



# Prevalence of and Risk Factors Associated with Parasitic, Bacterial and Viral Infections among Children with Gastrointestinal Illness in Bamrasnaradura Infectious Diseases Institute

Nuchalita Awae<sup>1,\*</sup>, Duangnate Pipatsatitpong<sup>1</sup>

<sup>1</sup>*Department of Medical Technology, Faculty of Allied Health Sciences, Thammasat University, Pathum Thani 12120, Thailand*

Mathirut Mungthin<sup>2</sup>, Toon Ruang-areerate<sup>2</sup>, Saovanee Leelayoova<sup>2</sup>

<sup>2</sup>*Department of Parasitology, Phramongkutklao College of Medicine, Bangkok 10400, Thailand*

Ratchaneewan Aunpad<sup>3</sup>

<sup>3</sup>*Faculty of Allied Health Sciences, Thammasat University, Pathum Thani 12120, Thailand*

Pawita Suwanvattana<sup>4</sup>, Unchana Thawornwan<sup>4</sup>

<sup>4</sup>*Department of Disease Control, Bamrasnaradura Infectious Diseases Institute, Ministry of public health, Nonthaburi 11000, Thailand*

Received 21 May 2018; Received in revised form 22 June 2018

Accepted 22 June 2018; Available online 30 September 2018

## ABSTRACT

Children living in developing countries are particularly susceptible to infectious gastrointestinal illnesses because of poor standards of hygiene and sanitation. Infectious gastrointestinal illnesses in Thailand remain the major health problems in children. This study aimed to determine the prevalence of intestinal infections and risk factors among the children with gastrointestinal illnesses at Bamrasnaradura Infectious Diseases Institute during June 2016 to September 2016. Stool samples were collected from eighty two children and examined for parasitic, bacterial, and viral infections. Demographic data and risk factors were collected using standardized questionnaire. Descriptive statistics were used to analyze the prevalence and demographic data. Risk factors were analyzed using binary logistic regression.

The prevalence of parasitic infections was 26.8% (22/82) whereas the prevalences of bacterial infections and viral infections were 14.5% (10/69) and 9.8% (6/61), respectively. The most intestinal parasitic, bacterial and viral infections were *Blastocystis* sp. (15.9%), *Salmonella* spp. (8.7%) and Rotavirus (9.8%), respectively. Binary logistic regression showed a significant association of intestinal infections in children who lived with many young children in the same house (odds ratio: 2.6, 95% CI: 1.0–6.9,  $p=0.045$ ). These findings indicate that living conditions and personal hygiene of children should be improved in order to prevent these infectious gastrointestinal illnesses.

**Keywords:** Prevalence; Infectious gastrointestinal illnesses; Children; *Blastocystis*; *Salmonella*; Rotavirus

## 1. Introduction

Intestinal infections remain a major public health problem in Thailand. A wide range of microorganisms including parasites, bacteria and viruses can cause intestinal disorders. Pathogenic organisms usually found in contaminated water and food. It is also commonly found in unimproved sanitation and lacking of hygiene [1-2]. Clinical symptoms of gastrointestinal disorders can be asymptomatic to severe gastrointestinal disorders in humans such as abdominal pain, nausea, vomiting, fatigue and diarrhea [1]. Diarrhea in children is still an important problem in public health. According to the WHO, diarrhea is the second leading cause of the death in children under five years old. Each year around 525,000 children were killed [1]. It is very important to diagnose the causes of diarrhea as earliest as possible. Therefore, this study aimed to determine the prevalence and risk associations of intestinal infections (parasites, bacteria and viruses) in children with gastrointestinal disorders at the Pediatric Inpatient Department, Bamrasnaradura Infectious Diseases Institute.

## 2. Materials and Methods

### 2.1 Study population and study design

A cross-sectional survey was carried out from June 2016 to September 2016, included gastrointestinal symptomatic children at Pediatric Inpatient Department, Bamrasnaradura Infectious Diseases Institute. A total of 82 children admitted to and diagnosed with gastrointestinal disorders were recruited. All children's stool samples were tested for parasite examination. However, other tests were depended on doctor's requirements. Their parents were asked to sign an assent form. This study was reviewed and approved by the Ethical Committees of Thammasat University and Bamrasnaradura Infectious Diseases Institute.

### 2.2 Stool collection and examination

Sterile plastic containers and Carry-Blair transport medium tubes were distributed to the nurses at Pediatric Inpatient Department. Standard procedures for stool specimen collection were informed to the nurses. All stool samples were carried in ice boxes and transported immediately to the Microbiology and Virology laboratories at Bamrasnaradura Infectious Diseases Institute for bacterial and viral identification.

For bacterial identification, fecal specimens were cultured directly on Blood agar base no 2, MacConkey agar, *Salmonella-Shigella* agar (Oxoid, Thermo Fisher Scientific, UK) and TCBS agar (Eiken Chemical, Japan) at 37°C for 24

hours. To identify *Campylobacter*, fecal samples were also cultured on *Campylobacter* blood-free agar with CCDA selective supplement, Sheep blood agar base no.2 with Skirrow selective supplement (Oxoid, Thermo Fisher Scientific, UK) and incubated at 37°C for 48 hours under microaerophilic conditions (10% CO<sub>2</sub>, 5% O<sub>2</sub>, 85% N<sub>2</sub>). All suspected pathogenic bacteria colonies on agar plates were identified using microbiology laboratory process.

For viral identification, fecal specimens were investigated Rotavirus and Adenovirus using Rida Quick rotavirus/adenovirus Combi test (R-Biopharm AG, Darmstadt, Germany).

For parasite investigation, stool samples were examined by wet smear preparation in normal saline solution. Each stool sample was cultured in Jones' medium supplemented with 10% horse serum and incubated at 37°C for 48–72 hours to identify *Blastocystis* sp.. All specimens were then processed using the phosphate-buffered saline (PBS)-ethyl acetate sediment concentration technique and microscopically examined. Each stool specimen was also examined for *Cryptosporidium* spp., *Cystoisospora belli*, *Cyclospora cayetanensis* using modified acid-fast staining and microsporidia using Gram-chromotrope staining.

### 2.3 Questionnaire

To determine the risk factors, a standardized questionnaire was prepared to elicited information on the demographic data (age and gender) medical backgrounds (diarrhea and dysentery, abdominal pain, stomach pain, bloating, vomiting & nausea), environmental sanitation and living condition characteristics (contact with domestic animals and area around the house). For infants, signs and symptoms were asked from their parents and observed from medical records.

### 2.4 Statistical analysis

Prevalence and risk factors for intestinal infections were evaluated. Prevalences and demographic data were analyzed and presented as frequencies and percentages. Independent risk associations were analyzed using binary logistic regression. The level of significance was set at 5%.

## 3. Results and Discussion.

Of eighty two individual children, who had gastrointestinal disorder and admitted at Pediatric Inpatient Department, Bamrasnaradura Infectious Diseases Institute, there were 49 (59.8%) male and 33 (40.2%) female. The pediatric patients aged 2 months–17 years (Mean  $\pm$  SD: 2.4  $\pm$  2.9 years). For data analysis, children were divided into 2 groups (age < 7 yr,  $\geq$  7 yr). Children were less than 7 years of age, their behaviors such as crawling, walking and playing had higher chance for intestinal infection.

### 3.1 The prevalences of intestinal infections.

The prevalence of intestinal parasitic infections was 26.8% (22/82), while 14.5% (10/69) and 9.8% (6/61) were positive for bacterial and viral infections, respectively. The most common parasite found in these children was *Blastocystis* sp. (15.9%). *Salmonella* spp. was the most common bacterial infection (8.7%). Rotavirus was positive in 6 cases (9.8%). One patient had co-infection with *Blastocystis* sp. and *Salmonella* spp. The prevalences of intestinal infections are shown in Table 1.

**Table 1.** The prevalences of intestinal infections.

Microorganisms	N	(%)
<b>Parasite infection (N=82)</b>		
<i>Blastocystis</i> sp.*	13	(15.9%)
<i>Ascaris lumbricoides</i>	4	(4.9%)
Hookworms	4	(4.9%)
<i>Trichuris trichiura</i>	1	(1.2%)
<b>Total</b>	<b>22</b>	<b>(26.8%)</b>
<b>Bacterial infection (N=69)</b>		
<i>Salmonella</i> spp.*	6	(8.7%)
<i>Aeromonas hydrophila</i>	1	(1.4%)
<i>Campylobacter jejuni</i>	1	(1.4%)
<i>Plesiomonas shigelloides</i>	1	(1.4%)
<i>Vibrio cholerae</i> NAG group 2	1	(1.4%)
<b>Total</b>	<b>10</b>	<b>(14.5%)</b>
<b>Viral Infection (N=61)</b>		
Rotavirus	6	(9.8%)
<b>Total</b>	<b>6</b>	<b>(9.8%)</b>

\*1 case was co-infection (*Blastocystis* sp. & *Salmonella* spp.)

### 3.2 Risk factors of intestinal infections.

Binary logistic regression was used to analyze independent risk factors of intestinal infections. The variables of infections influenced by demographic characteristics and environmental sanitations are shown in Table 2. Univariate analysis showed that children who lived with many young children in the same house had a significantly high risk of acquiring the intestinal infections with crude odds ratio of 2.6 and 95% CI of 1.0–6.9 ( $p=0.045$ ). There have no significant associations between intestinal infections and other factors such as gender, age groups, underlying disease (HIV, tumor, thalassemia, G6PD, nephrotic epilepsy and asthmas), putting hand in the mouth, contact domestic pet and area around the house ( $p > 0.05$ ).

**Table 2.** Univariate analysis of risk factors for intestinal infections.

Characteristics	No. of enroll subject	Prevalence of infection N (%)	Crude OR (95% CI)	P
Sex				
Male	49	24 (48.9)	1	0.393
Female	33	13 (39.4)	0.7 (0.3-1.7)	
Age groups				
Age < 7 years	74	34 (45.9)	1	0.650
Age ≥ 7 years	8	3 (37.5)	0.7 (0.2-3.2)	
Diarrhea symptom				
No	31	12 (38.7)	1	0.364
Yes	51	25 (49.0)	1.5 (0.6-3.8)	
Abdominal pain				
No	63	29 (46.0)	1	0.763
Yes	19	8 (42.1)	0.9 (0.3-2.4)	
Fever				
No	27	8 (29.6)	1	0.052
Yes	55	29 (52.7)	2.6 (0.9-7.0)	

<b>Underlying disease</b>					
No	72	30	(41.7)	1	
Yes	10	7	(70.0)	3.3 (0.8-13.7)	0.105
<b>Living with many young children in the same house</b>					
No	56	21	(37.5)	1	
Yes	26	16	(61.5)	2.6 (1.0-6.9)	0.045*
<b>Putting hand in the mouth</b>					
No	33	14	(42.4)	1	
Yes	49	23	(46.9)	1.2 (0.5-2.9)	0.687
<b>Contact domestic pet</b>					
No	52	20	(38.4)	1	
Yes	30	17	(56.6)	2.0 (0.8-5.2)	0.113
<b>Area around the house</b>					
Concrete	71	31	(43.6)	1	
Wetlands	11	6	(54.5)	1.5 (0.4-5.5)	0.502

**Note:** \* Significant at  $p$ -value  $< 0.05$ , OR = odds ratio; CI = confidence interval.

### 3.3 Discussion

In the present study, the predominant intestinal pathogenic protozoan and bacteria species were *Blastocystis* sp. (15.9 %) and *Salmonella* spp. (8.7%), respectively. One patient was co-infected with *Blastocystis* sp. and *Salmonella* spp. The prevalence of intestinal parasitic infections among children was 26.8% which was higher than the prevalence of bacterial and viral infections. These results were similar to those reported by Pipatsatitpong D. *et.al* study [3]. A cross-sectional survey showed predominant *Blastocystis* and *Salmonella* infections among children in a child care center, Bangkok, Thailand. *Blastocystis* sp. is one of the most common intestinal microorganisms found in Thai children. The prevalence studies of *Blastocystis* infection in Thai children were usually conducted in the communities or child care institutes. A few studies in child care institutes in Thailand showed the prevalence of *Blastocystis* infection ranged between

13.6% to 45.2% [3-7]. The same range of prevalence was also found in those studies of *Blastocystis* infection in school children [8-13]. The present study found the prevalence of *Blastocystis* infection in the hospitalized children who presented with gastrointestinal symptoms including diarrhea and abdominal pain. Although *Blastocystis* sp. has been considered as a pathogen [14-15], most of *Blastocystis* infection in humans are asymptomatic. In those who presented with clinical manifestations, the most common symptoms associated with *Blastocystis* infection are diarrhea, abdominal pain and vomiting. In this study, 84.6% (11/13) and 61.5% (8/13) of the children were positive for *Blastocystis* sp. had abdominal pain and diarrhea, respectively. However, no statistical association between *Blastocystis* infection and these symptoms was identified (data not shown).

The present study showed that *Salmonella* spp. was the most common bacterial causative agent in the children who

presented with gastrointestinal illnesses. A few studies in different period of time in Thailand indicated that *Salmonella* spp. remains one of the common causes of diarrhea in Thai children [16-18]. A study of at Bamrasnaradura Infectious Disease Institute, Thailand, from 1994 to 1996 showed that Nontyphoidal *Salmonella* spp. commonly caused diarrhea in young children especially the first 2 years of life [19]. *Salmonella* group B was the most common serogroup. A study of children with diarrhea caused by Nontyphoidal *Salmonella* spp. at Queen Sirikit National Institute of Child Health (QSNICH), Thailand in 2009 also showed the similar results [20]. Gastrointestinal illnesses caused by viral infections are also prevalent in Thailand. One of the common causes remains Rotavirus [16-18, 21].

Shared or different route of transmission may occur for different causative agents of gastrointestinal illnesses. The present study found that the independent risk association of gastrointestinal infections was living with many young children in the same house. It might be an evidence of person-to-person transmission. According to the most common microorganisms in the present study, i.e., *Blastocystis* sp., *Salmonella* spp. and Rotavirus, person-to-person transmission could occur [3, 6, 22-23]. One study reported that infected children had a higher risk of acquiring blastocystosis than those who lived in the rooms without infected children/child care workers [3]. A few studies showed that *Blastocystis* infection could be transmitted via person-to-person, waterborne, and zoonotic [24]. *Blastocystis* sp. is a common intestinal microorganism that can infect humans and a wide range of animals worldwide. To date, at least 17 subtypes of *Blastocystis* sp. have been reported. Since several subtypes can be detected both in humans and animals, zoonotic transmission has been proposed [15, 25]. In addition, waterborne is one of

the common routes of transmission of *Blastocystis* infection. A few studies showed the evidence of waterborne transmission of *Blastocystis* sp. in Thai communities [10, 26-27].

#### 4. Conclusion

These findings indicate that living conditions and personal hygiene of children should be improved in order to prevent these infectious gastrointestinal illnesses.

#### Acknowledgements

The authors would like to thank all participants, who enrolled in this study. For conflict of interests, the authors declare that they have no conflicts of interest in this study.

The authors gratefully acknowledge the financial support provided by Thammasat University under the TU Research Scholar.

#### References

- [1]. WHO. Diarrheal disease World Health Organization: WHO Media Centre; 2017 May [cited 2017 May 12] Available from: <http://www.who.int/mediacentre/factsheet/fs330/en/>.
- [2]. Bodhidatta L, Srijan A, Serichantalergs O, Bangtrakulnonth A, Wongstitwilairung B, McDaniel P, Mason CJ. Bacterial pathogens isolated from raw meat and poultry compared with pathogens isolated from children in the same area of rural Thailand. *Southeast Asian J Trop Med Public Health*. 2013 Mar; 44(2): 259-72.
- [3]. Pipatsatitpong D, Rangsin R, Leelayoova S, Naaglor T, Mungthin M. Incidence and risk factors of *Blastocystis* infection in an orphanage in Bangkok, Thailand. *Parasit Vectors*. 2012 Feb 14; 5: 37.
- [4]. Saksirisampant W, Nuchprayoon S, Wiwanitkit V, Yenthakam S, Ampavasiri A. Intestinal parasitic infestations among children in an orphanage in Pathum Thani province. *J Med Assoc Thai*. 2003 Jun;86 Suppl 2:S263-70.

- [5]. Thathaisong U, Siripattanapipong S, Mungthin M, Pipatsatitpong D, Tan-ariya P, Naaglor T, Leelayoova S. Identification of *Blastocystis* subtype 1 variants in the Home for Girls, Bangkok, Thailand. *Am J Trop Med Hyg*. 2013 Feb; 88(2): 352-8.
- [6]. Boondit J, Pipatsatitpong D, Mungthin M, Taamasri P, Tan-ariya P, Naaglor T, Leelayoova S. Incidence and risk factors of *Blastocystis* infection in orphans at the Babies' Home, Nonthaburi Province, Thailand. *J Med Assoc Thai*. 2014 Feb;97 Suppl 2:S52-9.
- [7]. Pipatsatitpong D, Leelayoova S, Mungthin M, Aunpad R, Naaglor T, Rangsin R. Prevalence and Risk Factors for *Blastocystis* Infection Among Children and Caregivers in a Child Care Center, Bangkok, Thailand. *Am J Trop Med Hyg*. 2015 Aug; 93(2): 310-5.
- [8]. Yaicharoen R, Ngrenngarmmlert W, Wongjindanon N, SriPOCHANG S, Kiatfuengfoo R. Infection of *Blastocystis hominis* in primary schoolchildren from Nakhon Pathom province, Thailand. *Trop Biomed*. 2006 Jun;23(1):117-22.
- [9]. Warunee N, Choomanee L, Sataporn P, Rapeeporn Y, Nuttapon W, Sompong S, Thongdee S, Bang-On S, Rachada K. Intestinal parasitic infections among school children in Thailand. *Trop Biomed*. 2007 Dec;24(2):83-8.
- [10]. Leelayoova S, Siripattanapipong S, Thathaisong U, Naaglor T, Taamasri P, Piyaraj P, Mungthin M. Drinking water: a possible source of *Blastocystis* spp. subtype 1 infection in schoolchildren of a rural community in central Thailand. *Am J Trop Med Hyg*. 2008 Sep;79(3):401-6.
- [11]. Kitvatanachai S, Rhongbutsri P. Intestinal parasitic infections in suburban government schools, Lak Hok subdistrict, Muang Pathum Thani, Thailand. *Asian Pac J Trop Med*. 2013 Sep;6(9):699-702.
- [12]. Sanprasert V, Srichaipon N, Bunkasem U, Srirunguang S, Nuchprayoon S. Prevalence of intestinal protozoan infections among children in Thailand: a Large-scale screening and comparative study of three standard detection methods. *Southeast Asian J Trop Med Public Health*. 2016 Nov;47(6):1123-33.
- [13]. Assavapongpaiboon B, Bunkasem U, Sanprasert V, Nuchprayoon S. A Cross-Sectional Study on Intestinal Parasitic Infections in Children in Suburban Public Primary Schools, Saraburi, the Central Region of Thailand. *Am J Trop Med Hyg*. 2018 Mar;98(3):763-767.
- [14]. Tan KS. New insights on classification, identification, and clinical relevance of *Blastocystis* spp. *Clin Microbiol Rev*. 2008 Oct;21(4):639-65.
- [15]. Roberts T, Stark D, Harkness J, Ellis J. Update on the pathogenic potential and treatment options for *Blastocystis* sp. *Gut Pathog*. 2014 May 28;6:17.
- [16]. Varavithya W, Vathanophas K, Bodhidatta L, Punyaratabandhu P, Sangchai R, Athipanyakom S, Wasi C, Echeverria P. Importance of salmonellae and *Campylobacter jejuni* in the etiology of diarrheal disease among children less than 5 years of age in a community in Bangkok, Thailand. *J Clin Microbiol*. 1990 Nov;28(11):2507-10.
- [17]. Suwatano O. Acute diarrhea in under five-year-old children admitted to King Mongkut Prachomklao Hospital, Phetchaburi province. *J Med Assoc Thai*. 1997 Jan;80(1):26-33.
- [18]. Bodhidatta L, McDaniel P, Sornsakrin S, Srijan A, Serichantalergs O, Mason CJ. Case-control study of diarrheal disease etiology in a remote rural area in Western Thailand. *Am J Trop Med Hyg*. 2010 Nov;83(5):1106-9.
- [19]. Moolasart P, Sangsujja J, Eampokalap B, Ratanasrithong M, Likansakul S. Nontyphoidal Salmonella diarrhea in Thai children: a study at Bamrasnaradura Hospital, Nonthaburi, Thailand. *J Med Assoc Thai*. 1997 Oct;80(10):613-8.
- [20]. Vithayasai N, Rampengan NH, Hattasingh W, Jennuvat S, Sirivichayakul C. Clinical features of gastrointestinal salmonellosis in children in Bangkok, Thailand. *Southeast Asian J Trop Med Public Health*. 2011 Jul;42(4):901-11.
- [21]. Kittigul L, Swangsri T, Pombubpa K, Howteerakul N, Diraphat P, Hirunpetcharat C. Rotavirus infection in children and adults with acute gastroenteritis in Thailand. *Southeast*

- Asian J Trop Med Public Health*. 2014 Jul;45(4):816-24.
- [22]. Fang ZY, Ye Q, Ho MS, Dong H, Qing S, Penaranda ME, Hung T, Wen L, Glass RI. Investigation of an outbreak of adult diarrhea rotavirus in China. *J Infect Dis*. 1989 Dec;160(6):948-53.
- [23]. Sirinavin S, Hotrakitya S, Suprasongsin C, Wannaying B, Pakeecheep S, Vorachit M. An outbreak of *Salmonella* urbana infection in neonatal nurseries. *J Hosp Infect*. 1991 Jul;18(3):231-8.
- [24]. Stensvold CR, Clark CG. Current status of *Blastocystis*: A personal view. *Parasitol Int*. 2016 Dec;65(6 Pt B):763-771.
- [25]. Yoshikawa H, Koyama Y, Tsuchiya E, Takami K. *Blastocystis* phylogeny among various isolates from humans to insects. *Parasitol Int*. 2016 Dec;65(6 PtB):750-759.
- [26]. Taamasri P, Mungthin M, Rangsin R, Tongupprakarn B, Areekul W, Leelayoova S. Transmission of intestinal blastocystosis related to the quality of drinking water. *Southeast Asian J Trop Med Public Health*. 2000 Mar;31(1):112-7.
- [27]. Leelayoova S, Rangsin R, Taamasri P, Naaglor T, Thathaisong U, Mungthin M. Evidence of waterborne transmission of *Blastocystis hominis*. *Am J Trop Med Hyg*. 2004 Jun;70(6):658-62.