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Progression of *Blastocystis* Infection in Experimental Mice

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ABSTRACT

The infectivity of Blastocystis cysts in the experimental mice model was further studied using isolates from human feces. Two groups of Swiss mice were orally infected with purified and concentrated Blastocystis cysts isolated from watery and formed human feces. Mice fecal Blastocystis cyst count by Direct Fecal Smear was performed daily post-inoculation for 10 days. Parasite intestinal shedding was detected as early as 2-3 days. Peak cystic count was observed after 4-7 days and declined thereafter. There was no significant difference in the infectivity of Blastocystis cysts isolated from watery or formed human feces.

Keywords: Blastocystis cyst, Infectivity, Swiss Mice, Inoculum, Direct Fecal Smear

INTRODUCTION

Blastocystis is one of the most prevalent intestinal parasites found in humans. It is prevalent worldwide with rates exceeding 50% in developing countries [1],[2]. The mode of infection is via the oral-fecal route and high percentages are persistent due to consumption of contaminated food and drink, overcrowding, and poor sanitation [3],[4].

The epidemiologic significance of *Blastocystis* remains controversial as this parasite has been detected in asymptomatic individuals and at the same time implicated in irritable bowel, gastrointestinal infections, abdominal pain, cramps, bloating, diarrhea, constipation, and hives [5]-[7]. It affects people of all ages and the immunocompromised. Zoonotic infections are highly probable as the parasite is widespread among all animals including insect vectors [8],[9].

Blastocystis is the only Stramenopile species studied and reported to cause human infections. Four morphologic forms of *Blastocystis* were viewed in feces: vacuolar, granular, amoeboid, and cyst forms [10],[11]. Experimental studies conducted in animals for infectivity revealed that the cyst form represented the transmissible and infective stage of the parasite [11],[12].

Researchers conducted several animal models to study the infectivity and pathogenicity of *Blastocystis* including the various subtypes, molecular biology, and cross-infections [13]-[15]. *Blastocystis* adhesion, colonization, and pathology in mice models, revealed the luminal, non-invasive nature of the parasite [16]. Another study on the pathogenicity of *Blastocystis* in the gastrointestinal tract of male Swiss mice was evaluated according to inoculum size and the period of infection [17]. None of these closely studied the daily progression of infection in mice models and the comparative infectivity of *Blastocystis* isolated from watery or formed human feces. To explore this line of inquiry, the infectivity of human-isolated *Blastocystis* cysts in orally infected Swiss mice and the period of laboratory detection was studied.

METHODOLOGY

A. Research Design

The study employed comparative quantitative research using *Blastocystis* isolates from human feces and subsequent microscopy of mice's fecal output 10 days post-inoculation.

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B. Sampling and Detection

From various diagnostic centers and hospital laboratories in Metro Manila, 25 fecal samples were randomly collected and evaluated for the presence of *Blastocystis* cysts. Feces of various consistencies were collected and transported to the Metro Clinical Laboratory. The microscopic evaluation of feces was performed by 2 trained Medical Laboratory Technologists with Direct Fecal Smear. The Direct Fecal Smear is the routine method used in fecalysis for the detection of parasites [18]. In a drop of Lugol's iodine on the slide, a sample of feces was emulsified evenly and examined microscopically in a standardized manner. The study obtained a permit to conduct fecal microscopic testing in the Metro Clinical Laboratory from May 15 – June 15, 2023.

C. Process

All the fecal specimens positive for *Blastocystis* cysts were grouped according to fecal consistency. Representative fecal samples were suspended in 2 tubes containing 10 ml of Ringer's solution. One final tube represents the *Blastocystis* cysts isolated from human watery fecal samples and another from formed fecal samples. To render it suitable for inoculation to experimental mice, the *Blastocystis* cysts suspension was concentrated and purified following the procedures of Toriano, 2022. *Blastocystis* cysts were concentrated and purified by fractional centrifugation at 2,500 rpm for 10 mins at room temperature. The supernatant was discarded, and the sediment was washed with Ringer's solution and centrifuged at 2,500 rpm for 5 minutes. This was repeated 3x to reduce the bacteria and debris in the purified suspension greatly. The final sediment was resuspended in Ringer's solution and microscopically checked for the presence of intact and viable cysts [19].

The concentrated and purified suspension was cultured in Ringer's Saline Serum (RSS) broth to keep the *Blastocystis* cysts viable for animal inoculation. On the day of animal inoculation, the *Blastocystis* cysts growing on the RSS broth medium were again purified and concentrated by fractional centrifugation. The sediments on the 2 tubes were separately resuspended in 2 ml of Ringer's solution [19]. These tubes represented *Blastocystis* cysts from a watery and formed human feces that were used to inoculate 2 groups of Swiss mice.

D. Swiss Mice Grouping and Inoculation

Fifteen (15) Swiss mice at about 10-12 weeks old were prepared. The mice were housed in partitioned cages to inhibit unnecessary contact with other mice groups. The mice were fed a normal diet of commercial pellets and given potable water ad libitum. The mice were maintained in a veterinary laboratory at 25°C under a 12-hour light/dark cycle [20]. The mice were pre-screened by fecalysis to ensure they were free of *Blastocystis* or any other intestinal parasitic infections. The 15 Swiss mice were divided into 3 groups – the negative control (5) and the 2 test groups: Swiss mice infected with *Blastocystis* cysts isolated from watery human feces (5) and formed human feces (5). A veterinarian evaluated the mice and assisted the researcher in the protocol.

The inoculum was checked microscopically to confirm the presence of intact and viable cysts. The inoculum was standardized to contain 10,000 cysts per mL using the Neubauer hemocytometer. Before inoculation, it was mixed by inversion 5x to ensure a consistent and even distribution in the Ringer's solution. From this 2 mL of purified *Blastocystis* cyst suspension, 50 uL is used as the inoculum volume to infect each one of the Swiss mice in the groups [19]. The inoculum was given orally through a feeding tube attached to a tuberculin syringe. Oral inoculation of purified *Blastocystis* cysts was performed in all mice groupings accordingly. The negative control group did not receive any inoculum.

E. Data Gathering Procedure

All the mice were monitored for the presence of intestinal infection or daily parasite fecal shedding for ten (10) days after inoculation. In a drop of Lugol's iodine on the slide, a sample of feces was emulsified evenly

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and examined microscopically [18]. *Blastocystis* cysts were counted in >10 fields and the average count per high power field (HPF) was tabulated.

F. Scope and Limitations

Blastocystis cysts were isolated from human feces in 2 consistencies only, watery and formed. Sampled soft and mucoid feces were negative. No data from the patients or the participating laboratory were required. Signs or symptoms of the fecal source were not included nor are they relevant to the objectives of the study. The period of fecal investigation lasted for 10 days. Fecalysis for cyst count was performed daily depending on the mice's fecal output.

RESULTS

Blastocystis cysts were detected in all of the post-inoculated mice in the 2 test groups: BWF – *Blastocystis* from Watery Feces, BFF – *Blastocystis* from Formed Feces (Table I). The tabulated numbers are the counted *Blastocystis* cysts microscopically detected in mice feces by Direct Fecal Smear method. Comparing the counts, some intestinal mice infections were lighter with <10 cysts/HPF in BWF 1,2 and BFF 2,3, and more severe with >10 cysts/HPF in BWF 3,4,5 and BFF 1,4,5. Sustained and continuous signs of mice intestinal infection were observed in BWF 2 and 5 which extended even up to Day 10 post-inoculation. There were days wherein the Swiss mice did not defecate, hence, no microscopic count was reported (N – not done). Expectedly, there were no *Blastocystis* cysts recovered in the feces of the negative mice control group.

The collective number of *Blastocystis* cysts per group of mice per day is presented in the bar graph (Table II). Noticeable is the bell curve made as the peak number is reached and the nearly similar spike in the 2 groups of mice. The number of *Blastocystis* cysts becomes apparent on Day 2-3 post-inoculation and recedes from Day 8. No *Blastocystis* cyst was observed in the feces of the 2nd group of mice (BFF) on Day 10 while it is scarce on the same day for the 1st group (BFW). The highest number detected was on Day 6 (45) in the 1st group and Day 7 (42) in the 2nd group.

The computed variation in the *Blastocystis* cysts number in the 2 test groups of mice generated a p-value of 0.236 (> 0.05 alpha), and a t critical of 2.26 with a lower t stat of 1.27 (Table III). The analysis of variance among the test groups was not significant. The data gathered in the lab experiment were nearly identical in the mice groups. Regardless of the source of *Blastocystis* cysts inoculum, whether from formed or watery feces, the observed number in orally infected mice did not vary significantly.

TABLE 1. POST-INOCULATION FECAL *BLASTOCYSTIS* COUNT IN SWISS MICE

Day	1	2	3	4	5	6	7	8	9	10
Group1 Mice (BWF, *Blastocystis* count / HPF) Inoculum: *Blastocystis cysts* from Watery feces										
BWF-1	0	N	2	9	8	6	3	N	3	0
BWF-2	0	4	6	7	N	N	8	N	4	2
BWF-3	N	2	N	12	10	15	12	14	N	N
BWF-4	N	0	0	3	8	10	7	6	N	0
BWF-5	0	0	4	6	N	14	12	N	4	3
Sub-total	0	6	12	37	26	45	42	20	11	5
Group 2 Mice (BFF, *Blastocystis* count / HPF) Inoculum: *Blastocystis cysts* from Formed feces										
BFF-1	0	3	11	12	N	N	8	4	0	N
BFF-2	N	0	3	N	7	9	6	N	0	N
BFF-3	N	0	N	0	2	4	7	4	N	0

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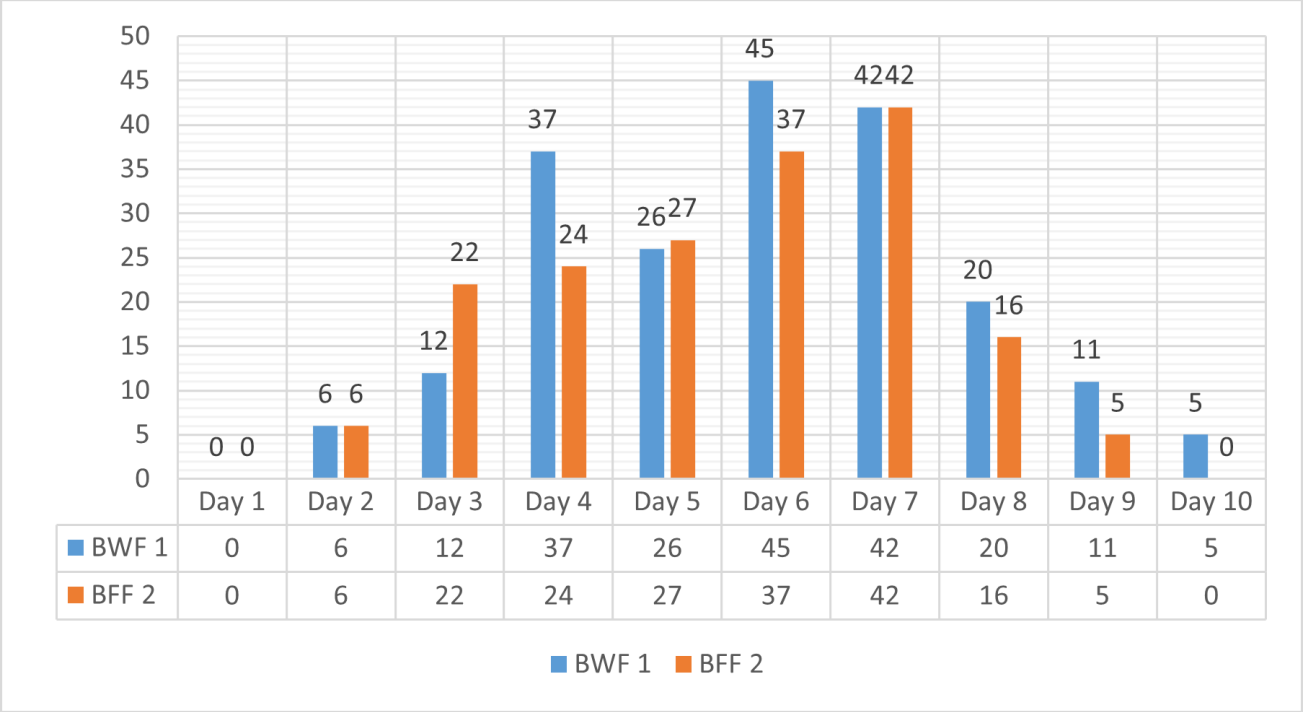
BFF-4	0	3	N	6	10	14	13	8	2	0
BFF-5	0	N	8	6	8	10	8	N	3	N
Sub-total	0	6	22	24	27	37	42	16	5	0
Group 3 Mice (C) Negative Control (*Blastocystis* count / HPF)										
C-01	0	0	N	0	0	0	0	N	0	0
C-02	N	0	0	0	N	N	0	0	0	N
C-03	N	N	0	0	0	0	N	0	0	0
C-04	0	0	0	N	0	0	N	0	0	0
C-05	0	N	0	N	0	0	0	0	N	0
Sub-total	0	0	0	0	0	0	0	0	0	0
Total	0	12	34	61	53	82	84	36	16	5

TABLE II. COMPARATIVE DAILY TOTAL NUMBER OF *BLASTOCYSTIS* CYSTS IN THE TEST GROUPS OF SWISS MICE



Legend: BWF – Group 1 Swiss mice inoculated with *Blastocystis* from Watery Feces

BFF – Group 2 Swiss mice inoculated with *Blastocystis* from Formed Feces

TABLE III. VARIATION IN *BLASTOCYSTIS* CYSTS COUNT

Mice Group	Mean	Variance	t Stat	t Critical	p-value	Analysis	Decision
Group 1 – BWF	20.4	266.49	1.27	2.26	0.236	Not significant	Accept Ho
Group 2 – BFF	17.9	226.1					

DISCUSSION

The findings showed that *Blastocystis* cysts were detected in all post-inoculated mice feces, a sign of intestinal infection and shedding of parasites. The positivity in feces became evident as early as 2-3 days. Late infection became apparent on Day 4 or 5 signifying that it takes more days for the parasite to proliferate

in the intestines of some mice. Notably, late evidence of infections happened in each of the groups of test mice infected with *Blastocystis* cysts isolated from watery and formed human feces. Such findings seconded the observations on Swiss mice using *Blastocystis* isolates from insects [19]. Interestingly, in a study of *Blastocystis*-infected mice, from the 2nd day of infection, vacuolar forms of the parasite were observed in only 60% of the test mice, in the remaining 40%, the infection was confirmed only in Day 7 [17]. Furthermore, when mice were intra-cecally inoculated, they were found to develop acute diarrhea and shed parasites for 2–3 days [16]. The highest *Blastocystis* counts were observed between 4-7 days in the groups of the test mice.

Characterizing the quality of mice feces over 10 days of observation, mice feces appeared formed, dark-colored, compact, and elongated as a rice grain prior to inoculation. It continued to appear unremarkable until Day 5 post-inoculation when the mice's fecal consistency became soft and eventually more watery with little fecal solids. As the number of mice fecal *Blastocystis* cysts increased, the mice's fecal consistency worsened. These fecal characteristics reflected the changes also observed in induced-diarrhea mice studies [21],[22].

The collective number of *Blastocystis* cysts per group of mice per day presented in a bar graph assumes a bell curve as the observed number of cysts exponentially rises and plummets. The cyst proliferation curve lasts for 3-4 days on its peak before it begins to decline. Despite the numbers, when analyzed statistically, there was no difference in the number of *Blastocystis* cysts observed in the fecal samples of the 2 groups of test mice infected with *Blastocystis* cysts isolated and purified from watery and formed human feces (p-value of 0.236). Because of the daily mice fecalysis monitoring, it was possible to plot the progression more closely - a characteristic of this study that makes it unique.

CONCLUSION

In conclusion, *Blastocystis* cysts are the infective stage of the parasite in mice causing observable proliferation in the number of cysts and intestinal shedding in the feces. The mode of infection in *Blastocystis* is by fecal-oral route as evidenced by the oral inoculation of purified *Blastocystis* cysts sourced from human feces. The sign of infection in feces is microscopically detectable by Direct Fecal Smear using Iodine. The earliest signs of infection become apparent in 2-3 days and reach the peak in 4-7 days. *Blastocystis* cysts isolated from either formed or watery feces of humans are equally infectious to mice.

Disclosure statement

The researcher has no conflict of interest to disclose.

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