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Risk Factors and Seasonality for Cryptosporidiosis Among Yemeni Children

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ABSTRACT

Few studies have been conducted on risk factors and seasonality for cryptosporidiosis from developing countries. The current study was conducted to determine prevalence, risk factors and seasonality for cryptosporidiosis among children in Sana'a city, Yemen. Using cross-sectional approach, a sample of 1253 diarrheic children aged 6-<12 years with mean 8.6+1.7 year were surveyed over a period of one year. Single fecal specimens were collected and modified Kinyoun's acid-fast staining of formalin- ether concentrate feces were examined for detection of Cryptosporidium. The rate of infection was 13.8% (173 of 1253), and mild oocysts-excretors of them represented the highest percentage(43.4%). Results indicated that, use of public and/ or well water for drinking, contact with animals, and mother's illiteracy were the risk factors that significantly associated with infection($P<0.05$) in diarrheic children. A higher incidence of infection was recorded during the rainy summer season through July to September. In addition, 13 specimens were collected from children undergoing chemotherapy for cancer; Cryptosporidium was detected in feces of 5(38.5%) and 6 (46.2%) children by Kinyoun's and immunochromatographic (ICT) techniques respectively.
Keywords: cryptosporidiosis, Yemeni children, risk factors, seasonality

INTRODUCTION

Cryptosporidium is a coccidian oocysts-forming protozoan which completes its life cycle both in human and animal. It is transmitted through zoonotic and anthroponotic transmission or via contaminated water or food, causing cryptosporidiosis[1]. This coccidian found in the brush border of the enterocytes of the small intestine in many vertebrate hosts, including humans. It has been found in association with diarrhoeal diseases especially among children in most parts of the world [2]. Approximately 12 species of the genus *Cryptosporidium* are now recognized; two species, *C. parvum* and *C.*

hominis are the major causing agents of human cryptosporidiosis [3]. The disease is recognized as a cause of self limited diarrhea in immunocompetent individuals or it is severe and even fatal in immunocompromised patients as those with HIV infection and patients receiving chemotherapy for cancer [4]. Contamination of drinking water by *Cryptosporidium* oocysts can result in major waterborne outbreaks of cryptosporidiosis [5]. It is now increasingly considered an important food-borne pathogen causing a disease of socioeconomic significance worldwide [6]. The potential for environmental contamination with this coccidian depends upon a variety of factors including the geographic distribution of the parasite, its seasonality, climate, number of infected hosts, human and/or animal activity, number of infective oocysts excreted, socioeconomic and ethnic differences in behavior, sanitation, safety of drinking water sources and supplies [7,8].

Cryptosporidium is under-diagnosed in most Yemeni hospital laboratories and few studies have estimated the problem of cryptosporidiosis, besides the lacking reports of its seasonality. Characterization of the epidemiology of cryptosporidiosis may identify geographic and sociodemographic risk factors that may contribute to the disease. The present study was designed to estimate some personal, familial, environmental risk factors and seasonality associated with cryptosporidiosis among diarrheic Yemeni children over a period of one year. Additionally, it was an attempt to assess the proportion of infection among diarrheic children undergoing chemotherapy for cancer in national oncology centre in Sana'a city.

SUBJECTS AND METHODS

This study was conducted during the period from February 2009 through to January 2010 in Sana'a city. Using Cross-sectional approach; 1253 fecal specimens were collected randomly from children with diarrhea, 724(57.8%) males and 529(42.2%) females, attending three Governmental hospitals; Al-Gomhoree, Al-Thawra, and Al-Sabeen. Children were aged six to less than twelve years. Information about each child were collected by means of questionnaire, filled with assistance of his parent when necessary. Data requested included some socio-demographic and environmental data as, name, age, gender, parent's education, crowding index(number of residents/room), animal contact, and drinking water source. Fresh single fecal specimen was collected from each child in a labeled plastic covered cup, about one ml of each specimen was placed in a labeled-tight bottle containing 3ml of 10% formalin and kept until being processed. To diagnose *Cryptosporidium* oocysts in fecal specimens, each specimen was subjected to formol-ether concentration technique. A thin smear was prepared from the sediment and examined using modified Kinyoun's acid fast staining technique [9]. The intensity of infection was estimated for positive samples by counting the number of oocysts in 100 different high power fields (hpf).Then the number of oocysts was divided by 100. Number of oocysts less than 3 /hpf was considered a mild infection, 3 to 8/hpf moderate, and more than 8 heavy [10]. In addition, 13 fecal samples were collected randomly(during January 2009) from diarrheic children aged 6-<12 years, undergoing chemotherapy for cancer in national oncology centre in Sana'a city. Each sample was subjected to formol-ether concentration technique and examined using both modified Kinyoun's method **[9]**, and Immunochromatographic assay for the identification of *Cryptosporidium* oocysts [11].

Modified Kinyoun's acid-fast staining technique (Kinyoun's cold method)
After concentration of fecal specimen in formol-ether technique, a thin smear was prepared from one drop of each fecal sample sediment and it was fixed in absolute methanol for 1 minute. Smear was stained with cold Kinyoun's carbol fuchsin for 5 minutes, the stain was rinsed off in 50% ethanol for about 3 seconds and then the stain rinsed off in clean tap water. Smear was decolorized using 1% sulfuric acid until no more color flooded from the smear. Decolorizer was rinsed off in tap water. Smear was counter stained with 0.3% malachite green for about 1 minute. Counter stain was rinsed off in tap water and blot to dry. Smear was examined microscopically using high dry objective to identify the oocysts and oil immersion objective to see the internal morphology. In positive sample, *Cryptosporidium* oocysts appeared as acid fast densely stained pink to red spherical structures measuring 4-6µm in diameter against a green background [9].

Immunochromatographic assay (ICT, Xpect^{Tm})
Qualitative chromatographic immunoassay was performed *in vitro* using Xpect^{Tm} REMEL,^{Inc}; USA kit(contains 20 test devices) according to the manufacturer′s instructions. Briefly, fecal specimen was added to conjugate containing colored microparticles linked to murine monoclonal antibodies specific for *Cryptosporidium* antigen. The specimen was considered positive if a complete red line of any intensity appeared at the *Cryptosporidium* test position [11].

Statistical analysis (MstatC): Chi-square (X^2) test was used for testing the association between categorical variables. A value of $P<0.05$ was considered significant. Arithmetic mean and standard deviation were used as descriptive measures.

RESULTS

From table (1); out of 1253 fecal specimens, 173 (13.8%) were positive for *Cryptosporidium* oocysts. The higher percentage of infection was among children aged 9 to<12 years (15.4%) compared to 12.0% among those aged 6 to < 9 years, but the difference was not statistically significant (X^2=0.33, $P > 0.05$). The relation of infection to gender, there was no significant difference between males and females. Percentage of infected children was significantly increased as the mother's educational level decreased(X^2= 8.45, $P < 0.05$). Similarly, Percentage of infection increased as the level of father's education decreased. However the difference was not significant (X^2 = 3.26, $P > 0.05$). It is clear from the table that, percentage of infection increased insignificantly as the crowding index increased. (X^2 = 1.63, $P> 0.05$). Relating to the presence of animals, percentage of infection was 10.4% among children who lived in compounds with no animals compared to 27.6% among those who lived in contact with animals, and the difference was significant (X^2 =8.52, $P < 0.05$). Concerning source of drinking water, children who drank public and /or well water were strongly associated with the risk of infection (24.6%) than those who drank tap water (10.4%) (X^2 = 19.9, $P< 0.05$). Among 13 children who were under chemotherapy for cancer; *Cryptosporidium* was detected in feces of 5(38.5%) and 6 (46.2%) children by Kinyoun's and ICT techniques respectively.

Relating to intensity of infection (Figure 1), mild infected children (<3 oocysts/ hpf) represented the highest percentage (43.4%) whereas, heavy infected children (> 8 oocysts / hpf) represented the lowest one (22.5%).

Monthly seasonal proportion of *Cryptosporidium* infection gradually increased from a minimum in March to a maximum between July and September 2009, then decreased in October. Peak was observed in September (Figure 2).

Table (1): Distribution of *Cryptosporidium* infection among children with diarrhea according to socio-demographic and environmental factors.

Variable	No. examined	*Cryptosporidium spp.*		Chi-Square X^2 (p.value)
		No.	%	
Age				0.33(0.564)
6-	598	72	12.0	
9- <12	655	101	15.4	
Gender				0.00(1.000)
Males	724	99	13.7	
Females	529	74	14.0	
Mother's education			19.3	8.45(0.015)*
Illiterate or read and write	579	112	10.6	
Primary or preparatory	482	51	5.2	
Secondary or university	192	10		
Father's education♥				3.26(0.195)
Illiterate or read and write	476	89	18.7	
Primary or preparatory	447	55	12.3	
Secondary or university	284	29	10.2	
Crowding index#				1.63(0.442)
< 2	139	12	8.6	
2-4	723	102	14.1	
> 4	391	59	15.1	
Presence of animals				8.52(0.004)*
Yes	246	68	27.6	
No	1007	105	10.4	
Drinking water source♥♥				19.9(0.000)*
Tap water	791	82	10.4	
Sieved water	104	3	2.9	
Public and / or well water	358	88	24.6	
Total	1253	173	13.8	

*P < 0.05, # No. of residents per room.

♥Excluding 46 children whose fathers were dead .

♥♥[Sieved water=filtered and/or bottled water, Public water= Pipes water outside home].

N.B. Room size = approximately 4x4 meter .

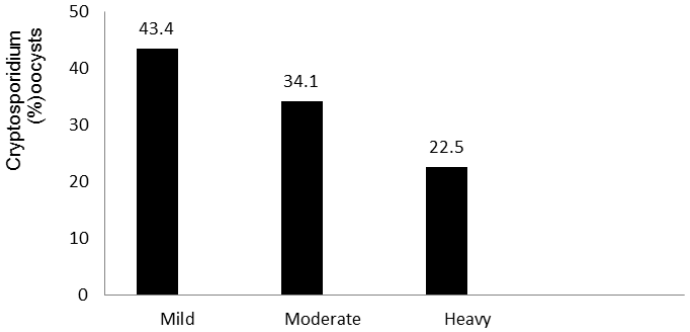
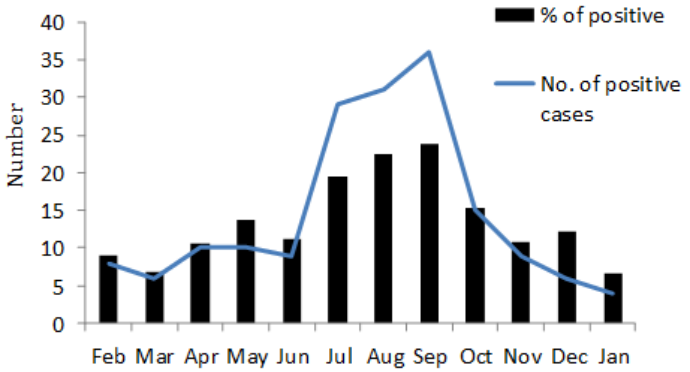


Figure (1): Distribution of *Cryptosporidium* infected diarrhoeic children according to intensity of infection.



Figure(2):Seasonality of cryptosporidiosis among children February 2009 to January 2010

DISCUSSION

Cryptosporidium is a leading cause of persistent diarrhea in developing countries. It can cause illness lasting longer than one to two weeks in previously healthy children or indefinitely in immunocompromised ones [12]. Prevalence data of *Cryptosporidium* are still underestimated in pediatric populations, due to a poor clinical valuation of pathognomic symptoms and to the absence of advanced laboratory tools in diagnostic routine panels[13]. This is first report of risk factors and seasonality for cryptosporidiosis in diarrheic children from Yemen. In the current study, 13.8% of diarrheic children had cryptosporidiosis. This rate is consistent with previous studies that reported 14% and 15% from Korea and Nigeria respectively [14,15]. Data from 16 case-control studies(1994-2003) indicate that, the average overall prevalence of infection in diarrheic immunocompetent patients in developing countries is 12.7% [16]. Slightly lower infection rates were reported from Kuwait(10%), [17] and from Palestine (11.6%) [18]. To the contrary, higher rates were reported form Jordan and Egypt, 37.3% and 27.9% respectively[19,20]. The rates of infection may have differed according to multiple factors; including type of the study, hospital or community based, socioeconomic, sanitary and environmental conditions. In addition to these the parasitological methods applied in each study could influence the outcome of results [21].

The relation of infection to socio-demographic and environmental factors**,** it is evident from table (1) that, percentage of infection increased insignificantly as the age increased. Thus, those aged 6-<9 represented the lowest percentage whereas, those aged nine to less than twelve years represented the highest percentage. It was reported that, percentage of infection increased gradually as the age increased and a strong tendency was observed that, the older the age the higher the infection rate. This suggested that, as children grow, they probably become active, energetic and are more susceptible to infection [14].

Considering gender, percentages of infection among boys and girls were similar. in agreement with the present work, uniform gender distribution of cryptosporidiosis had been reported by many surveys, where males and females were equally susceptible [22,23].

Regarding mother's education, percentage of infected children increased significantly as the mother's education level decreased. Nearly similar results were obtained from a study done among primary school diarrheic children in Egypt where, children of non-educated mothers had a higher risk of infection than those of educated mothers and the difference between the two groups was statistically significant[24]. Generally, the effect of maternal education on prevalence of parasitic infections is explained by Mata to include mother's knowledge of primary health care and the appropriate procedures for storage of food and water and handling of children's feces which is presumably a reflection of maternal education to decrease prevalence of parasites [25]. Illiterate fathers have low income, bad personal hygiene beside their ignorance about the prevention of intestinal parasitic infections [26].

Crowding index among children was insignificantly associated with the infection. This agrees with a previous study that reported no statistical difference between crowding index and cryptosporidiosis among household members. This was ascribed to two explanations; having a larger household size consisting of several working individuals may relate to increase household income and thus a higher standard of living, or, working household members are likely to use a more modern flush toilet at their place of work, thus minimizing their contribution to the household toilet [27].

Presence of animals was significantly associated with cryptosporidiosis. It has been an evident proof that the transmission of *Cryptosporidium* oocysts is increased through direct contact with animals[28]. The present result goes in accordance with a previous study in Egypt where, percentage of infection detected in animals attendants was 31.3% and it was deduced a positive association between percentage of infected animals and their attendants[29]. To the contrary, contact with cattle and home pets were not associated with cryptosporidiosis among diarrhoeic individuals in England [30]. This suggests that, animals may play a role in the spread of infection but other sources are not less important[28].

Concerning the source of drinking water, the majority of houses in Sana'a city had tap water connection inside their houses but potable water was not available all the time, as a result people tend to use public taps and / or wells as alternative sources of water supply. In the present study, use of public and/ or well water for drinking was statistically associated with infection. This is in agreement with another study that reported a high prevalence of human cryptosporidiosis. This was explained by the presence of polluted animals and / or humans excreta that may possibly contaminate the public taps[14].

Relating to intensity of infection (Figure 1), the majority of infected children in the present study was of mild degree(<3 oocysts/ hpf). Intensity of intestinal infection with *cryptosporidium* varies from host to another. It has been suggested that, excretion of oocysts by individuals is a host characteristic determined by the host immunity. The

severity of disease may be emphasized by the concomitant infection with other intestinal organisms[10,31]. Mild excretors children may be well-nourished and have a higher level of immunological resistance in whom replication of parasite is prevented to some extent [10].

In the current study, the highest proportion of infection occurred during the rainy summer season through July to September (Figure 2). Nearly similar result was reported during the rainy summer season (June–October), from Nepal [32]. In contrast, in Kuwait and Jordan, high incidence rate was reported in rainy winter season(January- April) [17,19]. Seasonal and temporal trends of infection varied from country to country. A seasonal incidence of infection is sometimes present, possibly corresponding to rainfall peaks, increased pollution from farm waste, or calving and lambing activities[13,33].

It can be concluded that, cryptosporidiosis is a health problem in Sana'a city and symptomatic children represent a small part of infection, further studies should be directed to asymptomatic infections, and study of *Cryptosporidium* genome may allow us to better understand host specificity and associated risk factors. Improvement of environmental sanitation especially proper sewage, and safe water supply are recommended. Ongoing public health measures and health education programs should be go on to focus on the proper care of domestic animals and the importance of hygienic practices.

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عوامل الإختطار والموسمية المرتبطة بالإصابة بخفيات الأبواغ الصغيرة بين الأطفال اليمنيين

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ملخص

قليلة تلك الدراسات التي أجريت على عوامل الإختطار والموسمية المرتبطة بالإصابة بخفيات الأبواغ الصغيرة في البلدان النامية. هدفت هذه الدراسة (للمرة الأولى) لتحديد مستوى الإصابة، عوامل الإختطار والموسمية المرتبطة بالإصابة بخفيات الأبواغ الصغيرة بين الاطفال في مدينة صنعاء باليمن. أجريت دراسة مقطعية على 1253 طفلا من الذكور والإناث يعانون من الإسهال تراوحت أعمارهم من ست سنوات إلى أقل من 12سنة ولفترة عام كامل. جمعت عينات برازية واحدة من كل طفل وطفلة وتم فحصها بطريقة الفورمالين-إيثر للترسيب بالطرد المركزي وطريقة التلوين البارد بصبغة كينيون زيل نيلسين المعدلة للكشف عن كيسات خفيات الأبواغ (الكربتوسبوريديم). معدل حدوث العدوى بخفيات الأبواغ بين الأطفال كانت 13.8% وكانت شدة الإصابة البسيطة هى الأعلى بين العينات الموجبة من الأطفال (43.4%). أظهرت النتائج أن مصادر مياه الشرب من الحنفيات العامة والأبار، الإحتكاك بالحيوانات والأمية في الأمهات مثلت عوامل إختطار هامة (وبدلالة إحصائية) لإنتشار العدوى بين الأطفال، وقد سجل معدل أكثر إرتفاعا للعدوى في الفصل المطير بين شهري يوليو وسبتمبر. بالإضافة الى ذلك، جمعت عينات برازية من 13طفلا يخضعون للعلاج الكيماوئي لإصابتهم بالسرطان (تراوحت أعمارهم من ست سنوات إلى أقل من 12سنة) لتحديد نسبة الإصابة بخفيات الأبواغ. وبإستخدام طريقتي صبغة كينيون المعدلة والفحص المناعي الكروماتوغرافي وجد أن نسبة الإصابة بينهم 38.5%، 46.2% على التوالي.

كلمات مفتاحية: الإصابة بخفيات الأبواغ الصغيرة، أطفال يمنيين، عوامل إختطار، موسمية