related factors were categorised into two based on previous studies on factors influencing transmission of malaria, a prevalent infectious disease in the present study area [27], sharing overlapping risk factors such as presence of poor drinking water source and lack of personal and community hygiene [28]. In the current study, residing < 1,500 m to the nearest stream and/or river water source, < 3,000 m to the nearest road, and < 2,500

to the nearest health facility in metres were used as reference points in the geospatial analyses.

Data analysis

Data were analysed using the statistical package for the social sciences (IBM® SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp., USA). Categorical data on factors associated with *G. duodenalis* based on patient responses were analysed using unadjusted as well as gender and age adjusted logistic regressions. Geospatial clustering of giardiasis including *G. duodenalis* assemblage and sub-assemblage infections with the environmental and anthropozoonotic risk factors was performed using ArcGIS® 10.3.1 software (ArcGIS® 10.3.1, Version 6.0. California, Esri: GIS Corp., USA) for generation of ArcMap using generalized estimating equations with an exchangeable working correlation matrix. The variables with a $P<0.1$ in univariate analysis were included in an initial multivariable model, while those with a $P>0.1$ were removed using backward elimination method. References groups used for the logistic regression analyses included those who were residing far and in low population density per square kilometre. The final model only retained variables that were significant at the level of $P<0.05$.

Ethical approval

This study was conducted in accordance with the tenets of human subjects' research as outlined in the Helsinki Declaration [29]. The current study obtained approvals from the Institutional Review Committee of the Masinde Muliro University of Science and Technology (MU/403012-V36), and the National Commission for Science, Technology, and Innovation (NACOSTI 436602). A permit for the study was obtained from the Ministry of Health, Busia county.

Results

Factors associated with Giardia duodenalis infection. Evaluation of factors associated with giardiasis is presented in Table 2. The use of stream and/or river water (unadjusted; uOR, 3.61; 95% CI, 1.79–7.29 and adjusted; aOR, 2.16; 95% CI, 1.24–4.61), and residence in households with cattle (uOR, 2.59; 95% CI, 1.28–5.22 and aOR, 1.85; 95% CI, 1.38–4.21), goats (OR, 2.97; 95% CI, 1.04–8.48) or poultry (uOR, 2.41; 95% CI, 1.21–4.80 and aOR, 2.13; 95% CI, 1.13–3.48) were associated with giardiasis.

Geospatial clustering of giardiasis with determinants of infection. Spatial evaluation of factors potentially driving transmission of *G. duodenalis* infection (Table 3) in this rural setting revealed that residing within the nearest 58 – 3,000 m to the market (uOR, 1.66; 95% CI, 1.47–2.92 and aOR, 1.73; 95% CI, 1.56–2.95), 0–1,500 m to the closest stream and/or river (uOR, 1.05; 95% CI, 1.72–5.26 and aOR, 1.16; 95% CI, 1.25–3.23), 7 – 3,000 m to the road (uOR, 1.93; 95% CI, 1.69–6.27 and aOR, 1.74; 95% CI, 1.22–4.41) or in a locality with a population density per square kilometre of 125-5,000 cattle (uOR, 2.36; 95% CI, 1.93–5.99 and aOR, 1.83; 95% CI, 1.35–5.93) or poultry (uOR, 1.46; 95% CI, 1.52–5.61 and aOR, 1.36; 95% CI, 1.23–3.72) were associated with higher probability of presenting with giardiasis.

Factors related to geospatial clustering of Giardia duodenalis assemblage infections. The association of selected geo-spatial risks with *G. duodenalis* assemblages is shown in (Table 4). Evaluation of spatial risk factors with assemblage infections indicated that residing within the nearest 0–1,500 m to the stream and/or river (uOR, 1.24; 95% CI, 1.35–4.34 and aOR, 1.14; 95% CI, 1.02–4.41) or in an area with >125 cattle per square kilometre (uOR, 1.78; 95% CI, 1.61–5.28 and aOR, 1.06; 95% CI, 1.06–6.63) were spatially correlated with assemblage A infections, whereas living in a household at 7 – 3,000 m to

Table 2 Factors associated with giardiasis

Characteristic	Negative	Positive	Unadjusted OR (95% CI)	*P*	Adjusted OR (95% CI)	**P*
Use of water from streams and/or rivers						
Yes	24	54	3.61 (1.79–7.29)	**< 0.001**	2.16 (1.24–4.61)	**0.026**
No	35	34	Ref			
Cattle						
Yes	19	45	2.59 (1.28–5.22)	**0.008**	1.85 (1.38–4.21)	**0.037**
No	40	43	Ref			
Goat						
Yes	14	19	2.97 (1.04–8.48)	**0.041**	2.21 (0.84–6.74)	0.058
No	45	69	Ref			
Poultry						
Yes	20	47	2.41 (1.21–4.80)	**0.012**	2.13 (1.13–3.48)	**0.022**
No	39	41	Ref			

Data presented are the number (n) of DNA samples analysed, and odds ratios (OR) with 95% confidence interval (CI). *P*, unadjusted and **P*, age and gender adjusted logistic regressions. Positive individuals with microscopy and/or polymerase chain reaction confirmed *G. duodenalis* infections, whereas negative individuals comprised negative *G. duodenalis* microscopy and/or polymerase chain reaction results for *G. duodenalis* infection. Ref., reference groups were individuals not exposed to the factor under analysis. Values in bold are significant *P*-values

Table 3 Geospatial association of giardiasis with possible risk factors

Geospatial variables	Negative	Positive	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Distance (metres) to:				
Markets				
58 – 3,000	27	69	1.66 (1.47–2.92) *	1.73 (1.56–2.95) *
>3,000	32	19	Ref.	
Stream and/or river water source				
0–1,500	30	62	1.05 (1.72–5.26) **	1.16 (1.25–3.23) *
>1,500	29	16	Ref.	
Road				
7 – 3,000	34	64	1.93 (1.69–6.27) *	1.74 (1.22–4.41) *
>3,000	25	24	Ref.	
Health facility				
125-2,500	35	46	1.17 (0.55–5.35)	1.04 (0.89–2.76)
>2,500	24	42	Ref.	
Population density per sq. km.				
Human				
>527	16	37	1.27 (0.62–2.23)	1.23 (0.62–3.17)
404–527	43	51	Ref.	
Cattle				
>125	22	59	2.36 (1.93–5.99) *	1.83 (1.35–5.93) *
26–125	37	29	Ref.	
Goats				
>500	4	9	1.78 (0.53–3.15)	1.02 (0.62–3.76)
101–500	55	79	Ref.	
Poultry				
>5,000	28	71	1.46 (1.52–5.61) *	1.36 (1.23–3.72) *
38 – 5,000	31	17	Ref	

Data are presented as number (n) of DNA samples analysed, and odds ratio (OR) with 95% confidence interval (CI) for geospatial associations of factors associated with risk of acquiring *G. duodenalis* infection. Positive individuals had microscopy and/or polymerase chain reaction confirmed *G. duodenalis* infection, while negative patients lacked *G. duodenalis* microscopy and/or polymerase chain reaction positive results. Ref, reference category. *$P<0.05$ and **$P<0.01$

the nearest road (uOR, 1.93; 95% CI, 1.58–6.48 and aOR, 1.87; 95% CI, 1.62–7.23), within a locality with cattle population density of > 125 per square kilometre (uOR, 1.31; 95% CI, 1.18–4.16 and aOR, 1.46; 95% CI, 1.28–6.13) or > 5,000 birds per square kilometre (uOR, 1.29; 95% CI, 1.08–4.09 and aOR, 1.18; 95% CI, 1.29–6.85) were associated with higher probability of assemblage B infections.

Table 4 Geospatial association of *Giardia duodenalis* assemblage with putative risk factors of infection

Geospatial variables	Negative	Positive	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Assemblage A, *n* = 18				
Distance (metres) to:				
Streams and/or rivers				
0–1,500	59	13	1.24 (1.35–4.34)*	1.14 (1.02–4.41)*
>1,500	21	5	Ref.	
Road				
7 – 3,000	21	8	1.52 (0.74–2.64)	1.34 (0.34–1.78)
>3,000	49	10	Ref.	
Population density per sq. km.				
Cattle				
>125	47	12	1.78 (1.61–5.28)*	1.06 (1.42–6.63)*
26–125	23	6	Ref.	
Assemblage B,*n* = 56				
Distance (metres) to:				
Road				
7 – 3,000	25	39	1.93 (1.58–6.48)*	1.87 (1.62–7.23)*
>3,000	7	17	Ref.	
Streams and/or rivers				
0–1,500	18	29	1.38 (0.98–3.57)	1.12 (0.73–2.64)
>1,500	14	27	Ref.	
Population density per sq. km.				
Cattle				
>125	20	39	1.31 (1.18–4.16)*	1.46 (1.28–6.13)*
26–125	12	17	Ref.	
Poultry				
>5,000	29	42	1.29 (1.08–4.09)**	2.98 (1.29–6.85)**
38 – 5,000	3	14	Ref.	

Data are presented as number (n) of patients, and odds ratio (OR) with 95% confidence interval (CI) for geospatial associations with risk factors for acquiring *G. duodenalis* infections. Positive individuals had confirmed *G. duodenalis* assemblage infection, while negative patients lacked the specified assemblage infections. Ref, reference category. *$P<0.05$ and **$P<0.01$

Factors clustering with Giardia duodenalis sub-assemblage infections. Analysis of selected spatial factors with *G. duodenalis* sub-assemblages (Table 5) showed that sub-assemblage AII infections clustered with residing at a distance 0–1,500 m to the nearest stream and/or river (uOR, 1.28; 95% CI, 1.11–4.94 and

Table 5 Geospatial association *Giardia duodenalis* sub-assemblages with risk factors

Geospatial variables	Negative	Positive	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Sub-assemblage AII, *n* = 7				
Distance (metres) to:				
Streams and/or rivers				
0–1,500	8	5	1.28 (1.11–4.94)*	1.51 (1.32–5.79)*
>1,500	3	2	Ref.	
Population density per sq. km.				
Cattle			1.43 (1.10–4.32)*	1.62 (1.26–5.67)*
>125	9	3	Ref.	
26–125	4	2		
Sub-assemblage BIII, *n* = 27				
Distance (metres) to:				
Road				
7 – 3,000	21	18	2.15 (1.74–7.65)**	2.04 (1.54–6.71)**
>3,000	8	9	Ref.	
Population density per sq. km				
Cattle				
>125	22	17	1.54 (1.17–3.86)*	1.22 (1.13–3.94)*
26–125	7	10	Ref.	
Sub-assemblage BIV, *n* = 15				
Population density per sq. km				
Poultry				
>5,000	31	11	2.24 (1.36–4.29)*	2.03 (10.42–8.38)*
38 – 5,000	10	4	Ref.	

Data are presented as number (n) of patients, and odds ratio (OR) with 95% confidence interval (CI) for geospatial associations with risk factors for having *G. duodenalis* infections. Positive individuals had confirmed *G. duodenalis* assemblage infection, while negative patients lacked the sub-assemblage infection were individuals lacking the specified sub-assemblage. Ref, reference category. *$P<0.05$ and **$P<0.01$

aOR, 1.51; 95% CI, 1.32–5.79) and in a locality with > 125 cattle density per square kilometre (uOR, 1.43; 95% CI, 1.10–4.32 and aOR, 1.62; 95% CI, 1.26–5.67). On the other hand, sub-assemblage BIII infections clustered with residence at a distance of 7 – 3,000 m to the nearest road (uOR, 2.15; 95% CI, 1.74–7.65 and aOR, 2.04; 95% CI, 1.54–6.71); or in an area with > 125 cattle population density per square kilometre (uOR, 1.54; 95% CI, 1.17–3.86 and aOR, 1.22; 95% CI, 1.13–3.94). In contrast, sub-assemblage BIV infections clustered with living within an area with > 5,000 bird population density per square kilometre (uOR, 2.24; 95% CI, 1.36–4.29 and aOR, 2.03; 95% CI, 1.42–8.38).

Clustering of giardiasis assemblages and sub-assemblages with spatial determinants of giardiasis. Spatial evaluation of factors potentially driving transmission of *G. duodenalis* infection (Fig. 3A) in this rural setting, revealed that residing within the nearest 58 – 3,000 m to the market, 0–1,500 m to the stream and/or river, 7 – 3,000 m to the nearest road, an area with cattle density per square kilometre > 125, or poultry density > 5,000 per square kilometre clustered with higher probability of having giardiasis.

The association of selected geo-spatial risks with *G. duodenalis* assemblages is shown in (Fig. 3B). This analysis shows that residing within a radius of 0–1,500 m to the stream and/or river in an area with > 125 cattle density per square kilometre clustered with greater odds for assemblage A infection. Additionally, residing in a household at 7 – 3,000 m to the nearest road, within a locality with cattle density of > 125 per square kilometre, and > 5,000 birds per square kilometre clustered with higher odds of having assemblage B infection.

Examination of selected spatial factors with *G. duodenalis* sub-assemblages (Fig. 3C) showed that residing within 0–1,500 m from the stream and/or river and/or in an area with > 125 cattle per square kilometre clustered with assemblage AII infections. Conversely, living at a distance of 7 – 3,000 m from the closest road or in a locality with > 125 cattle density per square kilometre clustered with sub-assemblage BIII infections. Likewise, residing in a region with > 5,000 birds per square kilometre clustered with greater probability of sub-assemblage BIV infection.

Discussion

Several environmental and anthropozoonotic factors associated with giardiasis have been documented across rural Africa. For instance, in rural Ethiopia and Ghana, the risk of giardiasis has been linked to the type of water source and the presence of domestic animals [13, 15]. Although no studies have extensively shown the clustering of giardiasis with environmental and anthropozoonotic risk factors in sub-Saharan Africa, earlier studies in Canada illustrated spatial clustering of giardiasis with livestock density [14]. The present study is the first to our knowledge from rural sub-Saharan Africa to report on spatial clustering of *G. duodenalis* assemblages and sub-assemblages with risk factors of the disease.

We have previously shown that *G. duodenalis* assemblage B is the predominant cause of giardiasis and is correlated with residing in a homestead with cattle and/or poultry [9]. In the current, study giardiasis was associated with usage of water from streams and/or rivers, and domestic animals such as cattle, goats, and/or

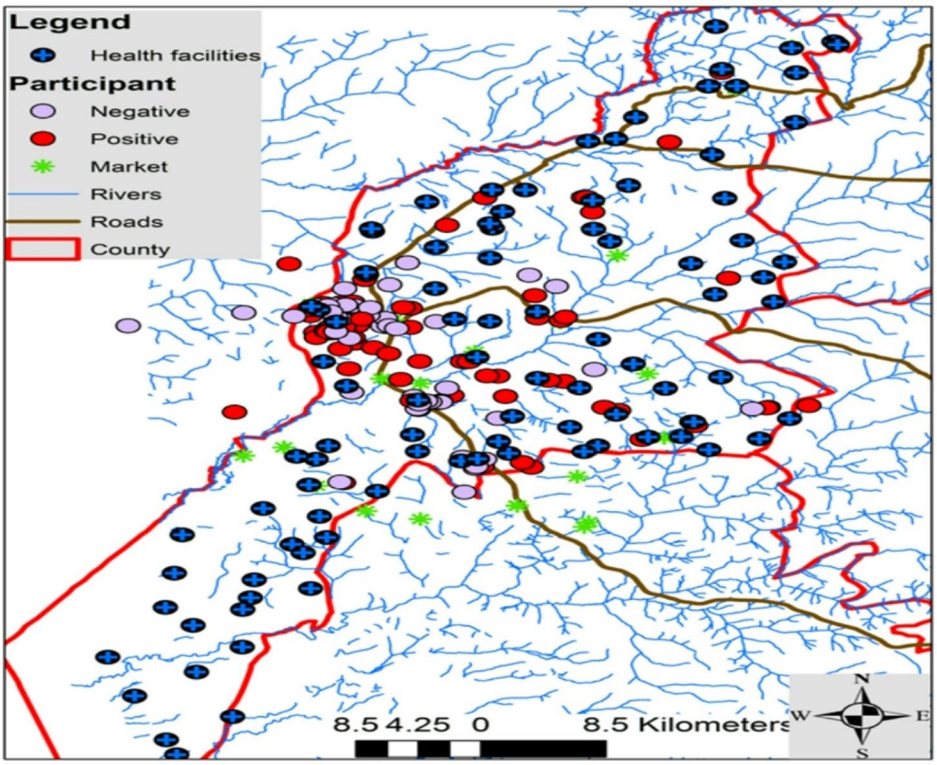

Fig. 3A Geospatial clustering of giardiasis with risk factors of giardiasis transmission. The distribution of giardiasis is represented by the coloured circles as follows purple, negative and red, positive of *G. duodenalis*. The determinants of giardiasis transmission are shown as blue plus sign, health facility; green star; market; blue line, river or stream; brown line, road; and red line, and county boundary

poultry after adjusting for gender and age. These results are consistent with those of other investigators indicating that having giardiasis is associated with residing in a homestead with cattle in Egypt, Ethiopia, and Tanzania [30-32], as well as with goats, ducks, and chicken in Côte d'Ivoire, Nigeria, and Tanzania [30, 33, 34]. Consistent with the current findings, studies in Kisumu, Kenya, detected high rates of *G. duodenalis* in stool samples of animals domicile to urban settings [35]. Overall, domestic animals form an important link in giardiasis transmission possibly through contaminating the environment with their faecal matter.

The spatial-clustering of giardiasis with distance from the household to the distance to the nearest market, stream and/or river water source or road, and residing in a locality with a high density of cattle or poultry, suggests a higher risk of *G. duodenalis* cyst contamination in these environments. In addition, it is a common practice for residents of this rural area to share households with small domestic animals especially poultry and calves with poor disposal of their faecal matter which can result in increased risk of contamination with giardia cysts from infected animals. Of note, is the fact that in rural settings there are no fixed demarcations and/or boundaries regarding households, farms, or water sources with most of these resources being communal and freely shared [36]. As such residents can access neighbours' homesteads, make contact with animals, and other farms belonging to other people, share markets, water sources, among other resources, therefore, poses a complex interplay of behavioural, environmental, and domestic animal-related factors in the transmission of giardiasis.

Further analysis indicating spatial clustering of assemblage A and sub-assemblage AII with distance to the nearest stream water and/or river water source and localities with larger cattle density; assemblages B and sub-assemblage BIII with nearest distance to the road and high cattle density; and sub-assemblage BIV with larger poultry density areas are consistent with our previous findings that these were the common genotypes of *G. duodenalis* infection in this rural area [9]. Similarly, clustering of anthropogenic sub-assemblage AI and BII with residing near roads is possibly attributable to limitations of roadside public toilets, comfort stops, eateries and/or cafes further resulting in contaminating the local environment adjacent to the roads. Besides, previous epidemiological and geospatial studies in Ethiopia and United States of America indicated a higher risk of acquiring *G. duodenalis* infection from drinking water obtained from contaminated rivers and private wells [11,37]. Likewise, multi-country enteric studies in children indicated a link between surface water sources and acquiring protozoal

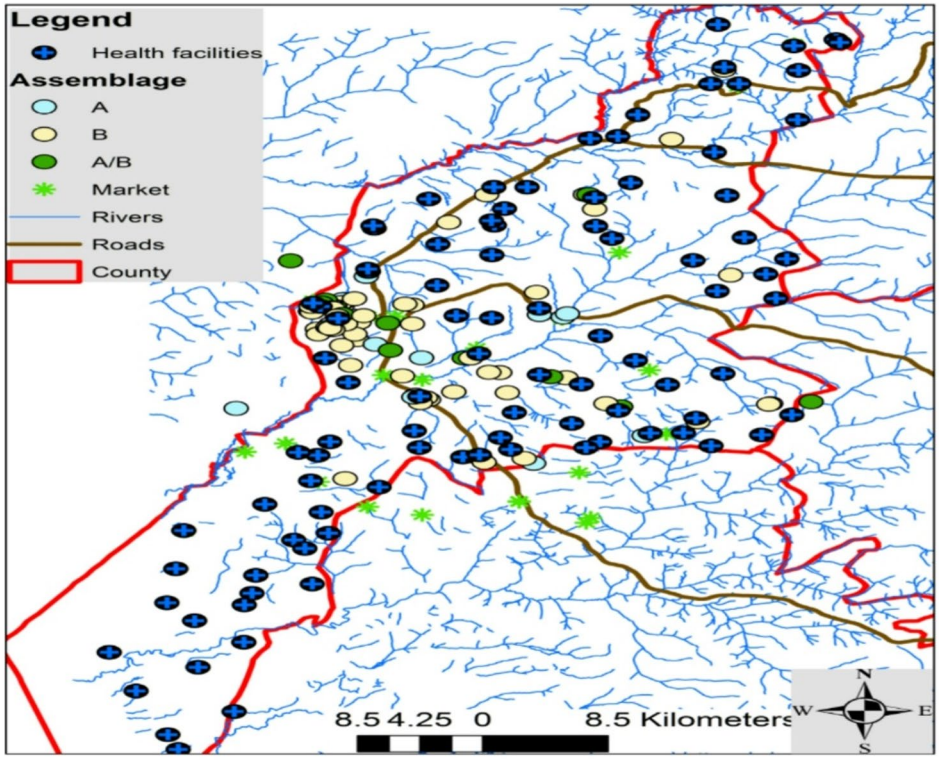

Fig. 3B Geospatial clustering of *G. duodenalis* assemblage infection with risk factors of giardiasis transmission. The distribution of the assemblages is represented by the coloured circles as follows light blue, assemblage A; yellow, assemblage B; and dark green, mixed assemblages A and B. The determinants of giardiasis transmission are shown as blue plus sign, health facility; green star; market; blue line, river or stream; brown line, road; and red line, county boundary

infections including *G. duodenalis* infection [38]. Additional studies in Norway indicated that large community outbreak of waterborne giardiasis was associated with consumption of water contaminated with leaking sewage pipes or that is insufficiently treated [39], suggesting that water safety is a key factor in the transmission both endemic and non-endemic low- and middle-income as well as high income countries.

The spatial clustering of *G. duodenalis* cysts with distance to the market suggests potential contamination of food products and market environments with food leftovers, roaming animals, effluents from eateries and slaughterhouses [40]. This is in part consistent with previous studies that detected *G. duodenalis* cysts in water samples, fresh produce samples, and soil samples [41], in horticultural products in Maputo markets, Mozambique [42]. The increased risk of acquiring giardiasis from these sources is possibly related to consumption of raw produce, especially without hand washing with safe water. Likewise, previous studies in Uganda showed that having large livestock herds was associated with high rates of human infection with cyst densities ranging from 110 to 270 cysts/g [43], as well as a correlation of having *G. duodenalis* infection with household ownership of cattle [44]. The clustering of giardiasis and the assemblage infections with poultry densities is consistent with our previous studies showing associations of these genotypes with residing in a homestead with poultry [9], and those of others showing a correlation between giardiasis and residing with poultry [10]. Given that poultry farming in the study area is mainly of indigenous breeds raised through the free-range system [45], increased exposures to the birds, their products and waste in homesteads in areas with large bird densities results in high environmental contamination and transmission of giardiasis. It is also possible that high density animal areas may lead to higher *Giardia* infections, not only to the animals but also to humans especially in rural settings with less water and waste infrastructure.

The limitations of the present study include the fact that temporal clustering of the genotypes was not examined even though *Giardia* cases, river water *Giardia* contamination, and diarrhoea cases are associated with surface run-offs and relief factors such as rainfall pattern, relative humidity, moisture, temperature, and flooding [46, 47]. In addition, mapping of sanitation, sewage, wastewater, and sludge treatment plants was not examined in this study despite studies in South Africa and

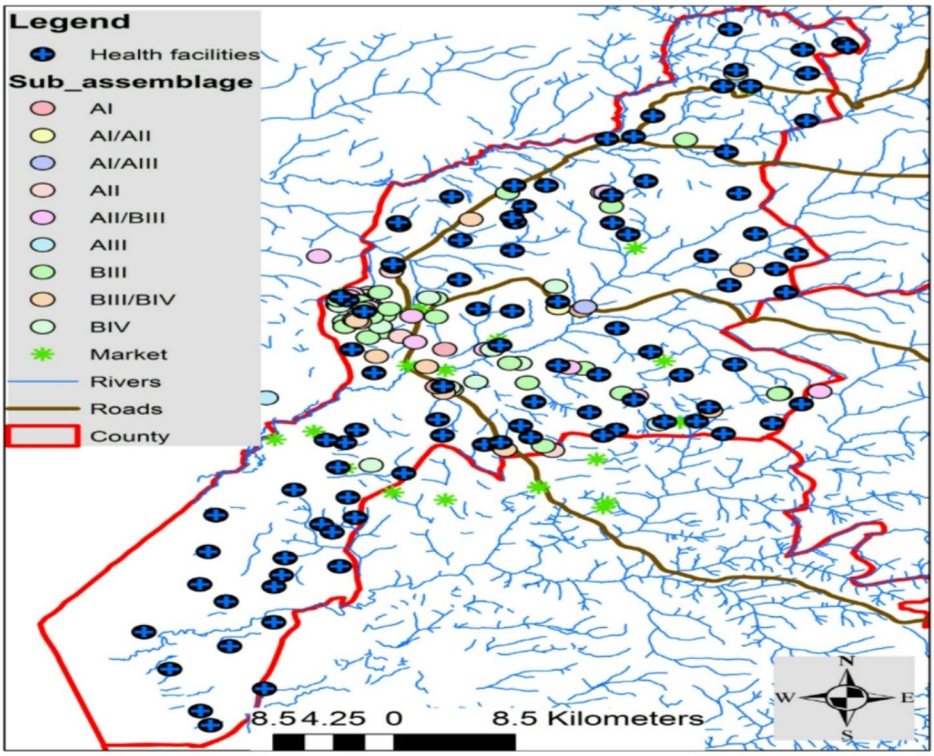

Fig. 3C Geospatial clustering of sub-assemblage infection with risk factors of giardiasis transmission. The distribution of the sub-assemblages is represented by the coloured circles as follows bright pink, sub-assemblages AI; baby yellow, mixed sub-assemblages AI/AII; bright sky, mixed sub-assemblages AI/AIII; baby pink, mixed sub-assemblages AII/BIII; bubble gum, sub-assemblages AIII; baby blue, sub-assemblages BIII; seaweed, sub-assemblages BIII; peach, mixed sub-assemblages BIII/BIV, and mint, sub-assemblages BIV. The determinants of giardiasis transmission are shown as blue plus signs, health facility; green star, market; blue line, river or stream; brown line, road; and red line, county boundary

Tunisia illustrating that these sites are also important sources of *G. duodenalis* assemblage A and B infections [48, 49]. Furthermore, previous five-year giardiasis incidence mapping studies in Colombia showed a higher rate in high population dense region of Bogota (17.7%) relative to Antioquia (10.9%), Atlantico (8.6%), and Risaralda (6.5%) [50], suggesting a concentration of multiple interacting factors in regions with huge populations. Moreover, sampling both domestic and wild animals to investigate the infection rates of *G. duodenalis* and with which assemblages and sub-assemblages could improve the understanding of the potential zoonotic transmission cycle. Future research should also incorporate remote sensing techniques, which offer powerful tools for integrating environmental, hydrological, and climatic variables into spatial analyses of giardiasis risk.

Conclusion

Giardiasis and the common assemblages (A and B) and sub-assemblages (AII, BIII, and BIV) differentially cluster with river and/or stream water source, living near a market or road, and cattle or poultry population density. Altogether, the results of this study suggest cyclic interactions between environmental and domestic animal-related factors in the transmission dynamics of giardiasis in this highly socially interactive population and rural setting. Future research should extend this work by integrating temporal analyses, remote sensing tools, and direct sampling of animal reservoirs to better disentangle environmental and zoonotic contributions to infection risk. For policy makers, our results highlight the importance of strengthening rural water infrastructure, improving livestock human interface management, and integrating environmental surveillance into routine public health strategies. Together, these measures can inform targeted interventions to reduce the burden of giardiasis in vulnerable communities.

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Author contributions
TW and RI conceptualized the study. RI, EB and JW performed laboratory analysis. TW and EB performed statistical analyses and co-drafted the manuscript. VB, PK, WS, and GJ interpreted and critically revised the manuscript. All authors have read and approved the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Competing interests
The authors declare no competing interests.

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