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Research Report

## **BLASTOCYSTIS AND OTHER INTESTINAL PARASITES INFECTIONS IN ELEMENTARY SCHOOL CHILDREN IN DUKUH VILLAGE, KARANGASEM DISTRICT, BALI**

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### **ABSTRACT**

*Blastocystis spp.* is the most common enteric parasitic infection found in several community surveys from developing countries. *Blastocystis* infections may cause gastrointestinal symptoms, but also cause extraintestinal symptoms such as urticaria and joint pain. *Blastocystis* infection can also be asymptomatic or a carrier. However, the prevalence of *Blastocystis* infection in children has not yet been fully investigated in Indonesia, particularly in Bali Province. This study aimed to determine the prevalence of *Blastocystis* and other intestinal parasites in elementary school children stools in Dukuh village, Karangasem regency. A cross sectional study was conducted in September 2016. A total of 103 school children stools were collected by informed consent and parasites were examined by microscopy with wet mounts method using Lugol's iodine solution. Thirty-five school children were infected with *Blastocystis* spp. (35/103, 34%) that consisted of a single infection (29/35, 82.9%) and mix infection with other parasites (6/35, 17.1%). The mix infections were *Blastocystis* spp. and hookworm infection (1/6, 16.7%), *Blastocystis* spp. and *Entamoeba coli* (1/6, 16.7%), *Blastocystis* spp. and *Giardia lamblia* (2/6, 33.3%), *Blastocystis* spp. and *Entamoeba histolytica/Entamoeba dispar* (1/6, 16.7%) and *Blastocystis* spp. and *Entamoeba histolytica/Entamoeba dispar* and *Giardia lamblia* (1/6, 16.7%). The vacuolar forms of *Blastocystis* were dominantly found, in which was non-infectious form, whereas the infectious form is the cyst form and *Blastocystis* density was observed less than 5 cells per field of view at 400 magnification in all cases. This study concluded that the high prevalence of *Blastocystis* infection in elementary school children in Dukuh Village, Karangasem District, Bali that were dominantly single infections and several mix infections with other intestinal parasites. The high prevalence of *Blastocystis* infection in elementary school children suggested that it needs proper prevention measures for the children in this study area.

**Keywords:** *Blastocystis* spp., intestinal parasites, prevalence, children, Bali

### **ABSTRAK**

*Blastocystis* spp. adalah infeksi parasit usus yang paling umum ditemukan di beberapa survei masyarakat dari negara-negara berkembang. Infeksi *Blastocystis* dapat menyebabkan gejala gastrointestinal, namun dapat juga menyebabkan gejala ekstraintestinal seperti urtikaria dan nyeri sendi. Infeksi *Blastocystis* dapat juga asimtomatik atau sebagai carrier. Namun, prevalensi infeksi *Blastocystis* pada anak belum sepenuhnya diketahui di Indonesia, khususnya di Provinsi Bali. Penelitian ini bertujuan untuk mengetahui prevalensi *Blastocystis* dan parasit usus lainnya pada anak-anak sekolah dasar di desa Dukuh, Kabupaten Karangasem. Penelitian cross sectional dilakukan pada bulan September 2016. Sebanyak 103 tinja anak sekolah dikumpulkan dan parasit diperiksa secara mikroskopis dengan metode preparat basah menggunakan larutan yodium Lugol. Tiga puluh lima anak sekolah terinfeksi *Blastocystis* spp. (35/103, 34%) yang terdiri dari satu infeksi tunggal (29/35, 82.9%) dan infeksi campuran dengan parasit lain (6/35, 17.1%). Infeksi campuran infeksi *Blastocystis* spp. dengan cacing tambang (1/6, 16.7%), *Blastocystis* spp. dengan *Entamoeba coli* (1/6, 16.7%), *Blastocystis* spp. dengan *Giardia lamblia* (2/6, 33.3%), dan *Blastocystis* spp. dengan *Entamoeba histolytica/Entamoeba dispar* (1/6,

16.7%) dan *Blastocystis* spp. dengan *Entamoeba histolytica*/ *Entamoeba dispar* dan *Giardia lamblia* (1/6, 16.7%). Bentuk vakuolar merupakan bentuk *Blastocystis* yang dominan ditemukan, yang mana bentuk ini adalah tidak infeksius, namun bentuk kista adalah bentuk infeksius, serta kepadatan *Blastocystis* diamati kurang dari 5 sel per lapang pandang pada pembesaran 400 yang ditemukan pada semua kasus. Penelitian ini menyimpulkan bahwa tingginya prevalensi infeksi *Blastocystis* pada anak sekolah dasar di Desa Dukuh, Kabupaten Karangasem, Bali yang mana infeksi tunggal adalah dominan ditemukan dan beberapa infeksi campuran dengan parasit usus lainnya. Prevalensi infeksi *Blastocystis* yang tinggi pada anak sekolah menunjukkan bahwa diperlukan tindakan pencegahan yang tepat untuk anak-anak di wilayah studi ini.

**Kata kunci:** *Blastocystis* spp, parasit usus, prevalensi, anak-anak, Bali

## INTRODUCTION

Diarrhea is still major cause of childhood morbidity and mortality in developing countries.<sup>1,2</sup> One of the most common intestinal protozoa found in several community survey is *Blastocystis* spp.<sup>3,4</sup> that able to cause diarrhea. *Blastocystis* spp. is an intestinal protozoan and most commonly reported human intestinal protozoan in children and adults in developing country, has a world-wide distribution with prevalence of 30% to 60 % in developing country and 1,5% to 20% in developed country.<sup>5–7</sup> The prevalence of *Blastocystis* infection is reported in Japan 0.5-1%, in Singapore 3.3%, but in developing countries the prevalence is much higher, as in Argentina 27.2%, in Egypt 33.3%, in Cuba 38.5%, in Brazil 40.9%, and in Indonesia 60%.<sup>6</sup> Pegelow<sup>8</sup> found that the incidence of *Blastocystis* infections in elementary school children in Sukaraja, West Java was 60%.<sup>3,8</sup> and in elementary school children in hygiene poor areas in Sumba Island found a prevalence of *Blastocystis* infections was 29.9%.<sup>9</sup>

The high prevalence of *Blastocystis* infection in children is attributed to many factors, particularly environmental and personal hygiene. This infection is mainly through fecal-oral route and belongs to water borne disease. The pathogenic potential is controversial because the infection can be asymptomatic.<sup>6</sup> *Blastocystis* infections may cause gastrointestinal (GI) symptoms, but may also cause extraintestinal symptoms such as urticaria and joint pain.<sup>7,10,11</sup> *Blastocystis* can cause irritable bowel syndrome (IBS) so that it can affect the occurrence of malnutrition. In addition, immunocompromised hosts are more susceptible to these organisms than immunocompetent hosts, which is common in HIV/AIDS patients and cancer patients.<sup>11,12</sup>

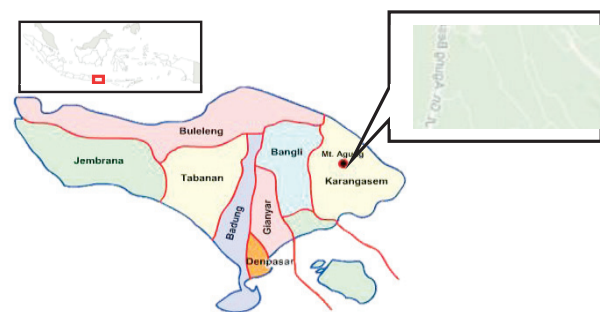
Bali island is a tropical region, where has some areas with poor hygiene, such as in Dukuh village, Karangasem district, *Blastocystis* infection prevalence has not been investigated. This study aimed to determine the prevalence of *Blastocystis* infection in this area with poor hygiene as one of the risk factors for parasitic infections, especially *Blastocystis*.

## MATERIAL AND METHOD

This cross-sectional study was conducted at elementary school in Dukuh village, Karangasem district, Bali Province,

Indonesia in September 2016. The Karangasem district is the eastern part of Bali island (Figure 1). This study area is selected as a research area due to associate with various risk factors of *Blastocystis* infection that are found in this village such as poor individual hygiene, lack of family latrines for defecation, lack of proper sanitation, poverty, close contact with animals, consuming contaminated food and water, dry natural conditions without any wellsprings and lack of clean water supply for daily living. Large rainy water reservoirs named ‘*cubang*’ are built at several points that serve to accommodate water during the rainy season.<sup>13</sup>

Residents in the Dukuh village there are those who keep animals without cages so that life together and close contact with animals (Figure 2), such as pigs, dogs, chickens, cows, rats and cockroaches. People share water resources with various animals.



**Figure 1.** Map of Bali Island. The study was conducted in Karangasem area (hatched)



**Figure 2.** People keep pigs, chickens and cows without cages

Stools were collected from 103 students (6-13 years old) at SD 2 Dukuh, an elementary school, by informed consent. The procedure for stool collection was thoroughly explained and clean plastic containers were distributed to each student on the day before specimen collection. All specimens were collected into wide-mouth, screw-capped containers and were immediately preserved in 10% formalin.

*Blastocystis* spp. and other intestinal parasites in stools were examined and identified by wet mounts method using Lugol iodine solution as described by World Health Organization (WHO) Manual of Basic Technique for a Health Laboratory.<sup>14</sup> Microscopic examinations of stool samples, which are based on morphology, are commonly used for identification of parasites. However, these methods are unable to differentiate subtype of *Blastocystis*.

Microscopic examination for the *Blastocystis* was viewed by magnification of 400 and 1000 times under a light microscope. *Blastocystis* is a polymorphic intestinal parasite that is common in humans. The four major morphologic forms: vacuolar (most common), cyst, granular, and amoeboid can be seen in stools.<sup>5</sup> To determine the density of *Blastocystis* was performed by counting the number of *Blastocystis* per field of view in 400 magnification.

This study was approved by the Ethical Committees, Faculty of Medicine, Universitas Airlangga.

## RESULT AND DISCUSSION

This study was conducted to determine the prevalence of *Blastocystis* infections in elementary school children located in Dukuh village, Karangasem District, Bali Province in September 2016. Stools were collected from 103 students, age from 6 to 13 years, and examined microscopically to determine *Blastocystis* infection by wet mount method using Lugol iodine solution. Samples examined found that (35/103, 34%) student stools infected with *Blastocystis*, asymptomatic infection. A single infection of *Blastocystis*

**Table 1.** *Blastocystis* and other intestinal parasite infections in elementary school student stools in Dukuh village

| Parasites   | Stools (N=103)             |
|---|----------------------------|
|   | Number of Infection (n, %) |
| <b>Single infection</b>   |                            |
| <i>Blastocystis</i> spp.  | 29 (28.1)                  |
| <b>Mix infections</b>   |                            |
| <i>Blastocystis</i> spp., hookworm  | 6 ( 5.9)                   |
| <i>Blastocystis</i> spp., <i>E. histolytica</i> /<br><i>E. dispar</i>                   | 1 ( 1.0)                   |
| <i>Blastocystis</i> spp, <i>E.coli</i>  | 1 ( 1.0)                   |
| <i>Blastocystis</i> spp, <i>G.lamblia</i>   | 1 ( 1.0)                   |
| <i>Blastocystis</i> spp, <i>E. histolytica</i> /<br><i>E. dispar</i> , <i>G.lamblia</i> | 2 ( 1.9)                   |
| <i>E. dispar</i> , <i>G.lamblia</i>   | 1 ( 1.0)                   |
| Total   | 35 (34.0)                  |

(29/35, 82.9%) dominantly detected than mix infections, *Blastocystis* and other parasites (6/35, 17.1%). Among mix infections, *Blastocystis* and *Giardia lamblia* were commonly observed (2/6, 33.3%) Therefore, the mix infection prevalence occurred less 10 % (6/103, 5.8%) (see on Table 1).

In the microscopic examination we found that the *Blastocystis* density image in the sample examined in Figure 3. and *Blastocystis* density was found to be less than 5 cells per field at 400 magnification in all cases (Table 2).

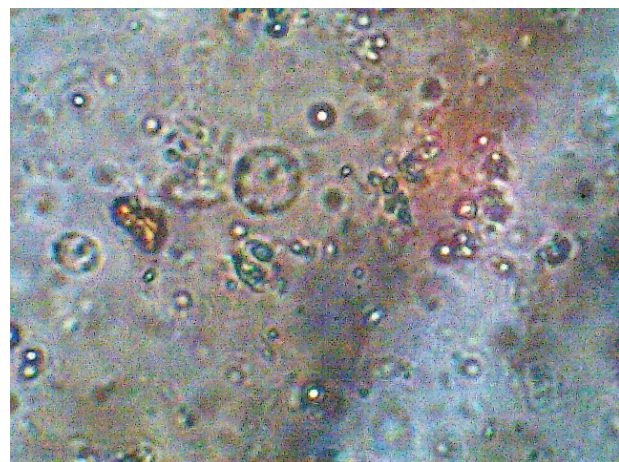
The dominant forms were vacuolar forms on microscopic examination of infected samples of *Blastocystis* (in Figure. 4).

The prevalence of *Blastocystis* infection in elementary school children at Dukuh village, Karangasem district was 34% that was similar with *Blastocystis* infection prevalence in Sumba island in 2016 (29.9%). Those prevalence of *Blastocystis* infection were commonly found in developing countries, 30-60% compared with industrialized countries<sup>5-7</sup>, and Indonesia is a developing country. It has been reported by Pegelow et al<sup>8</sup> that the incidence of *Blastocystis* infections reached to 60% in elementary school children in Sukaraja, West Java in 1997.<sup>3,8</sup>

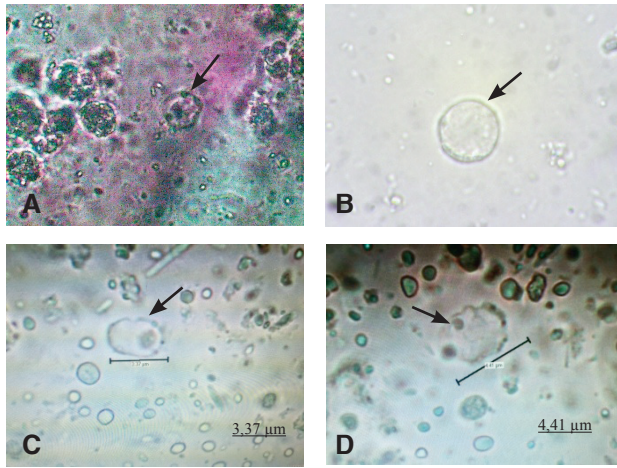
High prevalence of *Blastocystis* infection in elementary school children at Dukuh village, Karangasem district might due to the risk factors of *Blastocystis* infection, particularly

**Table 2.** *Blastocystis* density was calculated by the viewer with magnification of 400

| Number of <i>Blastocystis</i> per Field (40x Objective) | No. Infected Samples (n,%) |
|---|----------------------------|
| 1/field   | 23 (65.7)                  |
| 2/field   | 6 (17.1)                   |
| 1/3 field   | 1 (2.9)                    |
| 1/5 field   | 4 (11.4)                   |
| 1/10 field  | 1 (2.9)                    |
| Total   | 35 (100.0)                 |



**Figure 3.** *Blastocystis* density is examined under a light microscope at 400 magnification (*Blastocystis* is shown with arrows)



**Figure 4.** *Blastocystis* vacuolar form was found on microscopic examination using wet mount method with 1000 magnification (*Blastocystis* is shown with arrows)

the poor hygiene and sanitation in this study area such as sharing water resources (*cubang*) between human and animals, lack of family latrines for defecation, lack of proper sanitation, and close contact with animals (Fig.3). It is known that *Blastocystis* infection belongs to water borne diseases<sup>15,16</sup>, so the above factors are involving in the transmission of *Blastocystis* infection which is thoroughly the fecal-oral route by water contamination. Thus, it needs to study further the transmission of *Blastocystis* infection in this area by the examination of *Blastocystis* in animal stools and water resources (*cubang*).

In many epidemiologic surveys, *Blastocystis* is the most frequently isolated parasite, with a higher prevalence in underdeveloping countries, where poor hygiene, exposure to animals, and consumption of contaminated food or water are observed. It is more common than other protozoan parasites, *Giardia lamblia* and *Dientamoeba fragilis*.<sup>5</sup> In this study showed that hookworm, *Entamoeba coli*, *Giardia lamblia* and *E. histolytica/ Entamoeba dispar* were also detected together with *Blastocystis*, however, the prevalence of them were low (1%, 1%, 3% and 1% respectively). Even though, their prevalence was low, hookworm disease belongs to the neglected tropical disease<sup>17</sup> that causes anemia, which affect to the children growth and development<sup>18</sup>. So, it needs to be concerned for disease control and eradication, for example use the toilet for defecation, use the shoes or slipper during playing in the ground.

*Giardia lamblia* was more common obtained with *Blastocystis* than other intestinal protozoan and the cyst form, the infective form, of *Giardia lamblia* was dominantly found in student stools. The students seemed to be carrier hosts who can transmit the infective form through water contamination<sup>19,20</sup>. Health education needs to be conducted at elementary school in order to improve student behavior on use the proper toilet for defecation.

Mix infection between *Blastocystis* and *Entamoeba* in school children stools were found. Both protozoan parasites

cause water borne disease<sup>21</sup>. It means that the water at this study area is poor quality or contaminated water, thus the treated water for either cooking or drinking is required.

This study revealed that predominant intestinal parasite infection in the school children was *Blastocystis* infection without intestinal symptoms and density of *Blastocystis* observed by 400x magnifications was less than 5 parasites per field (see on Table 2). Previous studies suggested that more than 5 parasites per highpower field (40x objective) for wet mount or by oil immersion (100x objective) were associated with the presence of gastrointestinal disease<sup>6,22,23</sup> and treated by metronidazole.<sup>22,23</sup> In addition, the *Blastocystis* parasite seems to be more common in healthy individuals than in patients with inflammatory bowel disease and is associated with certain gut microbiota and health indicators. Although the parasite may elicit disease under certain conditions, the focus on *Blastocystis* infection may be shifting from a clinical to a public health viewpoint.<sup>24</sup>

A vacuolar form of *Blastocystis spp.* a polymorphic intestinal parasite, was dominantly detected in school children stools in Dukuh village. This form is one of *Blastocystis* forms in its life cycle that are, cyst, granular, and amoeboid in which can be seen in stool and axenic cultures.<sup>25,26</sup> Some studies proposed that morphologic features of *Blastocystis* have a role as predictors of clinical symptoms.<sup>25,26</sup> Unfortunately, this study did not investigate the subtype of *Blastocystis*, whereas the variability of intrasubtype may result in the production of effectors that contributes to *Blastocystis* pathogenicity in correlation with the presence of clinical symptoms.<sup>26</sup> Diverse morphologic features (vacuolar transiting to amoeboid), probably reflecting the progression from an asymptomatic to a symptomatic state, were observed in an asymptomatic subtype 3 carriers who later had symptoms. Searching for amoeboid forms might be helpful to presumptively screen symptomatic patients with subtype 3 or to follow up an asymptomatic subtype 3 carrier in case symptoms become evident before antiprotozoal treatment was attempted.<sup>26</sup> Diverse forms of *Blastocystis* have been reported by various researchers: vacuolar forms are observed in feces and in cultures, granular forms are considered degenerative products, and amoeboid stages of *Blastocystis* are detected whenever symptoms are reported.<sup>27,28</sup>

Beside vacuolar forms, we also found a few of granular forms but not amoeboid and cyst forms in school children stools. Both forms might be correlated to asymptomatic infection as the previous study reported that vacuolar forms and granular forms are found in people who have asymptomatic conditions, and when found in amoeboid form the possibility of progression from an asymptomatic to a symptomatic state.<sup>26</sup>

Our study revealed the first report of the high prevalence of *Blastocystis spp* in an elementary school children stools, Dukuh village, Karangasem district, Bali, with less density of *Blastocystis* and dominant vacuolar forms. It suggested that the water quality for drinking or cooking

needs to be improved, and also hygiene and sanitation be refined in this area. *Blastocystis* transmission, and the roles of morphologic features and variation within *Blastocystis* sub-types as predictor of symptomatic infection need to be elaborated further.

## CONCLUSION

The prevalence of *Blastocystis* infection was high in elementary school children in Dukuh village, Karangasem District, Bali that were dominantly single infections and several mix infections with other intestinal parasites. It suggested that needs proper prevention measures for the children in this area, particularly improving water quality. The result of this study could be used for other studies on the prevalence of *Blastocystis* infection in elementary school children, and for establishing policy in control and prevention programs of *Blastocystis* infection.

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