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Trend of *Cryptosporidium* Infection among Children below 24 Months in an Informal Urban Settlement, Kenya

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Abstract

Cryptosporidium infection is estimated to cause 2.9 million diarrheal cases yearly among children aged under 24 months in sub-Saharan Africa. Studies have shown long-term climatic variations can affect infectious diseases. The burden of cryptosporidiosis in rural areas of sub-Saharan Africa is well characterized. However, the trend of *Cryptosporidium* infection is not known, especially in informal urban settings. This study therefore sought to determine cryptosporidiosis trends, and further explore the association between year and *Cryptosporidium* infection among children below 24 months in Kibera urban informal settlement in Kenya. Data collected by the Kenya Medical Research Institute longitudinal study in Tabitha clinic in Kibera from 2009 to 2015 were used. At least 3000 children aged < 24 months receive free health care at the clinic. In the longitudinal study, children presenting with diarrhea were eligible for stool sample collection ($n = 477$), out of which 421 stool samples were tested using TaqMan^{TM} Array Card (TAC) polymerase chain reaction panel that included a target for *Cryptosporidium* genus. Data for the 421 children were included in the analysis. Logistic regression was used to explore the difference between the seven years and cryptosporidiosis. Overall, the pooled data indicated that 23.5% of the children who were tested had *Cryptosporidium* infection, with the highest proportions of *Cryptosporidium*-positive cases observed in 2015 (45.2%). The logistic regression results also indicated that children who were tested in the year 2015 were more likely to have *Cryptosporidium* infection (OR = 3.39; 95% CI: 1.44 - 7.96; $p = 0.005$) than those in 2009. Watery stool was also found to be an important symptom of cryptosporidiosis. There was a high prevalence of *Cryptosporidium* infection among young children, especially in the most recent year. Routine testing of *Cryptosporidium* infection using molecular methods, constant moni-

toring and identification of the infection sources is therefore necessary towards reducing the disease burden in the low resource settings.

Keywords

1. Introduction

Cryptosporidium is a protozoan parasite which is a common cause of gastrointestinal illnesses in children [1]. The current global statistics suggest that the burden of *Cryptosporidium* infection is between 15% - 25% among children with diarrhea [2]. This has shown a significant increase from earlier studies that were reported in a systematic review indicating a prevalence of 1% among children in high-income countries and 5% - 10% in low- and middle-income countries [3]. This increase could be as result of several factors including the environmental and behavioral change over time.

The clinical manifestations of *Cryptosporidium* infection in children are associated with their immunity status that may include profuse, non-bloody watery diarrhea that usually resolves within 48 hours. The severity of diarrheal illness could be exhibited by a diarrheal duration of 7 to 14 days or more in addition to symptoms such as fever, abdominal cramps, vomiting, lethargy, and general malaise [1] [4].

In sub-Saharan Africa, cryptosporidiosis is estimated to occur in 2.9 million children below two years annually, which also increases the mortality rate among children aged between 12 to 23 months [2]. Some studies in Africa have revealed that the prevalence of *Cryptosporidium* infection among children was 12.3%, 10% and 9% in Ethiopia, Botswana and Kenya, respectively [4] [5] [6]. However, most of these studies did not consider the trend of *Cryptosporidium* infection over time. Moreover, these positive cases were found to occur majorly during diarrheal outbreak periods and among children who were less than 5 years.

The World Health Organization (WHO), in 2004 integrated cryptosporidiosis into the neglected tropical diseases due to its occurrence among the children in low resourced settings, where inaccessibility to services, inadequate resources and harsh weather patterns are the main drivers [7]. In low resourced settings, particularly where cases and risk of deaths due to cryptosporidiosis are high, immunocompromised individuals and under-5 children are disproportionately affected with no antiprotozoal drug or vaccine for effective disease management currently existing [8].

One study in Kenya found that 46% of under-5 children who were *Cryptosporidium* positive were living in semi-urban areas [9]. This high prevalence of *Cryptosporidium* infection in semi-urban areas is worrying since Kenya has the

largest informal urban settlement in Africa, that is, Kibera slums. To develop public health preventive strategies, an understanding of the trend and the current situation was therefore necessary, and this informed the basis for conducting our study in an informal urban settlement in Kenya. Kibera slum being the largest slum in Africa increases the population at risk of *Cryptosporidium* infection due to several environmental hygiene factors such as lack of access to safe water for drinking and poor sanitation facilities [6] [10]. Therefore, this study was aimed to determine the trend of cryptosporidiosis, and further explore the association between the change in time (*i.e.* years) and *Cryptosporidium* infection among children below 24 months in Kibera informal urban settlement in Kenya.

2. Methods

2.1. Study Design and Data Source

A longitudinal data collected by Kenya Medical Research Institute (KEMRI) in Kibera's Gatwikira and Soweto villages among children were used. KEMRI in collaboration with Tabitha clinic offered free health care for the study participants who were under surveillance for several acute illnesses. The clinic and laboratory test results for children aged 24 months and below who were presented with acute diarrhea at Tabitha Clinic from 2009 to 2015 were used.

2.2. Sampling and Laboratory Processing

A convenience sampling technique was employed in the selection of children to be included in the study. A total of 22,660 children who were below 24 months (female = 11,265; male = 11,395) were identified as having visited the clinic from the year 2009 to 2015. Children who presented with symptoms of loose stools and had stool samples collected (n = 477) were then selected from the dataset. Out of the 477, those who were eligible for this study were only 421 whose samples were tested for cryptosporidiosis using enteric TaqMan^{TM} Array Card (TAC) real-time polymerase chain reaction (PCR) following the protocol described by Liu and colleagues [11].

2.3. Data Management and Analysis

Data containing children information including demographic, symptoms and laboratory test results for *Cryptosporidium* infection, were extracted from the database and saved in Microsoft Excel format before cleaning, checking for discrepancies and analyses. Descriptive statistics were used to determine the prevalence of *Cryptosporidium* infection by year, age, gender, and clinical characteristics in the study. The differences between categories of the variables were tested using Pearson's Chi-square test while logistic regression modeling technique was used to explore the association between the change in time (year) and *Cryptosporidium* infection among children below 24 months. Both the descriptive and inferential statistics were presented in the form of tables. All the analyses were

conducted using STATA version 13.0.

2.4. Ethical Consideration

Ethical approval was sought from the institutional ethics committee of the University of Eastern Africa, Baraton (UEAB) (UEAB/REC/03/10/2019) and the National Commission for Science, Technology and innovation (NACOSTI) (NACOSTI/P/19/2689). Permission to use the dataset was granted by KEMRI. For this study, there were no risks to the study participants since all the data were anonymized.

3. Findings

The analysis of the pooled data of 421 children indicated that close to a quarter of them had *Cryptosporidium* infection (23.5%) with those tested in the year 2015 presenting the highest proportion (45.2%) of infection (Table 1). Also, nearly a third of children who had watery stool (29.6%) had *Cryptosporidium* infection while a quarter of those who were vomiting tested positive for cryptosporidiosis. Moreover, 27.0% of the children who had stopped breastfeeding tested positive for *Cryptosporidium* infection, however the difference between the groups was not statistically significant.

The odds ratios (ORs) and 95% confidence intervals (CI) of the association between year and *Cryptosporidium* infection among children below 24 months in Kibera urban informal settlement in Kenya are presented in Table 2. The year was positively associated with cryptosporidiosis before and after adjustment. After adjusting for all the variables, a positive trend was exhibited as from the year 2013 to 2015 with the increased odds of 1.29, 1.42 and 3.39, respectively, though the years 2013 and 2014 were not statistically significant. However, the children who were tested in the year 2015 were more likely to have cryptosporidiosis (OR = 3.39; 95% CI: 1.44 - 7.96) than their counterparts who were tested in the year 2009. Watery stools remained a key symptom of *Cryptosporidium* infection with children who had watery stool being 3.04 times more likely to be infected (95% CI: 1.74 - 5.28). The children who were vomiting were however less likely to have *Cryptosporidium* infection but was marginally significant. Age, gender and presenting with fever were not significantly associated with cryptosporidiosis.

4. Discussion

This study was carried out in the largest informal urban settlement in Africa (*i.e.*, Kibera slums) to explore the trend of *Cryptosporidium* infection among children aged 24 months and below. We found that 45.2% of the children were *Cryptosporidium* positive in 2015—a higher prevalence detected using real-time molecular PCR method. Our results support the findings of a recent study which found a 46% prevalence of *Cryptosporidium* infection among under-5 children who were living in semi-urban areas [9]. The pooled data for the seven years however revealed a 23.5% prevalence. The prevalence found in this study was

higher than what had been previously reported in other studies carried out in urban (4%) and rural (9%) areas among the children below 24 months in Kenya [1] [9]. Other studies conducted in Africa have also reported a much lower prevalence (Egypt = 17% and Ethiopia = 12%) of *Cryptosporidium* infection among the under-five than what was found in this study [4] [12].

Table 1. Descriptive statistics of children aged 0 - 24 months, by *Cryptosporidium* infection.

Variables	*Cryptosporidium* lab results			
	Total *n*	Negative, *n* (%)	Positive, *n* (%)	*P-value*[a]
Total number	421	322 (76.5)	99 (23.5)	
Year				0.015
2009	79	60 (76.0)	19 (24.1)	
2010	92	79 (85.9)	13 (14.1)	
2011	58	45 (77.6)	13 (22.4)	
2012	36	28 (77.8)	8 (22.2)	
2013	43	32 (74.4)	11 (25.6)	
2014	71	55 (77.5)	16 (22.5)	
2015	42	23 (54.8)	19 (45.2)	
Age group				0.612
≤12 months	176	132 (75.0)	44 (25.0)	
13 - 18 months	140	106 (75.7)	34 (24.3)	
19 - 24 months	105	84 (80.0)	21 (20.0)	
Gender				0.197
Female	211	167 (79.2)	44 (20.8)	
Male	210	155 (73.8)	55 (26.2)	
Breastfeeding				0.598
No	37	27 (73.0)	10 (27.0)	
Yes	384	295 (76.8)	89 (23.2)	
Clinical presentation				
Had watery stool				0.001
No	191	160 (83.8)	31 (16.2)	
Yes	230	162 (70.4)	68 (29.6)	
Had fever (≥38.0˚C)				0.763
No	375	286 (76.3)	89 (23.7)	
Yes	46	36 (78.3)	10 (21.7)	
Had vomit				0.076
No	316	235 (74.4)	81 (25.6)	
Yes	105	87 (82.9)	18 (17.1)	

[a], *P*-value was obtained using Pearson Chi-Square to test the statistical difference between the categories.

Table 2. Logistic regression results of the *Cryptosporidium* infection of children aged 0 - 24 months.

Variables	Crude Model	Adjusted Model
	OR (95% CI)	OR (95% CI)
Year		
2009	1	1
2010	0.52 (0.24, 1.14)	0.52 (0.23, 1.16)
2011	0.91 (0.41, 2.04)	0.94 (0.41, 2.17)
2012	0.90 (0.35, 2.31)	0.98 (0.37, 2.61)
2013	1.09 (0.46, 2.56)	1.29 (0.52, 3.21)
2014	0.92 (0.43, 1.96)	1.42 (0.62, 3.29)
2015	2.61 (1.18, 5.79)**	3.39 (1.44, 7.96)***
Age group		
≤12 months	1	1
13 - 18 months	0.96 (0.57, 1.61)	0.99 (0.58, 1.72)
19 - 24 months	0.75 (0.42, 1.35)	0.75 (0.40, 1.40)
Gender		
Female	1	1
Male	1.35 (0.86, 2.12)	1.49 (0.92, 2.42)
Still breastfeeding		
No	1	1
Yes	0.81 (0.38, 1.75)	0.66 (0.30, 1.48)
Clinical presentation		
Had watery stool		
No	1	1
Yes	2.17 (1.34, 3.49)***	3.04 (1.74, 5.28)****
Had fever, ≥38.0˚C		
No	1	1
Yes	0.89 (0.43, 1.87)	0.95 (0.43, 2.11)
Had vomit		
No	1	1
Yes	0.60 (0.34, 1.06)*	0.55 (0.30, 1.01)*

OR, Odds ratio; CI, Confidence interval; * $p \leq 0.10$; ** $p \leq 0.05$; *** $p \leq 0.01$; **** $p \leq 0.001$.

Nonetheless, most of these studies in Africa used various conventional diagnostic techniques for detection of *Cryptosporidium* infection which are less sensitive compared to molecular diagnostic test that was employed in our study. This would therefore explain a much higher prevalence in this study than the rest that were conducted in other parts of Africa. Clearly, this would mean that

there has been an underreporting of the *Cryptosporidium* infections in Africa because of the diagnostic techniques that have been used before. Hence, it is necessary that future studies should consider using real-time molecular PCR method rather than the conventional diagnostic techniques. In addition, the different study design methods and study settings in the studies carried out in Africa could also explain the differences in prevalence that were found.

Furthermore, it is necessary to establish a surveillance system for *Cryptosporidium* infection within the country among other infectious diseases. Our study has revealed that there was an association between the change in time (year) and rate of cryptosporidiosis among children, with children who were born in the most recent years being more likely to be affected. Several public health intervention strategies are therefore necessary since the rates of infections are expected to be higher now than before. The reasons for this association and high infections in the recent years may be due to environmental degradation and low hygienic conditions in the informal urban settlement brought about by increased population over the years. Therefore, exerting more pressure on the existing water and sanitation facilities in the area.

This study also found that children who were presented with watery stool were 3.04 times more likely to test positive for *Cryptosporidium* infection. This association was also observed in a study in Pakistan [13]. Even though, this study found that children who were presented with vomiting were less likely to test positive, it remained marginally significant after adjustment. Other studies, however, found a positive association between vomiting and cryptosporidiosis [9] [12]. The lack of distinguishable clinical signs or symptoms of cryptosporidiosis in Kibera slums suggests that a detailed examination and screening for this infection should be performed especially in endemic areas [14].

This study had limitations and strengths. First, we used a secondary data that had the limitation of important variables such as the sources and treatment of drinking water, food handling and preparation practices as well as environmental and socioeconomic conditions. Therefore, we were unable to adjust for several important potential confounders. Secondly, this study could not establish whether the symptoms presented could have been as a result of other diarrheal infecting pathogens that are usually common among these children. Hence, the findings of this study should be interpreted with a lot of caution. However, some of the strengths in this study included the use of real-time molecular PCR method to diagnose cryptosporidiosis unlike other studies that used conventional diagnostic techniques. This gave accurate results of the infection. The other strength is also in relation to data that were collected over the seven-year period. We were able to explore the change in infection over time.

5. Conclusion

In this study, we found a positive trend in the *Cryptosporidium* infection among children below 24 months in Kibera urban informal settlement, with the preva-

lence being 45.2% in 2015. Moreover, we found an increase in the rate of infections in the most recent years. Routine testing of *Cryptosporidium* infection using molecular methods, constant monitoring and identification of the infection sources should be considered. This would necessitate the development and implementation of public health policies on health promotion and preventive strategies that will facilitate the reduction of the disease burden among the low-income populations. Future studies should therefore endeavor to explore the seasonality as well as the sources of cryptosporidiosis among children in Kibera.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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