

Quantitative microbial risk assessment related to urban wastewater and lagoon water reuse in Abidjan, Côte d'Ivoire

R. I. Yapo, B. Koné, B. Bonfoh, G. Cissé, J. Zinsstag and H. Nguyen-Viet

ABSTRACT

We assessed the infection risks related to the use of wastewater in Abidjan, Côte d'Ivoire, by using quantitative microbial risk assessment (QMRA). *Giardia lamblia* and *Escherichia coli* were isolated and identified in wastewater samples from the canal and lagoon. The exposure assessment was conducted using a cross-sectional survey by questionnaire with 150 individuals who were in contact with the wastewater during their daily activities of swimming, fishing, washing, and collecting materials for reuse. Risk was characterised using the Monte Carlo simulation with 10,000 iterations. Results showed high contamination of water by *G. lamblia* and *E. coli* (12.8 CFU/100 mL to 2.97×10^4 CFU/100 mL and from 0 cyst/L to 18.5 cysts/L, respectively). Estimates of yearly average infection risks for *E. coli* (90.07–99.90%, assuming that 8% of *E. coli* were *E. coli* O157:H7) and *G. lamblia* (9.4–34.78%) were much higher than the acceptable risk (10^{-4}). These results suggest the need for wastewater treatment plants, raising awareness in the population in contact with urban wastewater and lagoon water. Our study also showed that QMRA is appropriate to study health risks in settings with limited data and budget resources.

Key words | diarrhoea, *E. coli*, *G. lamblia*, QMRA, risk assessment, wastewater

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INTRODUCTION

Like other large cities in developing countries, Abidjan, the economic capital of Côte d'Ivoire, is undergoing rapid urbanisation, characterised by strong demographic expansion and concentration of industries (Koné *et al.* 2006). Demographic, economic and urban growth are the causes of different types of environmental pollution, among which the production of solid, liquid and gaseous waste contribute to negative consequences for the health of exposed populations (Cissé 1997; Cissé *et al.* 2002; Koné 2008). In Yopougon municipality, Abidjan, untreated urban wastewater is discharged into the

Ebrié Lagoon via sewers and rainwater drainage canals (JICA 2000; Kouadio 2000; Koné *et al.* 2006; Dongo *et al.* 2008).

Wastewater is a natural vector for microorganisms and the propagation of diseases. Mixing these waters with surface waters in which people swim, or use for production of vegetables that are consumed raw, presents a public health risk. Transported faecal matter may contain pathogens, such as *Salmonella*, *Giardia*, *Cryptosporidium*, *Entamoeba* and *Rotavirus*, which may be associated with respiratory

infections, gastroenteritis and more serious pathologies like haemolytic-uraemic syndrome (Westrell *et al.* 2004).

Quantitative microbial risk assessment (QMRA) is an analytical method which allows quantitative characterisation and estimation of potential risk for the health of the population exposed due to microorganisms. It allows the quantification of the infection risk and assessment of critical aspects in the food chain as a whole and within sanitation systems (Haas *et al.* 1999; Howard *et al.* 2007; Mara *et al.* 2007).

QMRA has been used to assess the health risks associated with consuming wastewater-irrigated food crops and vegetables (Pettersen *et al.* 2001; Hamilton *et al.* 2006; Mara *et al.* 2007; Seidu *et al.* 2008; Labite *et al.* 2010; Pavione *et al.* 2013). It has also served as a basis for the elaboration of World Health Organization (WHO) guidelines for drinking water quality and safety of water bodies, as well as the guidelines for the safe use of wastewater in agriculture and aquaculture (WHO 2006a, b).

Use of urban wastewater and lagoon water in agriculture and for other human activities is widespread in Yopougon municipality. In the canal, some people recover objects from the wastewater, while others use this water to wash plastic bags which will then be sold and recycled at an

industrial level (Silué *et al.* 2007). The population living close to the lagoon fish, swim and wash dishes in the lagoon water.

This study aims to evaluate the microbiological quality of urban wastewater and lagoon water using QMRA to quantify microbial infection risks linked to their use.

METHODS

Study site

The study was undertaken in the Uniwax catchment in Yopougon municipality (Figure 1). With an area of about 153 km² and an estimated population of 1,000,000 inhabitants (INS 2001), this municipality is the largest and most populated in Abidjan. Its southern part includes a very long lagoon coast along which several peri-urban villages are set, two of which are Azito and Béago. These villages are close to the outlet of the largest municipal wastewater drainage canal.

The Uniwax catchment has 459,000 inhabitants (INS 2001) on its 2,500 hectares and the flow rate of wastewater at

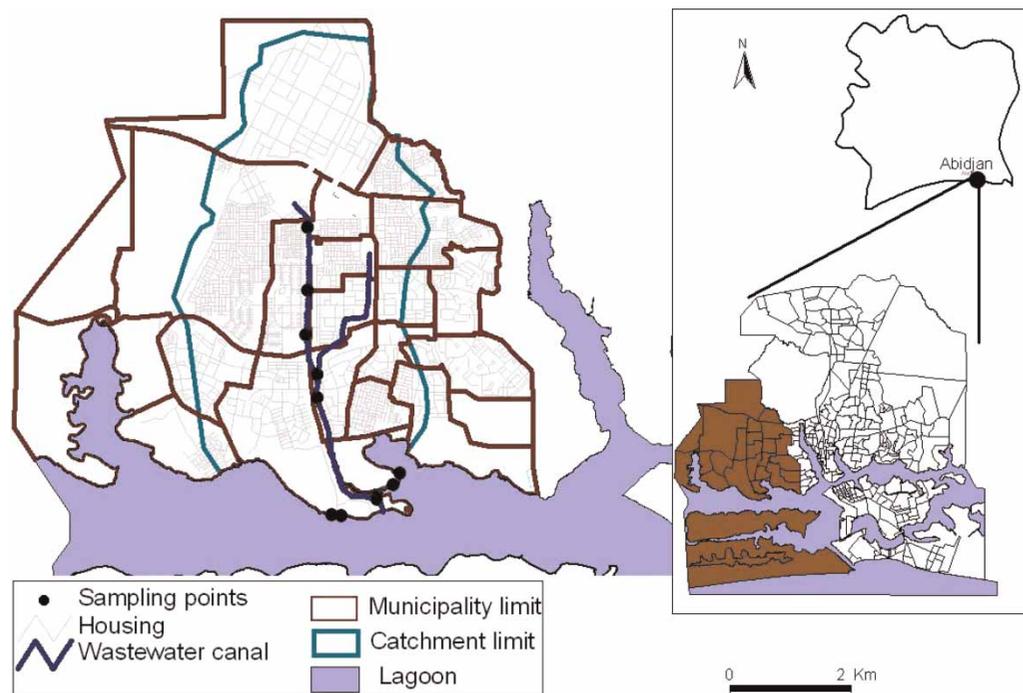


Figure 1 | Map of Yopougon municipality.

its outlet is about 47,500 m³/day (Koné 2008). The wastewater from this catchment runs for almost 8 km from the industrial zone in the north, to the Ebrié Lagoon in the south. The wastewater is collected in open drains, the most important canal being the Uniwax canal. The rainwater and the urban wastewater (from industries and households) are discharged into the lagoon via this canal, without any prior treatment.

Sampling and risk assessment

QMRA, as described by Haas *et al.* (1999), was used. This method comprises four steps: hazard identification, exposure assessment, dose-response assessment and risk characterisation. Data collection occurs during the hazard identification and exposure assessment steps.

Hazard identification

Diarrhoea is one of the causes of morbidity due to inadequate sanitation. An estimated 1.7 million people, among whom 90% are children of less than 5 years of age, die each day from diarrhoeal diseases (Mathers *et al.* 2006). Two diarrhoea-causing organisms *Giardia lamblia* and *Escherichia coli* were selected and studied. Both organisms are very resistant in aquatic environments and are common causes of diarrhoeal illnesses, and have often been targeted in the framework of other QMRA studies (Haas *et al.* 1999; Furness *et al.* 2000; Haas 2002). A high prevalence of *E. coli* has previously been reported in some regions of Côte d'Ivoire (Heckendorn *et al.* 2002; Matthys *et al.* 2007), and in a district of Abidjan in several water sources and dairy products sold close to schools (Dunne *et al.* 2001; Dadie *et al.* 2010). However, as not all *E. coli* strains are pathogenic, in this study we used *E. coli* O157:H7 as reference pathogen. As we did not analyse *E. coli* O157:H7 directly, we calculated the concentration of *E. coli* O157:H7 from that of *E. coli* by assuming 8% of *E. coli* were *E. coli* O157:H7. This assumption has been used by other authors in the past (Haas *et al.* 1999; Howard *et al.* 2006).

Other studies reported the presence of *G. lamblia* in Côte d'Ivoire (Adou-Bryn *et al.* 2001; Matthys *et al.* 2007), in particular in the semi-mountainous area of Man, where there was a prevalence of 17.5% (Ouattara *et al.* 2008).

Exposure assessment

The aim of the exposure assessment was to estimate concentrations and doses of pathogens likely to cause infections in the human body. Four parameters were measured: pathogen concentration in wastewater and lagoon water, number of people exposed, frequency of exposure and volume of water ingested during exposure.

The sampling method and the laboratory analysis were performed according to the guidelines of the Association Française de Normalisation (AFNOR 2001). Ten water sampling points were chosen, six in the Uniwax canal (P₁ to P₆) and four in the Ebrié Lagoon, close to the villages of Azito and Béago (P₇ to P₁₀) (Figure 1). The sampling points corresponded to places where human activities involved direct contact with water. Samples were taken on four days in July 2009, with an interval of 7 days between each sampling. In total, 24 samples from the canal (wastewater) and 16 samples of water from the lagoon were collected. The samples for the *E. coli* analysis were collected in 250 mL polyethylene flasks and kept in a cooler until they reached the laboratory. The samples for *G. lamblia* analysis were collected in 1 L flasks and transferred to the laboratory.

For *E. coli* detection, one or several decimal dilutions were made in the laboratory by adding 1 mL of sample to 9 mL of buffered peptone water, and organisms were enumerated on eosin methylene blue agar, followed by confirmative tests with the reduced set of tests recommended by Le Minor.

The *Giardia* cysts were studied and isolated according to the sodium acetate formalin (SAF) technique (AFNOR 2001). After 2 to 6 hours of settling of the 1 L sample, the sediment was collected and added to 10 mL SAF solution, and centrifuged for 5 min at 2,000 r.p.m. The precipitate was then mixed with 7 mL NaCl solution and 3 mL ether. This was again centrifuged for 5 min at 2,000 r.p.m. The cysts were then identified and enumerated with an optical microscope at a magnification of 50 ×.

The number of people exposed and the frequency of exposure were estimated in a cross-sectional survey by using a questionnaire targeting people who engaged in activities in the canal or lagoon waters. Systematic sampling was done along the canal and the lagoon villages of Azito and Béago with a total of 150 participants who were interviewed on their activities related to wastewater contact and reuse. The

variables are: the identity of sampled individuals, the size of the population exposed, the exposure frequency, the behaviour in relation to water and the perception of the risk. Activities such as (1) swimming, (2) fishing, (3) washing plastic bags and (4) collecting plastic bags were selected as main exposure scenarios. Collecting and washing plastic bags in the wastewater canal are common practices in Youpogon.

The volume of water ingested during each type of exposure was estimated from the literature (WHO 1998; Genthe & Rodda 1999; Haas *et al.* 1999). The volume of water ingested involuntarily while washing plastic bags in the canal was based on that for irrigation or laundry activities and was assumed to be 10 mL, and the volume of water ingested by people swimming or fishing in the lagoon was assumed to be 100 mL per exposure. Finally, calculating the percentage of people in contact with the canal's wastewater and the lagoon water allowed assessment of the risk for the population as a whole.

Dose-response assessment

The objective of this step was to define a relationship between the level of exposure to microorganisms and the probability that it will cause a deleterious effect. Dose-response assessment is a mathematical method which generally uses the Beta-Poisson model and the exponential model. The Beta-Poisson model has been adapted to data obtained on bacteria (*E. coli*) and the exponential model was adequate for data on protozoa like *G. lamblia* (Haas *et al.* 1999; Haas 2001; Westrell *et al.* 2004). According to these authors, in the case of a single exposure, the exponential and the Beta-Poisson models are expressed as

$$P_{\text{inf}} = 1 - \exp(-D/k) \quad (\text{exponential model})$$

$$P_{\text{inf}} = 1 - [1 + D/N_{50}(2^{1/\alpha} - 1)]^{-\alpha} \quad (\text{Beta-Poisson model})$$

where P_{inf} is the probability of infection, D is the microbial dose ingested, N_{50} is the median infection dose representing the number of organisms that will infect 50% of the exposed population [$N_{50} = \beta/(2^{1/\alpha} - 1)$] and k , α and β are constants.

For *G. lamblia*, k is 50.23 (Rendtorff 1954; Teunis *et al.* 1996). For *E. coli* O157:H7, we used the most recent dose-response proposed by Teunis *et al.* (2008) from outbreak

data: $\alpha = 0.373$, $\beta = 39.71$ which implies $N_{50} = 7.336$ (Teunis *et al.* 2008).

Risk characterisation

The risk characterisation consists of calculating the annual infection probability (P). It is linked to multiple exposures per person (Sakaji & Funamizu 1998) and was calculated as

$$P = 1 - (1 - P_{\text{inf}})^n$$

where P is the annual probability of infection, P_{inf} is the probability of infection for a single exposure to a dose D of organisms and n is the frequency of exposure, i.e. the number of days per year during which a person is exposed to a dose D of pathogenic agents.

In the current study, infection risk was calculated using a stochastic approach, with inputs as probability density functions of each parameter if data were available, and plausible for fitting a probability density function and Monte Carlo simulation with 10,000 iterations. Calculations showed that concentrations of pathogens in different replicates and the frequency of the event where people are exposed to wastewater fit best to a negative binomial distribution. Monte Carlo simulation gave a range of possible risks, including average and worst-case scenarios. The output of the model was again with a Monte Carlo simulation with 10,000 iterations. Results were expressed as the risk of infection, per person, per single exposure, as well as by yearly risk with multiple exposures. In the latter, the risk was presented by mean, minimum, maximum and standard deviation of 10,000 simulation values of risk.

Statistical data analysis

Mean and standard deviations of physico-chemical and microbiological parameters were calculated. The statistical analysis and calculations from the questionnaire data were done using Excel. A correlation study between the pathogen concentrations and environmental parameters (temperature, pH and dissolved oxygen) was conducted. @Risk version 5.0.1 of Palisade Corporation 2008 was used for fitting the probability density function and running Monte Carlo simulations to calculate infection risk.

RESULTS

Concentration of pathogens in water

Table 1 presents concentrations of *E. coli* and *G. lamblia* at the different sampling points, as well as measurements of physico-chemical parameters (temperature, pH, conductivity and dissolved oxygen).

There was high concentration of *E. coli* (12.8 CFU/100 mL to 2.97×10^4 CFU/100 mL) and *G. lamblia* (0–18.5 cysts/L) in both the urban canal wastewater (P₁ to P₆) and the lagoon water (P₇ to P₁₀). The pollution was not homogenous from one sampling point to another one along the canal or the lagoon. There was a positive correlation between the *E. coli* concentration and the number of *G. lamblia* cysts ($r = 0.52$, $P < 0.05$, Pearson correlation). However, neither the *E. coli* nor the *G. lamblia* concentrations were correlated to the temperature or the pH of the water.

Exposure to hazards

The volumes of water ingested during each exposure scenario are presented in Table 2. It appeared that the main risk-prone activities in the population were the washing of plastic bags, with 42% of the population being exposed, followed by the collection of plastic bags and fishing, both 22%, and finally swimming, 16%. The average annual exposure frequency was calculated on the basis of the number of weekly exposures (Table 2). Overall, the exposure frequency related to fishing was the lowest (257 day/year), compared with swimming (280 day/year), washing bags (278 day/year) and collecting plastic bags (279 day/year).

Based on the questionnaire, 84% of sampled individuals conducted activities directly in contact with wastewater from the canal and lagoon water. Nearly all these individuals (99%) did not have or use any protection measures. The remaining 1% used only gloves. Fishing and swimming

Table 1 | Results of physico-chemical and microbiological analysis of urban wastewater and lagoon water in Yopougon

Sampling point ^a	<i>E. coli</i> (CFU/100 mL)	<i>G. lamblia</i> ^b (cysts/L)	Temperature (°C)	pH	Oxygen (mg/L)	Conductivity (µS/cm)
Canal	P ₁	$3.6.10^3 \pm 5,423$	0.0 ± 0	29.1 ± 0.2	9.4 ± 0.5	2.4 ± 0.3
	P ₂	$6.1.10^3 \pm 5,094$	0.5 ± 1	28.5 ± 0.9	9.1 ± 1.2	0.8 ± 0.8
	P ₃	$2.6.10^3 \pm 3,193$	9.5 ± 15	28.2 ± 0.5	8.6 ± 2.1	0.6 ± 0.9
	P ₄	$2.1.10^4 \pm 9.1$	18.5 ± 16	28.2 ± 0.4	7.9 ± 1.1	0.1 ± 0.0
	P ₅	$3.0.10^4 \pm 8.9$	17.5 ± 12	27.5 ± 0.3	7.3 ± 0.2	0.1 ± 0.0
	P ₆	$1.3.10^4 \pm 2.8$	14.8 ± 2	30.5 ± 1.7	7.5 ± 0.1	0.1 ± 0.1
Lagoon	P ₇	12.8 ± 15	0.8 ± 1	27.9 ± 1.0	7.2 ± 0.2	3.5 ± 0.6
	P ₈	176.0 ± 170	0.0 ± 0	28.5 ± 0.9	7.1 ± 0.0	3.0 ± 0.4
	P ₉	164.0 ± 158	0.3 ± 0.5	28.7 ± 1.4	7.0 ± 0.1	2.8 ± 0.8
	P ₁₀	76.8 ± 82	0.0 ± 0	29.1 ± 1.0	7.1 ± 0.3	3.1 ± 1.2

^aAt each of the sampling points from P1 to P10, four samples were collected.

^bDetection limit was 1 cyst/1 L of water analysed by SAF method.

Table 2 | Exposure and scenarios

Activity/scenarios	Frequency (days/year)	Amount of ingested water (mL/exposure)	Percentage of the population exposed (%)	Exposed group of people
Swimming ^a	280	100	16	Men, children
Fishing ^a	257	100	22	Men, children
Washing plastic bags ^b	278	10	42	Women, men
Collecting plastic bags ^b	279	10	22	Women, men

^aWHO (1998); Genthe & Rodda (1999); Haas et al. (1999).

^bAdapted from Genthe & Rodda (1999).

were done without any particular protective measures. Concerning accidental ingestion of water during activities, 67% of sampled people stated that they involuntarily ingested water from the canal and the lagoon. However, this information was difficult to verify, thus the volume of water consumed was estimated from literature sources.

Estimates of yearly average infection risks for *E. coli* (90.07–99.90%, assuming that 8% of *E. coli* were *E. coli* O157:H7) and *G. lamblia* (9.42–34.78%) were much higher than the acceptable risk (10^{-4}).

Infection risk

Table 3 presents the probability density function of different parameters used in the risk models. The mean annual *E. coli* (i.e. *E. coli* O157:H7) infection risk varied between 90.07 and 99.9%; and was between 9.42 and 34.78% for *G. lamblia*, depending on the activity (Table 4). Furthermore, the mean risk related to activities involving urban wastewater was estimated at 34.78%, whereas it was lower in lagoon waters, with a value of 8.66% for *G. lamblia*. Overall, the activities undertaken in the canal presented a higher risk than those undertaken in the lagoon.

Table 3 | Probability density functions of different parameters used in the risk models

Scenario	Parameters of probability density functions ^a		<i>G. lamblia</i>	Frequency of exposure
	<i>E. coli</i> O157:H7			
Swimming	Negative binomial (1, 0.099379) ^b		Negative binomial (1, 0.80000)	RiskNegBin (9, 0.031096)
Fishing	Negative binomial (1, 0.099379)		Negative binomial (1, 0.80000)	RiskNegBin (5, 0.019065)
Washing plastic bags	Negative binomial (1, 0.00098953)		Negative Binomial (1, 0.089888)	RiskNegBin (14, 0.047977)
Collecting plastic bags	Negative binomial (1, 0.00098953)		Negative Binomial (1, 0.089888)	RiskNegBin (14, 0.047756)

^aProbability density function, fitted with all replicate values of real measurements of *E. coli* O157:H7 (estimated as 8% of *E. coli* analysed) and *G. lamblia* as well as the frequency of exposure for protozoa in water and vegetables.

^bValues in parentheses are values of two parameters r (>0) and P ($\in 0-1$) of the negative binominal distribution.

Table 4 | Yearly risk of infection for different scenarios

Scenario	<i>E. coli</i>				<i>G. lamblia</i>			
	Min (%)	Mean (%)	Max (%)	Standard deviation	Min (%)	Mean (%)	Max (%)	Standard deviation
Swimming	0	90.07	100	29.91	0	9.42	98.21	20.05
Fishing	0	90.07	100	29.91	0	8.66	99.37	18.98
Washing plastic bags	0	99.90	100	3.16	0	34.63	99.99	26.07
Collecting plastic bags	0	99.90	100	3.16	0	34.78	99.89	26.08

DISCUSSION

This paper which is part of a larger study on combined health and environmental assessment (Nguyen-Viet *et al.* 2009) successfully applies QMRA in the context of West Africa to assess the health risks related to wastewater reuse in Abidjan, Côte d'Ivoire. A similar study was conducted with success in Southeast Asia to estimate the health risk of wastewater contact in Thailand (Ferrer *et al.* 2012). Combining exposure assessment data from the literature and from actual measurements, this study shows that QMRA is appropriate to study health risks in developing countries as it can detect very low levels of disease risk and can complement epidemiological studies.

The microbiological analysis in this study showed the presence of *E. coli* and *G. lamblia* in the canal wastewater and lagoon waters. The presence of these organisms reveals faecal contamination due not only to defaecation near the lagoon and canal drainage wastewater, but also to the discharges of household garbage and wastewater (greywater and blackwater) (Koné 2008). The results are consistent with those of Guamry *et al.* (2007), who showed that parasite eggs found in wastewater in Morocco revealed faecal contamination.

In contrast, the amount of *G. lamblia* (average of 15.1 cysts/L) was higher than that found in the wastewater of the city of Sidi Yahia, Morocco (estimated 5.43 cysts /L) (Sylla & Belghyti 2008). The low concentration of microorganisms recorded upstream from the canal could be explained by the fact that this wastewater was predominantly industrial and chemical pollution was probably present. Indeed, the pH of the water upstream ($P_{1,2}$) was higher (9.26 ± 0.86) than that downstream (7.79 ± 1.2 ; $P_{4,5,6}$). A similar trend was observed for conductivity, salinity and redox potential (Table 1).

These results confirm those obtained by Sylla & Belghyti (2008) for wastewater in Morocco and Koné (2008) in Côte d'Ivoire on the same site as the present study. The high numbers of pathogens sampled upstream versus downstream may be related to housing density and quantity of domestic effluent that reached the canal. Inadequate sanitation in Yopougon resulted in high microbial contamination of the canal. The immediate vicinity of the canal included many poor neighbourhoods located beyond the urbanised area of the town (Dongo *et al.* 2008), thus explaining the almost non-existent health infrastructure, which is the reason for defaecation observed along the canal, and the direct discharge of domestic garbage and industrial and hospital wastewater into the environment.

The concentration of *E. coli* in the lagoon water was lower than the WHO guidelines (500 CFU/100 mL) for swimming waters, suggesting that the quality of the lagoon water would be acceptable for swimming and fishing activities (Festy *et al.* 2003; WHO 2003a, b). However, an increase in the pollution of the canal waters would cause a high contamination risk for individuals who are in contact with these lagoon waters.

The annual mean infection probability of *E. coli* and *G. lamblia* varied from 90.07 to 99.90% and from 9.42 to 34.78%, respectively, depending on exposure activities and places (Table 4). Generally, these values exceeded the acceptable risk of 10^{-4} per year. Collecting and washing plastic bags in the wastewater canal are common practices in Yopougon and are shown to be associated with the highest risk of infection. When generalising the risk of infection to the community of the study site, the annual cases of *E. coli* infections would be 379 people for those in direct contact with the canal wastewater and 974 people for lagoon water out of the

population of 1 million inhabitants. As mentioned above, protective measures were rarely used during exposure to wastewater in the canal; this may contribute to higher risks for workers in contact with wastewater, in particular when collecting and washing plastic bags. Simple interventions from a public health point of view, such as wearing gloves and boots when working or improve hygienic practice such as cleansing hands could have an extended impact on the health of workers. In addition to the infection risk, the main health problems reported by respondents were: malaria (76%), skin diseases (68%), diarrhoea and other gastrointestinal infections (51%), and lung infections (5%). Studies carried out by other colleagues on the same site showed the same health problems (Koné *et al.* 2006; Silué *et al.* 2007).

The probability of an *E. coli* infection at the sampling points could be influenced by the duration of exposure and the concentration of pathogens. In contrast to *E. coli*, the concentration of *G. lamblia* would have more influence on the probability of an infection. The annual infection risks were similar to those obtained by Hamilton *et al.* (2006) for enteroviruses after consumption of vegetables irrigated with pre-treated wastewater 1 day before harvesting (0.1% to 10%). The volume of water that was involuntarily ingested by people washing plastic bags was an assumption based on the value for that involuntarily ingested during irrigation, due to the similarity of the mode of contact with wastewater during both types of activities. We also note a similarity between our results and those found by Seidu *et al.* (2008) in Ghana. Their analysis showed that annual infection risks by *Ascaris* associated with the use of wastewater from sewers and streams were 8.2×10^{-2} and 8.4×10^{-2} , respectively. In addition to the use of wastewater and the consumption of products irrigated with wastewater, the contamination of soils has been shown to be a non-negligible risk factor (Mara *et al.* 2007).

CONCLUSIONS

Using *E. coli* and *G. lamblia* as indicators, the current study highlighted the poor quality of the canal and lagoon water which is negatively influenced by the discharges from Yopougon municipality. Levels of *E. coli* and *G. lamblia* in canal water exceeded the acceptable limits for swimming and reuse of water for crop irrigation. Consequently, the

infection risks caused by these micro-organisms in both the canal and lagoon were high from 90.07 to 99.90% and from 9.42 to 34.78%, respectively, for *E. coli* O157:H7 and *G. lamblia*. This study has assessed the real risk of people who use urban wastewater for various activities in Côte d'Ivoire. In particular, collecting and washing plastic bags – the most common activities in the canal in Youpogon – constitute an important health risk to workers, which needs interventions from different perspectives to reduce health risk. Our study also showed that QMRA is appropriate to study health risks in settings with limited resources.

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