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Concentration and risk assessment of *Cryptosporidium* infection associated with exposure to the Njoro River, Njoro Sub-County, Nakuru, Kenya

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Abstract

Background *Cryptosporidium* is a gastrointestinal pathogen. The oocysts are transmitted through the environment, and drinking contaminated water is one particular route. There is heavy pollution of *Cryptosporidium* in Njoro River, the main source of drinking water for humans and animals around the watershed. However, there is no information on the parasite concentration and estimated health risk exposed to these populations. This study determined the level of contamination and risk of infection by *Cryptosporidium* parasites in Njoro River. Water samples were collected monthly from three ecological sites along Njoro River for twelve months. *Cryptosporidium* oocysts were concentrated from these water samples using calcium carbonate flocculation method, examined and counted using epifluorescent microscopy. Quantitative microbial risk assessment was applied to estimate the health risk of *Cryptosporidium* infection in Njoro River using a beta-Poisson dose–response model.

Results The concentration of *Cryptosporidium* parasites in Njoro River is 0.936 ± 0.73 oocysts/litre. However, this concentration fluctuates with ecological site of the river; highest concentration occurs at downstream (1.325 ± 0.73), followed by midstream (0.917 ± 0.74) and least at upstream (0.567 ± 0.54). Concentration of *Cryptosporidium* in the river is higher during wet than dry seasons, with the difference in mean concentrations between the two seasons being significant ($t_{(34)} = -6.101$, $p < 0.01$). There was a negative correlation between *Cryptosporidium* concentration, temperature and pH, while a strong positive correlation existed between *Cryptosporidium* concentration and turbidity. The daily probability of infection by *Cryptosporidium* in Njoro River watershed is 0.25, while the annual risk is 0.99.

Conclusions Njoro River is heavily polluted with *Cryptosporidium* parasites. This exposes both the humans and animals that drink water from this river to a high risk of cryptosporidiosis, a potentially fatal infection particularly in immunocompromised individuals.

Keywords *Cryptosporidium*, Njoro River, Water pollution, Infection risk

Background

Cryptosporidium is a protozoan parasite (Phylum Apicomplexa, Class Gregarinomorphea, Sub-class Cryptogregaria (Levine, 2018; Ryan et al., 2016). It is a gastrointestinal pathogen of both humans and animals, and causes a severe diarrheal disease, especially in immunocompromised humans (Halliez & Buret, 2015; Webb, 2019). It is shed in feaces in form of an oocyst which is protected from the environment by a hard shell

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(Arrowood, 2019; Leitch & He, 2011). Cryptosporidiosis is transmitted through the feacal-oral route via drinking water contaminated with oocysts. Outbreaks have commonly been associated with person-to-person and waterborne transmission. However, both foodborne (Gharpure et al., 2019) and zoonotic (Essendi et al., 2021) transmission have also been documented in recent past. Waterborne *Cryptosporidium* outbreaks have been reported, both on small scale and large scale. The largest outbreak was recorded in Milwaukee, Wisconsin in 1993, and affected an estimated population of 403,000 people (Corso et al., 2003; Salinsky, 2016; William et al., 1994). Such outbreaks disrupt families, communities, governments, businesses and cause enormous economic losses (Chyzheuskaya et al., 2017).

Infection with *Cryptosporidium* organism can also contribute to premature deaths of immunosuppressed individuals (El-Sayed & Fathy, 2019; Hunter & Nichols, 2002). For this reason, analysis of *Cryptosporidium* oocysts in water bodies such as rivers, lakes, reservoirs, and occasionally in treated water, has been of great public interest. Conventional water disinfection methods have proven futile in killing *Cryptosporidium* oocysts, and even the best water filters may allow a few organisms to pass through in treated water (Castro-Hermida et al., 2006; Daugschies et al., 2013; Murphy & Arrowood, 2019; Quilez et al., 2005). However, the health risks associated with drinking water obtained from public water supplies, contaminated with small numbers of oocysts, is unknown.

Quantitative Microbial Risk assessment (QMRA) has become a widely used tool for assessing the risk of infection from microbial pathogens (Haas et al., 2014; WHO, 2017). QMRA evaluates the risks posed by pathogens in water sources using four steps, which includes: (1) hazard assessment, (2) an exposure assessment, (3) dose–response assessment, and (4) a risk characterization. Different models such as Poisson dose–response model and exponential dose–response model have been developed to estimate the risk of infection using pathogen concentrations (Haas et al., 2014; QMRAwiki, 2017).

Njoro River is the main source of drinking water for both humans and domestic animals within the river watershed. Residents also use this water for washing, bathing, and cooking while children swim in the river (Merimba, 2021; Yillia et al., 2008). However, this river is polluted by waterborne pathogens shed by domestic animals, which can then infect humans (Jenkins & Maina-Gichaba, 2009). The present study aimed at determining *Cryptosporidium* parasite concentrations and estimated the risk of infection in Njoro River watershed. Knowledge of the estimated risk of *Cryptosporidium* infection in the watershed will guide in development of cryptosporidiosis control programs in Njoro Sub-County, Nakuru County in Kenya.

Methods

Study area

This study was conducted in Njoro River (Fig. 1), in Njoro Sub-County, Nakuru County, Kenya. Njoro River lies between longitudes 35°05′ E and 36°05′ E, and latitudes 0°15′ S and 0°25′ S (Mainuri & Owino, 2013). The river originates in the Eastern Mau and terminates in Lake Nakuru covering about 60 km in length.

Study design

This was a spatial–temporal study which involved microscopic examination and enumeration of *Cryptosporidium* oocysts contained in concentrated water samples. Water samples were collected monthly from three ecological sites along Njoro River; upstream (Neissuit), midstream (Ngata) and downstream (Kaptembwo) over a period of 12 months (between June 2019 and May 2020). The study also explored the effect of seasonality (dry and wet seasons) and various physico-chemical variables (temperature, pH and turbidity) on the concentration of *Cryptosporidium* along the three sampling sites of Njoro River. A beta-Poisson dose–response model was applied to evaluate the daily and annual infection risk of cryptosporidiosis in Njoro River watershed.

Water sampling

Twenty litres of water were collected monthly, from a depth of 20–30 cm below water surface, from each of the three sampling sites along Njoro River, by use of a Van Dorn water sampler. In total, 36 water samples were collected from the river, comprising of 12 samples from each sampling site. The pH, water turbidity and surface temperature of the water were recorded at the water collection site using multiprobe universal meter (Model HQ40d, @HACH Company, UK). Samples were transported to Biological Science laboratory at Egerton University for analysis within 12 h.

Concentration of *Cryptosporidium* oocysts

Calcium carbonate flocculation technique was used to concentrate water samples as described by Vesey et al. (1993). 100 ml of calcium chloride solution was added to 10 L of well-shaken sample and mixed well. To this mixture, 100 ml of sodium hydrogen carbonate solution was added and mixed well. The pH of the mixture was raised to approximately 9.0 by adding 100 ml of sodium hydroxide solution and mixed well. The precipitate of calcium carbonate was left to settle for a minimum of 4 h. The supernatant liquid was then aspirated leaving calcium carbonate residue undisturbed. Carefully, 200 ml 10%

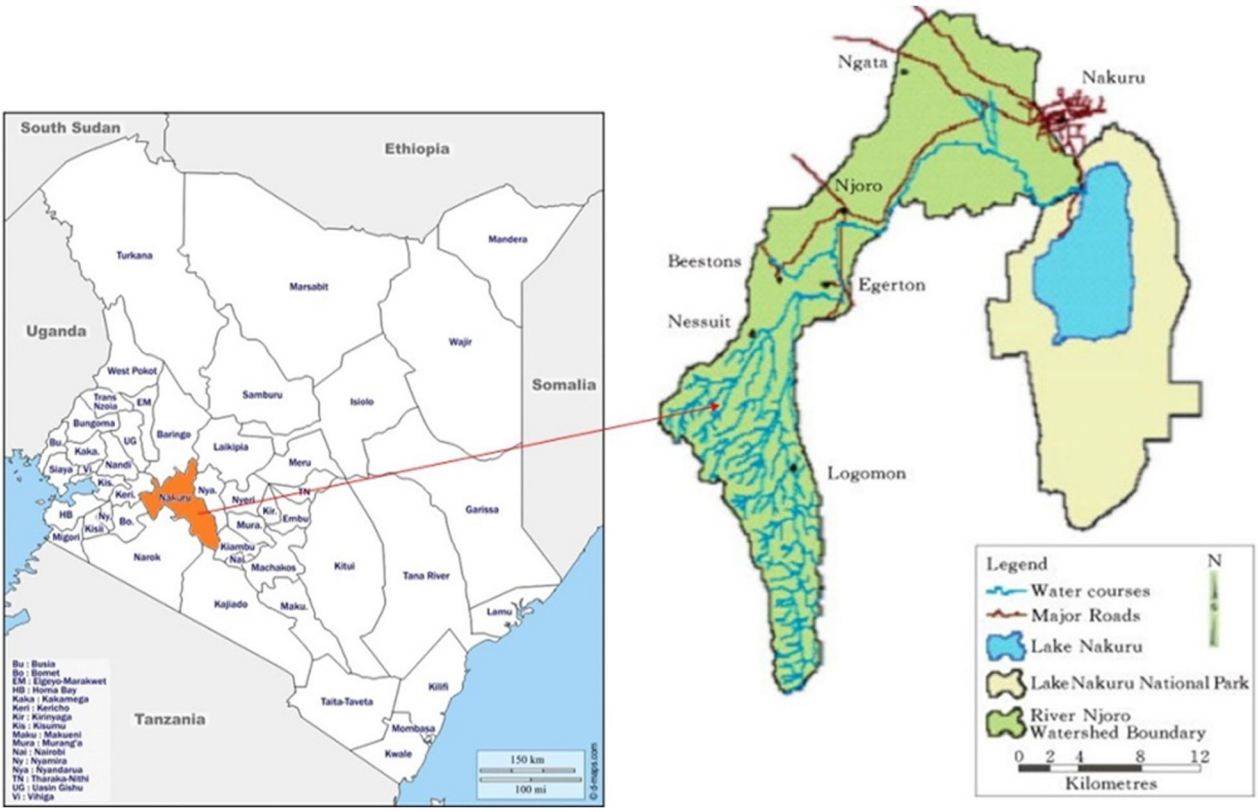

Fig. 1 Geographical location of River Njoro in Njoro Sub-County. River Njoro originates from the Eastern part of Mau forest and terminates into Lake Nakuru. It covers about 60 km in length (Mainuri & Owino, 2013)

(w/v) sulphamic acid solution was added, to completely dissolve the calcium carbonate precipitate. The sulphamic acid solution was added slowly in approximately 50 ml aliquots, to avoid excessive effervescence. At the same time, the mixture was gently shaken, tilted and rotated to ensure that all the calcium carbonate precipitate dissolved.

When the calcium carbonate had dissolved, the resulting sample was transferred into a 1000 ml centrifuge bottle and 100 ml of detergent solution (polyoxyethylene (20) sorbitanmonooleate) added and shaken vigorously to ensure any particulate matter got suspended in the solution and did not adhere to the sides of the container. The mixture was then transferred into the 1000 ml centrifuge bottle. This process was repeated with a further 100 ml quantity of detergent solution to ensure all particulate matter got transferred to the 1000 ml centrifuge bottle. Using 1 M sodium hydroxide solution, the pH of the mixture was adjusted to a value between of 2.5 and 3.5. Finally, the pH of the mixture was adjusted with 0.1 M sodium hydroxide solution to a value of between 5.5 and 6.5 by ensuring the mixture was continuously mixed throughout this process.

The sample was then concentrated further by centrifugation at 7200 rcf for 12 min at room temperature. The tube from the centrifuge was removed, and the supernatant liquid carefully discarded, leaving sufficient liquid to just cover the resulting pellet of particulate matter. The tube was shaken vigorously to re-suspend the particulate matter and the suspension transferred to a 50 ml centrifuge tube. Approximately 20 ml of detergent solution was added to the 1000 ml centrifuge bottle and rinsed to re-suspend any remaining particulate matter. This was transferred to the 50-ml centrifuge bottle and made to approximately 50 ml with water.

The suspension was centrifuged at 1050 rcf for 10 min at room temperature. The 50-ml tube from the centrifuge was removed, and the supernatant liquid discarded, ensuring particulate matter was not removed or discarded. The volume, V_P ml, of particulate material in the tube was estimated and recorded. Water was then added to the centrifuge tube and made to a known total volume, V_S ml. The tube was vortexed to re-suspend the pellet of particulate material and the suspension was ready to proceed directly to the purification stage and microscopic examination.

Microscopic identification of *Cryptosporidium* spp.

Concentrated water samples were subjected to microscopic examination and enumeration using epifluorescent microscopy after immunofluorescent staining of oocysts to define size and shape. Nuclear fluorochrome 4′, 6-diamidino-2-phenylindole (DAPI) was used to stain nuclei of oocyst sporozoites, and differential interface contrast (DIC) microscopy used to determine the internal morphology of oocysts. The use of DAPI and DIC microscopy in conjunction with IFA reduced the false positive and false negative results from raw water samples and final water samples (Shimizu et al., 2012; Smith et al., 2002).

Seasonal variation in *Cryptosporidium* concentrations

This study compared mean concentration of oocysts in Njoro River during dry and wet seasons. This was done to investigate seasonal variations in mean concentration of oocysts in the river and to study effects of weather patterns on the prevalence of oocysts. Annual weather pattern was determined using meteorological data obtained from a weather station located near River Njoro, Egerton University weather station, as shown in Table 1. (https://en.climate-data.org/africa/kenya/nakuru/njoro-765723/).

Cryptosporidium risk assessment

A Quantitative Microbial Risk Assessment tool was used to determine the risk of *Cryptosporidium* infection in Njoro River. This involved four steps: The first step entailed a description of the problem setting (Haas et al., 2014; QMRAwiki, 2017). *Cryptosporidium* parasites infect both humans and animals causing acute gastroenteritis, manifested with abdominal pains and diarrhoea. In immunosuppressed individuals, the parasite causes prolonged infections that can be fatal (Webb, 2019). The second step involved exposure assessment. Agricultural activities which involve use of animal dung as manure and unhygienic practices such as defecation of humans in forests within Njoro River watershed lead to runoff of manure in the river. These, coupled with direct disposal of industrial wastes into the river results in high concentrations of *Cryptosporidium* in Njoro River. Since both humans and domestic animals drink the contaminated water from River Njoro, humans are exposed to a high probability of cryptosporidiosis infection (Yillia et al., 2008). The third step involved dose–response assessment. The average *Cryptosporidium* dose consumed was obtained from concentration of *Cryptosporidium* oocysts in Njoro River, evaluated in the study, while the amount of surface water consumed per day was taken as 2 L, in accordance with the accepted reference value for a person weighing 60 kg (WHO/UNICEF, 2019). This dose was used as input in a dose–response model to predict the probability of *Cryptosporidium* infection. The mean dose of *Cryptosporidium* per exposure per day was determined using Eq. (1) below (Haas et al., 2014; QMRAwiki, 2017):

$$\text{Exposure dose} = C * q \tag{1}$$

where C is the concentration of *Cryptosporidium* in River Njoro (oocysts/l), q is the amount of surface water ingested per day (l/d).

The dose–response assessment in a QMRA estimates the risk of a response (illness, infection or death) given the dose of pathogen (Haas et al., 2014). This study applied a Beta-Poisson dose–response model, Eq. 2, to calculate probability of infection of an individual, given *Cryptosporidium* concentrations obtained in Njoro River and the dose an individual consumes per day. The values α and β are determined to be 0.115 and 0.176 for *Cryptosporidium* (Teunis et al., 2002).

$$P_{\text{inf /single}} = 1 - \left(1 + \frac{\text{Exposure dose}}{\beta}\right)^{-\alpha} \tag{2}$$

where $P_{\text{inf/single}}$ is the one day probability of infection, *Exposure dose* is the ingested dose on one day (in oocysts per day), α is set to 0.115, β is set to 0.176.

The final step is risk characterization. The probability of infection after drinking two litres of water from Njoro River in one day was then used to estimate annual risk of *Cryptosporidium* infection in Njoro Sub-County.

Risk characterization was achieved using Eq. 3 as follows (Haas et al., 2014):

Table 1 Table showing Njoro River watershed annual weather pattern (obtained from Egerton University weather station)

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Avg. Tempt (°C)	16.9	17.8	17.8	16.8	16	15.2	14.5	14.5	15.3	15.8	15.5	15.9
Min. Tempt°C	11.5	11.6	11.8	11.7	11.2	10.3	9.7	9.7	9.7	10.7	11.3	11.4
Max. Tempt °C	22.6	24.2	24.1	22.3	21.4	20.5	19.6	19.8	21	21.3	20.4	21
Precipitation/rainfall (mm)	21	21	53	109	74	53	61	78	53	94	100	45
Humidity (%)	52	45	51	66	69	69	71	72	65	66	72	63
Rainy days (Nos.)	3	2	5	9	7	7	9	9	6	9	11	6

$$P_{\text{inf /combined}} = 1 - (1 - P_{\text{inf /single}})^{N} \quad (3)$$

where $P_{\text{inf/combined}}$ is the probability of one or more infections over N exposure events, $P_{\text{inf}}/single$ is the single event probability of infection and N is 365 (the days in a year).

Data analysis

Data analysis for this study was conducted using SPSS version 20. The number of oocysts detected per litre was calculated by average of counts of three slides upon applying Eqs. 4 and 5 (Eveline et al., 2002; Schaefer, 2003):

$$\text{Number of oocysts in pellet} = \frac{\text{No. of oocysts in an analyzed drop * total mL of the pellet}}{\text{Volume of drop analyzed}} \quad (4)$$

$$\text{Number of oocysts/L} = \frac{\text{No. of oocysts in pellet}}{\text{No. of litres flocculated}} \quad (5)$$

The total number of oocysts observed divided by total volume of water investigated was used to calculate mean concentration of oocysts per site and month of the year. Mean differences between sites were determined using analysis of variance (ANOVA) followed by Tukey's post hoc test, to establish the influence of study sites; and independent sample *t*-test to ascertain influence of seasons. Spearman's rho nonparametric correlation analysis was used to study the relationship between physicochemical variables (Temperature, pH and turbidity) and oocysts concentration. Prior to analysis, the data was tested for suitability of parametric analysis by being subjected to Shapiro–Wilk test for normality and the Levene's test for homogeneity of variance tests.

Results

Mean oocyst concentration at sampling sites

The descriptive data for oocysts concentration at each of the three sampling sites along Njoro River and mean oocysts concentration for entire river structure are presented in Table 2.

Mean concentration of oocysts was 0.567 ± 0.54, 0.917 ± 0.74 and 1.325 ± 0.73 cells per litre at upstream, midstream and downstream sites, respectively. Overall mean concentration of *Cryptosporidium* oocysts in Njoro River is 0.936 ± 0.73 oocysts/litre.

From the data, it is evident that mean concentration of *Cryptosporidium* spp. increases downstream (Fig. 2).

The analysis of variance indicated that there existed significant difference in oocysts concentration among sites ($F_{(2, 33)} = 3.751$, $p = 0.034$) (Table 3).

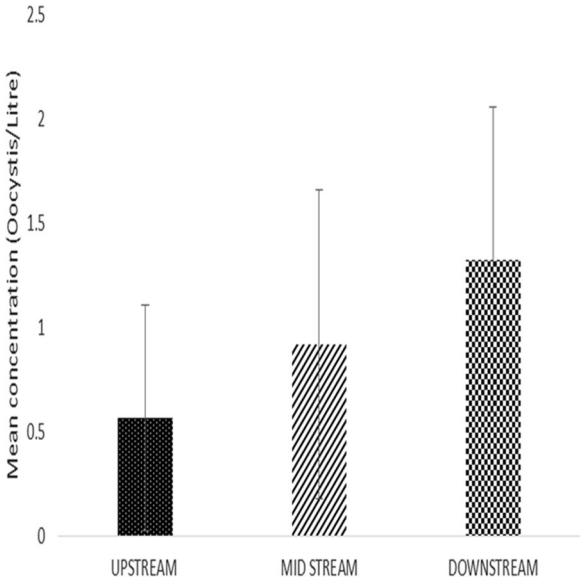

Fig. 2 Bar graph of mean oocyst concentration at sampling sites. Y axis represents the mean oocyst concentrations; X axis represents the sampling sites. Mean oocyst concentration is higher at the bottom stream and least upstream

Table 2 Descriptive statistics of *Cryptosporidium* oocysts concentration along Njoro River, Kenya

	No	Means	Standard deviation	Std. Error	95% confidence interval for mean		Min	Max
					Lower bound	Upper bound		
Upstream	12	.567	.5449	.1573	.22	.913	0	1.5
Midstream	12	.917	.742	.2142	.445	1.388	0	2.1
Downstream	12	1.325	.7313	.2111	.86	1.79	.2	2.4
Total	36	.936	.7302	.1217	.689	1.183	0	2.4

Table 3 ANOVA table showing mean concentration between upstream, midstream and downstream sites along Njoro River

	Sum of squares	*df*	Mean square	*F*	Sig
Between groups	3.457	2	1.729	3.751	.034
Within groups	15.206	33	.461		
Total	18.663	35			

Tukey's multiple comparison showed that significant difference existed in oocysts concentration between upstream and downstream ($p<0.05$); where the oocyst concentration in downstream site was significantly higher than concentration at the upstream site. However, significant differences were not observed between upstream and midstream as well as downstream and midstream ($p>0.05$) (Table 4).

Seasonality variation of oocyst concentration

T test results showed that mean concentration of oocysts is higher during wet season compared to dry season and difference in mean concentration of oocyst between the two seasons was significant ($t_{(34)}=-6.101$, $p<0.01$) (Table 5). This is further illustrated by a box plot (Fig. 3).

Effect of environmental variables on *Cryptosporidium* concentration in River Njoro

There was a strong negative correlation between oocysts concentration and temperature ($r=-0.784$, $p<0.05$.). Similarly, there was a strong negative correlation ($r=-0.866$, $p<0.05$) between pH and oocysts concentration. On the contrary, turbidity showed significant positive correlation with oocysts concentration ($r=0.890$, $p<0.05$) (Table 6).

Risk of infection from *Cryptosporidium*

From the results, the estimated daily risk of *Cryptosporidium* infection in Njoro River watershed is 0.246, while the annual risk is 0.99 (Table 7).

Discussion

The mean concentration of *Cryptosporidium* oocysts in Njoro River is 0.936 oocysts/litre. These findings are consistent with other documented studies which reported

Table 4 Tukey's multiple comparisons of mean concentrations of *Cryptosporidium* oocysts at upstream, midstream and downstream sites

(I) Stream section	(J) Stream section	Mean difference (*I*–*J*)	Std. Error	Sig	95% confidence interval	
					Lower bound	Upper bound
Upstream	Midstream	– .35	.2771	.426	– 1.03	.33
	Downstream	– .7583	.2771	.026*	– 1.438	– .078
Midstream	Upstream	.35	.2771	.426	– .33	1.03
	Downstream	– .4083	.2771	.316	– 1.088	.272
Downstream	Upstream	.7583	.2771	.026*	.078	1.438
	Midstream	.4083	.2771	.316	– .272	1.088

*The mean difference is significant at the 0.05 level

Table 5 Table showing independent sample *t*-test for comparison of mean concentration of oocysts in Njoro River during dry and wet seasons

	Levene's test for equality of variances		*t*-test for equality of means						
	F	Sig	*t*	*Df*	Sig(2-tailed	Mean difference	Std. error difference	95% confidence interval	
								Lower	Upper
Season									
Equal variances assumed	9.47	.004	– 6.101	34	.000	– .1042	.181	– .472	– .7364
Equal variances assumed			– 7.774	33.5	.000	– .1042	.142	– .393	– .8153

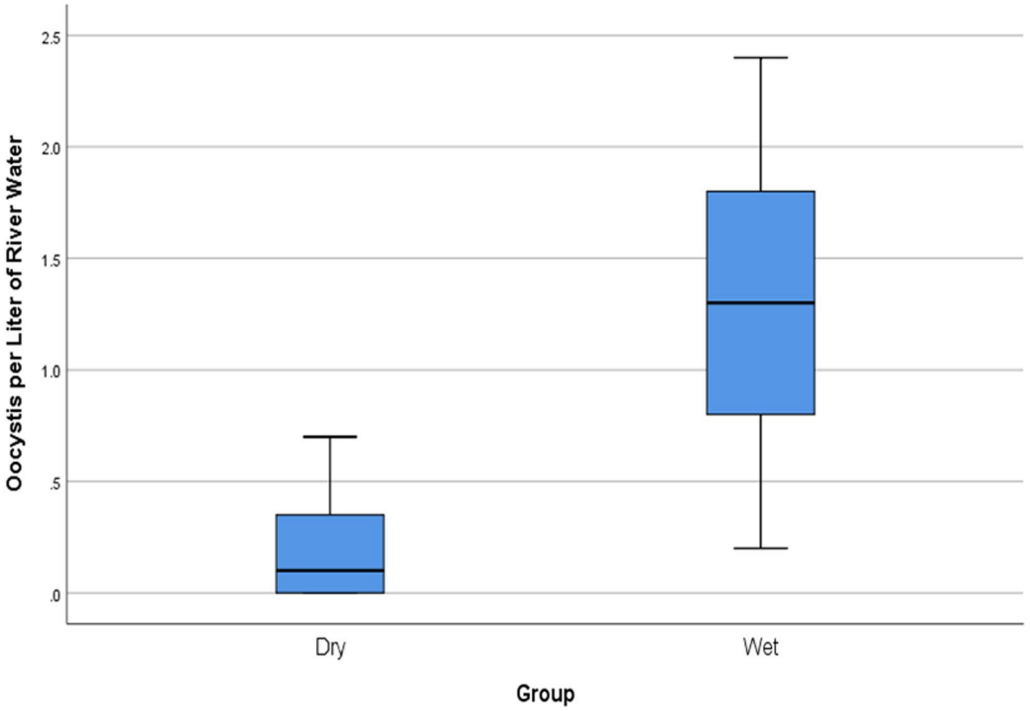

Fig. 3 Box plot showing seasonal variation of oocysts concentration in River Njoro. Y axis represents the pooled *Cryptosporidium* oocyst concentrations; X axis represents the pooled dry months and wet months. Mean concentrations of *Cryptosporidium* oocysts is higher during the wet season and lower during the dry season

Table 6 Spearman's correlation analysis between *Cryptosporidium* oocyst concentration and pH, temperature and turbidity

			Temp (°C)	pH	Turbidity (NTU)
Spearman's rho	Conc. (Oocyst/litre)	Correlation coefficient	– .784**	– .866**	.89**
		Sig. (2-tailed)	.000	.000	.000
		N	36	36	36

Table 7 Table showing daily and annual probability of *Cryptosporidium* infection in Njoro River watershed

Site	Concentration of *Cryptosporidium*	Exposure dose	Daily risk	Annual risk
Upstream	0.567	1.134	0.206	0.99
Midstream	0.917	1.834	0.244	0.99
Down-stream	1.325	2.65	0.274	0.99
Whole river	0.936	1.872	0.246	0.99

that concentrations of *Cryptosporidium* in surface waters range from 0.01 to 100 oocysts/litre (Hashimoto et al., 2002; Karim et al., 2010; LeChevallier, 2004; Rose, 1997). In a recent Malaysian survey, Lesley et al. (2017) demonstrated that *Cryptosporidium* oocysts in environmental water samples occur in the range of 0.1–2.7 oocysts/litre. Njoro River watershed comprises of forested, agricultural lands and urban settlements (Mainuri & Owino, 2013). This watershed is polluted by human and domestic animal feacal matter, previously infected by cryptosporidiosis. *Cryptosporidium* oocysts eventually flow into Njoro River, through surface runoff, especially during rainy seasons. Also, direct contamination of Njoro River may occur when domestic animals defecate into the river while drinking water.

Tukey's multiple comparison showed that a significant difference existed in oocysts concentration between upstream (Neissuit) and downstream (Kaptembwo) where concentration in downstream site (1.325 oocysts/l) was significantly higher than concentration at upstream site (0.567 oocysts/l). A similar observation was previously observed in Kenya by Muchiri et al. (2009) and most recently in Malaysia by Lesley et al. (2017). Kaptembwo is an urban region,

prone to disposal of significant amounts of both domestic and industrial wastes, which then get washed into Njoro River. Numerous surveys have identified sewage effluent as a source of *Cryptosporidium oocysts* which contaminate rivers (Montemayor et al., 2005; Sahasrabhojanee, 2017; Squire & Ryan, 2017). Previous studies have demonstrated higher levels of *Cryptosporidium* oocysts in urbanized waters compared to pristine waters (Cacciò & Chalmers, 2016; Lucie et al., 2019). These findings are consistent with WHO Guidelines for Drinking Water Quality which estimates an average *Cryptosporidium* concentration of 1 oocyst per litre in polluted rivers (WHO, 2017).

In this study, the upstream region, Neissuit, is characterized by little and dispersed human settlement and agricultural activities. Domestic and wild animals are dominant in this region. However, there is no direct input of human or livestock wastes into Njoro River. This probably explains the low concentration of *Cryptosporidium* parasites (0.567 oocysts/l) detected in water samples collected from this ecological site compared to other sampled sites. *Cryptosporidium* concentrations obtained in Neissuit are however higher compared to the estimated levels of 0.01 oocysts per litre, expected in less anthropogenically impacted waters as per the WHO Guidelines for Drinking Water Quality (WHO, 2017).

The mid-stream region, Ngata, is moderately polluted. This region is characterized by villages whose residents undertake extensive agricultural activities. Also, feacal wastes are collected and waste treatment done before being discharged into the river. In this region, *Cryptosporidium* concentration is much higher (0.917 oocysts/l) compared to the upstream region. Furthermore, the concentration of *Cryptosporidium* in the midstream region is higher compared to estimated levels of 0.1 oocysts per litre, expected in moderately polluted waters as per the WHO Guidelines for Drinking Water Quality (WHO, 2017).

The present study did not observe any significant differences in *Cryptosporidium* concentrations between upstream and midstream as well as downstream and midstream ($p > 0.05$). This could probably be due to similarity in the nature of socio-economic activities practiced by residents of these regions.

The results of this study are in agreement with the hypothesis of Ikiroma and Pollock (2020) that weather influences seasonal variations of *Cryptosporidium* oocysts levels in water surfaces. According to the data obtained from a local meteorological station at Egerton University, Njoro River watershed receives heavy rainfall between April and November. This coincides with an increase in concentration of *Cryptosporidium* oocysts in Njoro River, with a peak observed in August. This may be due to shedding of large amounts of oocysts by animals on land, which are readily washed into the river. Once oocysts have been washed into Njoro River, they undergo sedimentation and resuspension (King & Monis, 2006; Mohammed, 2020). Oocyst mobilization, sedimentation and resuspension have been reported as the main mechanisms responsible for higher *Cryptosporidium* oocysts concentrations during rainfall seasons (Chalmers et al., 2021; Liu et al., 2010; Searcy et al., 2006). However, during dry seasons, from November to March, mean concentration of *Cryptosporidium* oocysts in Njoro River declines, probably due to lesser surface runoff into the river, hence minimal river flow. Several authors have shown that concentration of *Cryptosporidium* in rivers can be 10–100-fold higher during heavy rainfall and snowmelt than during non-event situations (Ferguson et al., 2004; Gertler et al., 2015; Kistemann et al., 2002). Peak precipitation or snowmelt events may not only lead to increased run off but also to rapid movement of oocysts from source to rivers or groundwater wells (Medema & Stuyfzand, 2020; Zahedi et al., 2016).

This study investigated effect of environmental variables such as pH, temperature and turbidity on concentration of *Cryptosporidium* parasites in Njoro River. Fluctuations in these physico-chemical parameters in river systems have been found to occur in response to anthropogenic influence (Tebkew et al., 2021). For instance, agricultural runoff and wastes from urban areas which find their way into rivers could alter the physical and chemical properties of receiving water bodies (Norman & Michel, 2000; Yang et al., 2010). The altered water quality then affect populations of organisms living in water (Tebkew et al., 2021). Enhanced anthropogenic disturbance causes variations in levels of temperature, pH and turbidity in rivers (Dutta et al., 2018; Lintern, 2017). Results of this study indicate significant relationships between oocysts concentration and physico-chemical variables. Specifically, a strong negative correlation exists between oocysts concentration and temperature ($r = -0.784$). This implies that as temperature increases, oocysts concentration declines. Cebrián (2017) documented that higher temperatures inactivates different microorganisms. Survival time of *Cryptosporidium* parasites has also been shown to decrease as temperature increases (King et al., 2005; Li et al., 2010; Pokorny et al., 2002; Squire & Ryan, 2017). Temperature affects both the reaction kinetics and survival of *C. parvum* in the environment (King et al., 2007; Maria et al., 2019; Peng et al., 2008). Previous studies demonstrated that temperature values of 30–50 °C reduce viability of *Cryptosporidium* by melting the oocyst cell wall fatty acids and hydrocarbons (Fayer & Nerad, 1996; Jenkins et al., 2010; King et al.,

2005). Furthermore, high temperatures can enhance excystation of *C. parvum* oocysts (Gómez-Couso et al., 2009; Pecková et al., 2016).

From this study, a strong negative correlation ($r = -0.866$) was observed between pH and *Cryptosporidium* oocysts levels. This relationship implies that a high pH condition does not favour survival of oocysts in Njoro River. These results are consistent with those of Mohammed (2020) and Reinoso et al. (2008) both of which indicated that higher water pH destroys *Cryptosporidium* oocysts.

Turbidity showed significant positive correlation with oocysts concentration ($r = 0.890$). This relationship implies that turbidity, mainly caused by the amount of suspended particles in water, favours occurrence of oocysts. Njoro River is mainly driven by surface runoff; therefore, positive correlation between this variable emphasizes the contribution of surface runoff (exacerbated by land disturbance in the Njoro river catchment) in increasing concentration of oocysts in river Njoro waters.

Results of this study indicate that individuals who drink water directly from Njoro River have an estimated daily and annual risk of 0.25 and 0.99, respectively, with regard to *Cryptosporidium* infection. This annual estimated risk of infection is similar to that obtained in Mexico by Mota et al. (2009), but much higher compared to the risk in other European countries such as France and England, 0.58 and 0.57, respectively (Pouillot et al., 2004). A study by Dennis de Raaij (2017) mapped countries surrounding Lake Victoria (Kenya, Tanzania, Uganda and Rwanda) as hotspots of *Cryptosporidium* infection, with the annual risk ranging between 90 and 100%. Infection risk obtained in this study is higher than the world standard regulation of annual risk probability of *Cryptosporidium*, 1×10^{-4} (Balderrama-Carmona et al., 2017). This high risk can be attributed to heavy pollution of Njoro River in three ways; allowing of livestock to directly drink water in the river, some of which defecate shedding *Cryptosporidium* oocysts in the water. Also, in some areas, humans defecate in forests and use animal dung as manure within the watershed. This leads to runoff into the river. Finally, there is unhygienic disposal of sewage and industrial wastes in the river, especially at Kaptembwo. Higher infection risk may occur in the elderly as a result of declined immunity and in infants due to frequent exposure to contaminated water and food during weaning period, at home and school (Hlavsa et al., 2012). The risk of infection may also be higher in females than in males due to increased exposure; women are responsible for multiple uses of water resources (Sayal, 2019; Tombang et al., 2019; Wambu & Kindiki, 2015).

Conclusions

With the following assumptions in mind; one, that the average reference water consumption rate of 2 L per person per day, secondly, homogeneity in *Cryptosporidium* distribution, and lastly the viability and infectivity of the recovered Oocysts, this study made some serious conclusions. Firstly, there was a high pollution of Njoro River with *Cryptosporidium* parasites. Secondly, the concentration of parasites increased with the river structure; from upstream to downstream and varied with both seasonality and physico-chemical variables. Lastly, this pollution exposes residents who drink water from the river to an annual cryptosporidiosis high infection risk of 0.99. Therefore, this study recommends adequate boiling and treatment of water, sourced from Njoro River, before consumption. Also, public education on personal hygiene practices, use of latrines and proper disposal and treatment of sewage should be enforced in order to reduce this risk among vulnerable populations.

Abbreviations

ANOVA	Analysis of variance
DAPI	4, 6-Diamidino-2-phenylindole
DIC	Differential interface contrast
IFA	Indirect fluorescence assay
P_{inf}	Probability of infection
QMRA	Quantitative microbial risk analysis
UK	United Kingdom
UNICEF	United Nations International Children's Emergency Fund
WHO	World Health Organization

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Author contributions

WME, CIM and EOO contributed to the study conception and design. Material preparation, data collection and analysis were performed by WME and EOO. The first draft of the manuscript was written by WME, and all authors critically reviewed all the versions of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All raw datasets generated and analysed were converted and arranged in table formats as shown in the "Results" section but are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests
The authors declare that they have no competing interests.

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