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Prevalence of Cryptosporidiosis infection among children under 5- years in Cotonou, Benin

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Cryptosporidiosis is recognized as one of the leading causes of childhood diarrhea in developing countries, but no data has been published, so far, on the prevalence among children with diarrhea in Benin. The aim of the present study was to assess, for the first time, the prevalence of *Cryptosporidium* spp. infection in Cotonou, Benin. A prospective study involving children younger than 5 years of age (n = 104) hospitalized or consulted in the paediatric departments of three hospitals between September 2015 and May 2016 was carried out. *Cryptosporidium* oocysts were detected by means of a smear of sedimented stool stained by the modified Ziehl-Neelsen technique and were found in 5.8% of the studied population. Neither age, non-use of drinking water, nor nutritional status influenced the presence of cryptosporidiosis in the study, but 5 of the 6 children infected were less than two years of age. In conclusion, this first study showed that infection with *Cryptosporidium* spp. could be a cause of diarrhea in a not insignificant proportion in Cotonou. Studies with larger numbers of patients, conducted for a longer time and in rural areas would be necessary to estimate the real prevalence of cryptosporidiosis and to assess the risk factors.

Key words: Cryptosporidiosis, prevalence, children, Benin.

INTRODUCTION

Diarrheal disease, a leading cause of mortality and morbidity in young children, is estimated to cause more than 760000 annual deaths among children of < 5 years of age (WHO, 1986), with 72% of these deaths occurring

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in children of < 2 years of age (Walker et al., 2013). Sub-Saharan Africa accounts for half of all global childhood deaths from diarrheal diseases (Walker et al., 2013). In Benin, according to health statistics (Ministry of Health, 2014), diarrhea is responsible for 3.1% of hospitalizations. Diarrheal diseases can be caused by various bacteria, viruses and parasites. Among the parasitic causes, cryptosporidiosis is recognized as one of the leading causes of childhood diarrhea in developing countries (Davis et al., 2002; Fletcher et al., 2012). Initially recognized in highly immunocompromised people infected with HIV, cryptosporidiosis may also be of major public health importance in young children. It has dramatic adverse effects on child growth and development and causes increased mortality in developing countries where HIV, poverty, and lack of sanitation and infrastructure increase the risk of cryptosporidial waterborne infection. Transmission occurs via the fecal-oral route from human and animal reservoirs. The symptoms of acute cryptosporidiosis include severe watery diarrhea, eventually leading to dehydration, malabsorption and malnutrition. In immunocompetent hosts, cryptosporidiosis is usually self-limiting, but in developing countries, it contributes to persistent diarrhea in children (Huang et al., 2006). Thus, studies conducted in sub-Saharan Africa among young children showed prevalence that vary from one country to another and may reach 35% for *Cryptosporidium* spp. (Kassi et al., 2004; Gay-Andrieu et al., 2007; Mbae et al., 2013; Tellevik et al., 2015; Nassar et al., 2016). However, the burden of cryptosporidiosis, can be underestimated due to the presence of many silent asymptomatic carriers and poor performance of traditional diagnostic procedures in many laboratories which can result in misdiagnosis and mistreatment with serious and possibly fatal outcomes, especially in young children. In Benin, the prevalence of cryptosporidiosis in HIV infected people is reported as 10.8% (Loko et al., 2008). However, this pathogen is not often sought as etiology during diarrhea in children in Benin and no information is available on the prevalence of crypto-sporidiosis in children. The present study investigated the prevalence of *Cryptosporidium* infection among young children with diarrhea in Benin and attempted to identify risk factors for infection. The results will be used as a database in the orientation of the management of diarrhea in children.

MATERIALS AND METHODS

Study site, sample and data collection

The study was performed between September 2015 and May 2016, primarily during the dry season, in three hospitals, namely the Centre National Hospitalier et Universitaire Hubert Koutoucou MAGA (CNHU/HKM), the Centre Hospitalier Universitaire de la mère et de l'enfantlagune (CHUMEL) and the Mènontin district hospital, all located in Cotonou in the south of Benin. Laboratory analyses were carried out in the Laboratory of Parasitology of CNHU/HKM. The study was conducted among children < 5 years of age admitted with diarrhea in one of the three hospitals involved. Written informed consent was obtained from one of the child's parents. Diarrhea was defined as three or more watery stools within 24 h. An episode of diarrhea was considered over when two consecutive days passed without diarrhea. An episode of acute diarrhea was defined as lasting between 24 h and less than 14 days. Persistent diarrhea was defined as diarrhea for 14 days or more. Children were categorized as having normal nutritional status, or either mild or severe malnutrition using weight -for length Z score according to WHO criteria (WHO, 1986). The HIV status of each child was sought in the registry. HIV tests were conducted for those for whom the status was not known. All children admitted for paediatric consultation or who were hospitalized, and who met the inclusion criteria, were tested for oocysts of cryptosopridia in a stool specimen that was collected and sent to the Parasitology Laboratory of the CNHU. For sites other than the CNHU, the stool samples were kept at 4°C prior to transportation in cold boxes. A standardized questionnaire was used for collection of demographic and clinical information. In the laboratory, direct stool examination and Ritchie technique were systematically performed, and the presence of *Cryptosporidium* oocysts was assessed by means of a smear from the sediment which was stained by the modified Ziehl-Neelsen technique (Deluol, 1999). A double reading was made for each smear by experienced technicians of the parasitology laboratory of CNHU, the national reference centre for parasitology. In case of discrepancy, a third reading was made by another technician for the validation of the final result. Samples having red oocysts of 4 to 5 µm in diameter were considered positive for *Cryptosporidium* infection.

Data analysis

Data were handled and analyzed using Excel software and Epi Info 2000 software. Qualitative variables were described in terms of number and percentage. For quantitative variables, averages and standard deviations were calculated. Proportional comparisons were made using the Chi-square test, with $P < 0.05$ being considered statistically significant.

Ethics statement

The Ethical Committee of the School of Medicine and Health Sciences, University of Benin, gave the ethical approval for the study. The study was approved by the respective hospital authorities at the three study hospitals. Written informed consent was obtained from the parents or guardian of all the children enrolled in the study.

RESULTS

A total of 104 children of less than 5 years of age were recruited into the study, where the mean of ages was 15 ± 12.03 month. The female sex was the most represented and the majority of the children had a normal nutritional status. Only three of the study children attended school, and more than half of their parents had a primary level of education. Majority of the children used drinking water and latrines: 82.7% and 81.7% respectively (Table 1). All the children were HIV negative. With regards to clinical symptoms, there was fever in most of the cases as well as vomiting, while dehydration and abdominal pain were found in smaller proportions

Table 1. Some socio-demographic variables related to children (n = 104) examined for crypotosporidiosis in Cotonou, Benin

Variable	Number examined (%)
Age in years	
<2	86(82.7)
2-5	18(17.3)
Sex	
Male	51(49)
Female	53(51)
Ratio weight for length	
Normal nutritional status	86(82.7)
Moderate malnutrition	6 (5.8)
Severe malnutrition	5(4.8)
Overweight	7(6.7)
Schooling of children	
Yes	3(2.9)
No	101(97.1)
Parent level of education	
Primary school	60(57.7)
Secondary school	33(31.7)
University	11(10.6)
Use of drinking water	
Yes	86 (82.7)
No	18(17.3)
Use of latrine	
Yes	85 (81.7)
No	19(18.3)

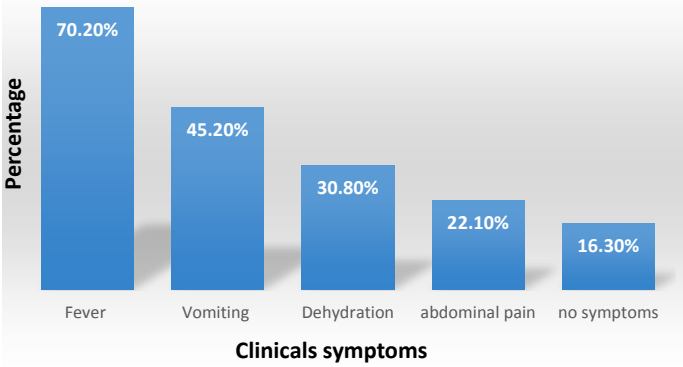

Figure 1. Clinical symptoms associated with diarrhea among children (n = 104) of Cotonou, Benin.

Table 2. Characteristics of stool collected from children (n = 104) in Cotonou, Benin.

Variable	Number examined (%)
Evolution of diarrhea	
Acute	91 (87.5)
Chronic	13 (12.5)
Number of stools/per day	
≤ 5	73 (70.2)
>5	31(29.8)
Stool consistency	
Liquid	56(53.8)
Semi-liquid	48(46.2)

(Figure 1). Table 2 shows the characteristics of diarrhea, where the majority of children had acute diarrhea and 70.2% had less than 5 stools per day. Of the 104 children examined, 6 had oocysts of *Cryptosporidium* spp. in their stool, giving a prevalence of 5.8%. Neither age, non-use of drinking water, nor nutritional status influenced the occurrence of cryptosporidiosis in this study (Table 3).

DISCUSSION

During the study period, 104 children of less than five years with diarrhea were recruited, a smaller study population as compared to studies in Niger and Senegal (Gay-Andrieu et al., 2007; Faye et al., 2013). The difference could be explained by the fact that in Senegal, the study focused on children aged 0 to 15 years, while in Niger, both diarrheal and non-diarrheal children were concerned. The predominance of children under 2 years of age in the study population could be explained by the fact that this is an age group more vulnerable to diarrhea because basic hygiene rules are neither known nor respected. This high proportion of children under 2 years of age also explains why very few children in our study attended school, because the age of schooling in Benin is generally around 3 years. Identifying school attendance for children relates to the fact that the peak of parasitism occurs at the age at which children are sent to kindergarten and primary schools when promiscuity, community games and contact with dirty soil favour contamination. Moreover, the rate of malnourished children in this study population (10.6%) is lower than the rate found at the national level, which was 36% for children less than 5 years (unpublished data from the Ministry of Health). This is probably due to the city of Cotonou being the site of our study, the economic capital of Benin, where children are better fed than in rural areas where malnutrition rates could be higher (INSAE, 2012).

The prevalence of cryptosporidia oocysts in the study population was 5.8%. This comparatively low prevalence

Table 3. Some risk factors and cryptosporidiosis infection among children (n = 104) of Cotonou, Benin (n = number infected; N = number examined; % = prevalence of infection; RP = prevalence report).

	N	n	%	RP [IC 95%]	p.value
Age in years					
<2	86	5	5.8	1.05 [0.01-10.00]	0.966
2-5	18	1	5.6		
Use of drinking water					
Yes	86	5	5.8	0.95 [0.88-10.15]	0.966
No	18	1	5.6		
Nutritional status					
Normal	86	4	4.7	4.1 [0.75-22.45]	0.824
Moderate malnutrition	12	2	16.7		

could be explained in several ways. The study was conducted in Cotonou, a city where the prevalence of intestinal parasitic infections has decreased due to improved access to drinking water and latrine use, which reduces fecal contamination rates, thus limiting occurrence of parasitosis in general and cryptosporidiosis in particular. Cryptosporidiosis is a disease related to hydrofecal contamination, which justifies exploring the study population's habits with regard to the use of drinking water. The small size of our sample due to the study period may also explain this low prevalence. Indeed, this study was conducted largely in the dry season which characterized by low rainfall, a period thus not favoring the multiplication of oocysts. Studies have shown that the prevalence of cryptosporidiosis is greater in the rainy season (Duong et al., 1995; Tumwine et al., 2003; Jagai et al., 2009; Siwila et al., 2011; Mbae et al., 2013; Tellevik et al., 2015), the rains causing the dissemination of fecal matter that contaminates drinking water, fruit and vegetables. This low prevalence could be due to methodology, using microscopy which can be less sensitive than PCR, that we were unable to take account because of limited financial resources. A study of a larger size conducted in the rainy season, and taking into account rural environments, could lead to stronger conclusions regarding the prevalence of cryptosporidiosis in children in Benin, because taking into account all the above, the figure of 5.8% reported here could clearly be a low estimate of overall prevalence. Significant variation between generally more affected rural environments compared with urban environments has been noted by others (Lu et al., 2008). Health authorities will need to sensitize medical laboratories to the need to examine stools for oocysts of *Cryptosporidium* spp., a simple and inexpensive exercise, in order to improve the management of diarrhea in children under 5 years of age. Hospital-based diagnostic laboratories in Benin currently do not routinely look for this parasite. It should be noted that infection with *Cryptosporidium*s spp. as a direct cause of diarrhea in the children in this study is important to consider because no other parasitic infection was detected. Kassi et al. (2004) in Abidjan found that in 80% of carriers of *Cryptosporidium* spp., it was the only parasitic infection found. Similarly, Hojling et al. (1984) reported that, in carriers of cryptosporidia, it is often a mono-infection. The prevalence reported here is close to the 5.5% found by Gay-Andrieu et al. (2007) in Niger, the 5.6% found by Areeshi et al. (2008) in Madagascar and the 4.5% reported by Faye et al. (2013) in Senegal. On the other hand, it is lower than that found in Abidjan, Tanzania, Ethiopia and Rwanda (Kassi et al., 2004; Kabayiza et al., 2014; Tellevik et al., 2015; Wegayehu et al., 2016), respectively 7.7, 10.4, 9.4 and 7.2%. In the Kassi et al. (2004) study in Abidjan, 18.5% of the children included were infected with HIV, while in Tanzania, the study was conducted only in children under 2 years of age and from August to July, a rainy season favouring the spread of oocysts. High prevalence have also been found in countries with high average rainfall such as Gabon and Nigeria (Duong et al., 1995; Nassar et al., 2016), with 31.6 and 38.3%, respectively.

The low prevalence of cryptosporidiosis in this study may explain the absence of risk factors for cryptosporidiosis. Age was not a risk factor; although, 5 out of 6 children who had cryptosporidiosis were less than two years of age, suggesting that cryptosporidiosis is more common in young children. This would be consistent with the results of several studies showing that cryptosporidiosis affects infants more frequently (Dieng et al., 1994; Perch et al., 2001; Gatei et al., 2006; Khalili and

Mardani, 2009; Mbae et al., 2013; Kotloff et al., 2013). Not using drinking water also does not appear to be related to the occurrence of cryptosporidiosis. The same observation was made by Sakar et al. (2013) in India, in a study aimed at verifying the risk of using municipal water sources and bottled water in the occurrence of cryptosporidiosis. Other studies have nevertheless shown the involvement of water type in the occurrence of cryptosporidiosis. This is the case in the epidemic in Milwaukee caused by contamination of the city's water system (MacKenzie et al., 1994) and a study in Sudan that showed a higher prevalence of cryptosporidiosis in residents of rural areas who do not have access to safe drinking water (Sim et al., 2015). These findings suggest that the use of water purification systems is important for preventing *Cryptosporidium* infection among inhabitants of rural areas. All children with cryptosporidiosis in the study population were seronegative for HIV. Studies in Tanzania, Kenya and Uganda found that infection with *Cryptosporidium* was found more frequently in HIV-infected children (Tumwine et al., 2005; Mbae et al., 2013; Tellevik et al., 2015).

However, *Cryptosporidium* should not be ignored as a cause of diarrhea in small children not known to be HIV-positive, as the GEMS-study found that it was an important pathogen at all sites regardless of HIV sero-prevalence, and the second most common pathogen causing diarrhea in infants (Lu et al., 2008). A relationship between malnutrition and cryptosporidiosis was also not found in this study due to the very few malnourished children. On the other hand, Gay-Andrieu et al. (2007) in Niger found a high proportion of malnourished children among children with cryptosporidia (10/12 or 83%). Also, Tellevick et al. (2015) in Tanzania showed that children who had stunted growth had a significantly higher risk of being infected with *Cryptosporidium*. Molloy et al. (2011) and Yones et al. (2015) also found association between stunting and *Cryptosporidium* infection among Nigerian and Egyptian children respectively, which supports the findings that stunted children had a significantly higher risk of being infected with *Cryptosporidium*.

Conclusion

This first study, carried out in children less than five years of age with diarrhea, showed that the prevalence of cryptosporiodiosis was estimated at 5.8%, a possible underestimation. A study with a larger sample size and of a longer duration is needed not only to determine the real prevalence of this parasitosis but also to assess the associated risk factors in Benin.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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