

# Prevalence of *Cryptosporidium* spp. infection among children admitted to Hospital Tengku Ampuan Afzan

Asady Abdullah, MD<sup>1</sup>, Soraya Ismail, PhD<sup>2</sup>, Marsitah, BSc<sup>3</sup>, Oothuman Pakeer, PhD<sup>2</sup>

<sup>1</sup>Department of Microbiology, Kabul University of Medical Sciences, Karte Sakhi, 3rd district, Kabul, Afghanistan, <sup>2</sup>Department of Basic Medical Sciences, Kulliyah of Medicine, International Islamic University Malaysia, Bandar Indera Mahkota, Kuantan, Pahang Darul Makmur, <sup>3</sup>Department of Pathology, Hospital Tengku Ampuan Afzan, Kuantan, Pahang

## ABSTRACT

**Introduction:** *Cryptosporidium* spp. is identified as an important cause of diarrhoeal morbidity and mortality worldwide particularly in children below five years of age and immunocompromised individuals. Infections are present among cattle and humans. Until now, there is no report on its prevalence in humans in Kuantan. The aim of this study is to record the prevalence and associated risk factors of *Cryptosporidium* spp. infection among children admitted to Hospital Tengku Ampuan Afzan (HTAA), Kuantan.

**Methods:** This is a cross-sectional study conducted among children admitted to the Paediatrics ward in HTAA between December 2017 and May 2018. Faecal samples were examined using wet smear and Modified Ziehl-Neelsen (MZN) staining techniques. Data on demography and hygiene practices was collected using a pretested questionnaire, and analysed using SPSS version 22.

**Results:** One hundred thirty five children (95.6% were of Malay ethnicity) were included in the study. The overall prevalence of *Cryptosporidium* spp. infection was 25.2%. The prevalence was slightly higher in females (28.1%) than males (23.1%). The prevalence was higher than expected. This study showed that some risk factors namely children's age and trash disposal methods were significantly associated with *Cryptosporidium* spp. infection ( $p < 0.05$ ).

**Conclusion:** Higher prevalence could possibly be due to an outbreak of this infection or until now undetected.

## KEY WORDS:

*Cryptosporidium*, Hospital Tengku Ampuan Afzan, Kuantan, Parasites, Protozoan

## INTRODUCTION

*Cryptosporidium* spp. is a protozoan parasite, which infects humans and a variety of animals like cattle and cats.<sup>1</sup> The infection is more common in developing countries (up to 10%) compared to developed countries (<1% to 3%).<sup>2</sup> This protozoan has a complex life cycle mainly in epithelial cells of the gastrointestinal tract of these hosts and is an important cause of morbidity and mortality worldwide.<sup>2</sup> Studies confirmed the importance of *Cryptosporidium* spp. as a cause of watery diarrhoea and malnutrition in children. The

diarrhoea caused is usually self-limiting in immune-competent individuals, but may be chronic and life-threatening in immunocompromised persons.<sup>3</sup>

The first case of *Cryptosporidium* infection in Malaysia was reported in 1984 in a young man who presented with bloody diarrhoea.<sup>4</sup> In a community-based study in Kelantan (Malaysia) conducted among young children with diarrhoea, 11% were infected with *Cryptosporidium* spp.<sup>5</sup> The occurrence of cryptosporidiosis among paediatric patients ranged between 1-11%.<sup>6</sup>

The aim of this research was to study the prevalence and associated risk factors of *Cryptosporidium* spp. infection among children admitted to Hospital Tengku Ampuan Afzan (HTAA), Kuantan.

## MATERIALS AND METHODS

This is a cross-sectional study conducted in HTAA, a public hospital in Kuantan, Pahang, Malaysia. Data was collected from December 2017 to May 2018. Participants were selected from patients admitted to the Paediatrics ward. Potential participants were briefed about the objectives and methodology of the study. All children who admitted to the Paediatrics ward of HTAA and have signed the informed consent form were included in the study. Children with HIV/AIDS was excluded. Participants were admitted due to various medical conditions other than intestinal parasitic/*Cryptosporidium* infection. Upon agreement, informed consent was obtained. A pretested questionnaire designed to collect information on the demography, socio-economic status, and hygiene practices of the participants. Three experts from the IIUM assessed the content validity for the questionnaire and approved it to be used for the study. Before beginning data collection, the questionnaire was pilot tested among 20 randomly selected subjects and necessary changes were applied by expert parasitologists at the Department of Basic Medical Sciences, Kulliyah of Medicine, IIUM. Children above 12 years old were asked to fill up the questionnaire, as for the children below 12 years, their parents/guardians were asked to answer the questions.

The stool samples were collected immediately after defecation anytime during admission. Screw capped plastic containers with spatulas and gloves were given to the patients/parents for collection of samples. After collection of samples, they

This article was accepted: 15 August 2019

Corresponding Author: Soraya Ismail

Email: dr\_soraya@iium.edu.my

Table I: Socio-demographic characteristics of the subjects

Characteristics		Frequency (%) / mean (SD)
Age group	≤2 year	87 (64.4)
	>2 years	48 (35.6)
Gender	Male	78 (57.8)
	Female	57 (42.2)
Ethnicity	Malays	129 (95.6)
	Chinese	2 (1.5)
	Indians	1 (0.7)
	Orang Aslis	2 (1.5)
Residence	Kuantan	108 (80)
	Outside	20 (14.7)
	Average members per household (mean, SD)	5.34 ± 1.7
Household number	≤5 members	79 (62.7)
	>5 members	47 (37.3)
Household income (RM)	≤1000	14 (11.5)
	>1000	108 (88.5)
Father's education level	>6 years (Educated)	113 (91.9)
	≤6 years (Not educated)	10 (8.1)
Mother's education level	≤6 years (Not educated)	2 (1.6)
	>6 years (Educated)	121 (98.4)
Working fathers	Yes	112 (91.1)
	No	11 (8.9)
Working mothers	Yes	66 (53.7)
	No	57 (46.3)

Table II: Hygiene and environmental practices of the subjects

Practices		Frequency (%)
Source of drinking water	Tap water	61 (49.6)
	Filtered water	55 (44.7)
	Mineral water	2 (1.6)
	Hand pump	2 (1.6)
	Open well and river	3 (2.4)
Boiling water	Yes	74 (60.2)
	No	49 (39.8)
Defecation place	Pour flush inside house	116 (94.3)
	Indiscriminate (Outside house, river side)	7 (5.7)
Garbage disposal	Municipality	107 (87)
	Burning	16 (13)
Swimming habits	Regular	9 (7.3)
	Not regular	114 (92.7)
Close contact with domestic animals	Yes	49 (39.8)
	No	74 (60.2)
Poultry ownership	Yes	28 (22.8)
	No	95 (77.2)
Cat ownership	Yes	40 (32.5)
	No	83 (67.5)
Dog ownership	Yes	1 (0.7)
	No	122 (99.23)

Table III: Results of Chi-square and logistic regression analysis for factors significantly associated with *Cryptosporidium spp.* infection

Factors		Cryptosporidium spp.		Chi-square		Logistic regression	
		Yes n (%)	No n (%)	p	OR <sup>c</sup> (95 % CI)	p	AOR <sup>**</sup>
Subject's age	≤2 Years	15 (17.2)	72 (82.8)	0.004	1	0.167	1
	>2 Years	19 (39.6)	29 (60.4)				
Trash disposal	Municipality	25 (23.4)	82 (76.6)	0.08	1	0.046	1
	Burning	7 (43.8)	9 (56.3)				

\*Crude Odds Ratio \*\*Adjusted Odds Ratio 1= Reference category

were transported within three hours in an appropriate icebox to IIUM microbiology research laboratory for analysis. Macroscopic, direct wet mount, sedimentation and Modified Ziehl-Neelsen (MZN) staining techniques were used to screen for *Cryptosporidium* spp.

IBM SPSS Statistics version 22 for windows (SPSS Inc., Chicago, IL) were used for the statistical analysis. Risk factors related to the prevalence were analysed using Chi-square ( $\chi^2$ ) to determine if there is a statistical significance between the *Cryptosporidium* spp. infection and particular factors. Pearson's ( $\chi^2$ ) test on proportion was used to examine the crude associations between dependent and independent variables. Logistic regression analysis was carried out to determine the independent association of variables with the dependent variable, i.e., *Cryptosporidium* spp. infection. All variables with a significant level of  $p \leq 0.25$  in the univariate analysis were chosen for multivariate analysis.<sup>7</sup>

This research was registered in the National Medical Research Registry (NMRR), (Research ID # 36392, NMRR-17-1229-36392). Human ethical approval was obtained from the Medical Research and Ethics Committee (MREC), (Ref. (10, 11) KKM/NIHSEC/P17-1113), the study site (HTAA, CRC), and IIUM's Kulliyah Research Committee approvals were also obtained.

## RESULTS

A total of 135 children were recruited for the study. The age of the subjects ranged from <1-year-old to 14-year-old with a median of two (IQR=2) years; 91.9% of them were  $\leq 5$  years old. Male participants (57.8%) were slightly more than female (42.2%) and 95.6% were from Malay ethnicity. Most of the participants (85.7%) lived in Kuantan. The size of the households averaged  $5.34 \pm 1.7$  members, with 62.7% having five or less members. Majority of the participants' households (88.5%) had an income of  $\geq$  RM1000/month. Table I shows the socio-demographic characteristics of the subjects.

Almost half of the study population responded that they used tap water for drinking without using filtration and 44.7% used filtered water. Moreover, 60% of them boiled their water before consumption. Most (93.4%) of the tap water users boiled water before drinking, while the majority (77.4%) of filtered water users did not boil the water. Nearly 85% of households used municipality services for garbage disposals and the remaining (15%) reported to burn their garbage. Around 40% of the participants reported to have close contact with domestic animals, and of these 34% had close contact with cats and 24% with poultries. Table II shows hygiene and environmental practices of the subjects.

The overall prevalence of *Cryptosporidium* spp. infection was 25.2%. Table III shows the results of univariate (Chi-square) and binary logistic regression analysis for factors significantly associated with *Cryptosporidium* spp. infection. Subjects who were  $> 2$  years old had higher odds of getting *Cryptosporidium* spp. infection compared to those  $\leq 2$  years old (Odds Ratio, OR 3.1; 95% Confidence Intervals, 95%CI: 1.4-7.0). Subjects whose households disposed their garbage via burning had higher odds of getting *Cryptosporidium* spp.

infection than those households who used Municipality services for their garbage disposal (OR 4.3; 95%CI: 1.0-18.0).

## DISCUSSION

The prevalence of *Cryptosporidium* spp. infection in the study was (25.2%). Although the *Cryptosporidium* spp. is a well-established cause of sporadic gastroenteritis with outbreaks characterised by watery diarrhoea, abdominal pain, nausea, vomiting and low-grade fever, its occurrence is underdiagnosed and under-reported in many countries.<sup>8</sup>

In Malaysia, several studies on *Cryptosporidium* spp. infections were conducted during 1990s. The highest prevalence of *Cryptosporidium* spp. was reported among HIV infected intravenous drug users (23%)<sup>9</sup> and an Orang Asli community (20.1%).<sup>10</sup> A recent study in Malaysia has reported a prevalence of 4.62% among children below 12 years of age presenting with diarrhoea.<sup>11</sup>

In the current study, the prevalence of *Cryptosporidium* spp. infection was higher among children  $> 2$  years old. This probably reflects increased exposure to the protozoan due to poorer hygiene practices and a lack of or partial immunity in children. Infected children are also likely to transmit the infection to their parents via direct contact.<sup>8</sup> In developing countries, it has been reported that *Cryptosporidium* infects children, particularly  $< 5$  years of age and the infection is higher in children  $< 2$  years of age.<sup>11</sup>

Disposal of garbage via burning was found a significant contributor for *Cryptosporidium* spp. infection. This is in line with a study done by Lee et al.<sup>12</sup>

Analysis of our questionnaire noted that out of 116 subjects/households who had access to tap water, 96% of them either boiled or filtered the water before drinking, possibly because they thought that the quality of tap water is not suitable for drinking. Furthermore, 93.4% of the participants who used tap water also reported to boil water, whereas, only 23% of those subjects who used filtered water reported to boil their water before drinking. This indicates that 77% of the subjects/households used inefficient filter systems. Study shows that common filter systems do not successfully remove *Cryptosporidium* spp. oocysts from contaminated water because of their smaller size ( $4\text{-}6\mu\text{m}$ ).<sup>13</sup> Since there was no published study on the status of *Cryptosporidium* spp. infection among human population in Kuantan, therefore, we looked for reports of evidence of this infection in cattle population and water resources. In a study conducted to determine the prevalence of *Cryptosporidium* spp. among cattle in Kuantan, Pahang it was found that 15.9% of the animals were infected. The authors suggested that proper preventive measures must be applied in order to reduce infection among cattle, since the infected cattle may serve as reservoir to humans (possibly animal handlers).<sup>14</sup> In this study, no association could be drawn between cattle ownership and *Cryptosporidium* spp. infection.

Furthermore, *Cryptosporidium* spp. oocysts are reported to be ubiquitous in water sources in the state of Pahang, Malaysia which can survive for months in the harsh environments.<sup>15</sup>

Contamination of sources of water (for drinking, recreation and irrigation) probably plays a role in animals and human getting *Cryptosporidium* spp. infection.

This study shows a higher prevalence of *Cryptosporidium* spp. infection among children admitted to HTAA. More studies need to be conducted in order to find the prevalence of this infection among community population in the Kuantan and exact sources of the infection.

**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

**ACKNOWLEDGEMENTS**

The authors would like to thank Prof. Dr. Norhayati Moktar, formerly Head of Parasitology Department at UKM, Dr. Raden Shamilah Radin Hisam, of Institute for Medical Research, and Dr. Silva Kumar of HTAA. The funding for this study was provided by the Ministry of Higher Education Afghanistan through Higher Education Development project and International Islamic University Malaysia.

**REFERENCES**

1 Thompson RCA, Koh WH, Clode PL. *Cryptosporidium* - What Is It? Food and Waterborne Parasitology 2016; 4: 54-61.  
 2 Mahmoudi MR, Ongerth JE, Karanis P. *Cryptosporidium* and cryptosporidiosis: The Asian perspective. Int J Hyg Environ Health 2017; 220(7): 1098-109.

3 El-Malky MM, Mowafy NM, Zaghloul DA, Al-Harathi SA, El-Bali MA, Mohamed RT, et al. Prevalence of cryptosporidium species isolated from diarrheic children in Makkah, Saudi Arabia. Tropical Biomedicine 2018; 35(1): 76-81.  
 4 Che Ghani M, Abdullah MM Hashim MB. A case of cryptosporidiosis in a young, man presenting with bloody diarrhea. Journal of the Malaysia Society of Health 1984; 4: 80-1.  
 5 Lai K. Intestinal protozoan infections in Malaysia. Southeast Asian J Trop Med Public Health 1992; 23(4): 578-86.  
 6 Lim YA, Ahmad RA, Smith HV. Current status and future trends in *Cryptosporidium* and *Giardia* epidemiology in Malaysia. J Water Health. 2008 Jun;6(2):239-54.  
 7 Bendel RB, Afifi AA. Comparison of stopping rules in forward "stepwise" regression. Journal of the American Statistical Association 1977; 72: 46-53.  
 8 Cacciò SM, Chalmers RM. Human cryptosporidiosis in Europe. Clin Microbiol Infect 2016; 22(6): 471-80.  
 9 Kamel AG, Maning N, Arulmainathan S, Murad S, Nasuruddin A, Lai KP. Cryptosporidiosis among HIV positive intravenous drug users in Malaysia. Southeast Asian J Trop Med Public Health 1994; 25(4): 650-3.  
 10 Kamel A, Kasim MS, Lai K. Parasitic infections among Orang Asli community in Pangsun, Hulu Langat. Institute for Medical Research Annual Report 1994: 46-7.  
 11 Latif B, Rossle NF. Cryptosporidiosis among children with diarrhoea in three Asian countries: a review. Asian Pacific Journal of Tropical Biomedicine 2015; 5(11): 885-8.  
 12 Lee SC, Ngui R, Tan TK, Muhammad Aidil R, Lim YA. Neglected tropical diseases among two indigenous subtribes in Peninsular Malaysia: highlighting differences and co-infection of helminthiasis and sarcocystosis. PLoS One 2014; 9(9): e107980.  
 13 Zainutdin FK, Barudin MA, Jainul MA, Isa MLM, Yusof AM. The association of *Cryptosporidium* from three different points of Balok River and Kuantan River by using physico-chemical and heavy metal assessments. Asian Pacific Journal of Tropical Disease 2017; 7: 449-54.  
 14 Hisamuddin NH, Hashim N, Soffian SN, Amin MH, Wahab RA, Mohammad M, Isa ML et al. Identification of cryptosporidium from dairy cattle in Pahang, Malaysia. Korean J Parasitol 2016; 54(2): 197-200.