From Insects to Swiss Mice: Effect of *Blastocystis Species* in the Intestinal Tract

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ABSTRACT

Blastocystis is a widespread enteric protozoan of zoonotic importance, frequently transmitted via contaminated food and water. This study investigated the histopathological impact of *Blastocystis* on the intestinal architecture of Swiss mice. Isolates were obtained from the external surfaces and digestive tracts of the American cockroach (*Periplaneta americana*) and the housefly (*Musca domestica*). Following oral inoculation, mice were sacrificed at 10 days post-infection for histopathological analysis. Findings revealed significant mucosal disruption, with moderate to severe villi erosion observed in the majority of infected subjects. Pathological hallmarks included villi blunting, apical fragmentation, thinning of the intestinal mucosa, and exposure of the lamina propria. Notably, the severity of intestinal damage was comparable between isolates sourced from both insect vectors. These results characterize *Blastocystis* as a significant intestinal pathogen in a murine model and underscore the critical role of synanthropic insects as mechanical vectors in its transmission cycle.

Keywords: *Blastocystis*, Histopathology, Intestinal villi, Swiss mice, Synanthropic insects.

INTRODUCTION

Blastocystis, a unicellular protozoan parasite, has been extensively studied in various animal models, including rats, to understand its pathogenesis, transmission dynamics, and interaction with the host intestine [1,2]. Rats are widely used as an animal model for *Blastocystis* infection due to their anatomical and physiological similarities to humans, particularly in terms of gut colonization patterns [3]. Research has focused on the parasite's colonization, invasion, and interaction with the host intestine, although the full life cycle of *Blastocystis* remains a significant research gap [4,5].

The parasite is thought to infect the host through the fecal-oral route, where the cyst form is ingested and releases the trophozoite form in the intestine. The trophozoite is the most common form found in the intestine and is responsible for symptoms [6,7]. It can undergo binary fission or differentiate into the cyst form, which is excreted in feces [8,9]. The cyst form is highly resistant to environmental stress, allowing it to survive outside the host and be transmitted [4].

Blastocystis exhibits various morphological forms beyond trophozoite and cyst forms, including granular and amoeboid forms, although their significance remains unclear, recent evidence links the amoeboid stage to increased pathogenic potential [8]. Research has shown that *Blastocystis* colonizes the large intestine, cecum, and colon, leading to changes in the intestinal mucosa and submucosa [3,10]. Further investigation has found that *Blastocystis* invades the lamina propria, submucosa, and muscularis layers, causing significant tissue damage and inflammation [11,12].

The parasite also disrupts the intestinal microbiota, leading to an imbalance in gut flora and potentially contributing to disease pathogenesis [13,14,15]. Ultrastructural studies demonstrate that *Blastocystis* forms close associations with host epithelial cells, altering microvilli and the brush border [6,16]. The parasite also alters mucin and glycoprotein expression, contributing to changes in the intestinal mucosa and submucosa [17,18].

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Histopathological studies in mice reveal inflammation, edema, and damage to the intestinal epithelium, with severity varying by subtype and inoculum size [19,20]. Studies in animal intestines shed light on its pathogenesis, transmission dynamics, and interaction with the host intestine [21,22]. *Blastocystis* colonizes the large intestine, cecum, and colon, inducing an inflammatory response and exhibiting invasive behavior [24,25]. Larger inoculum triggers earlier responses, while smaller inoculum delays the response [26,27]. Adhesive isolates are better colonizers, leading to increased virulence and tissue damage [28,29]. *Blastocystis* infection in mice disrupts the intestinal microbiota, leading to gut flora imbalance and potential disease pathogenesis [30,31]. *Blastocystis*'s impact on cognitive function and behavior has also been explored, with one study linking infection to colonic hyperresponsiveness, anxiety, and depressive-like behavior in rats [32,33].

The complex interaction between *Blastocystis* and the host intestine warrants further study to elucidate infection mechanisms [1]. Several research gaps remain, including the pathogenesis, zoonotic potential, and development of effective animal models and diagnostic tools [8,23]. Addressing these gaps is crucial for improving our understanding of *Blastocystis* infection [4].

METHODOLOGY

Research Design

This study employed an experimental research design to investigate the histopathological effects of *Blastocysti*s isolated from insect vectors on the intestines of Swiss mice. *Blastocystis* was obtained from cockroaches and flies, and purified suspensions were used to infect groups of Swiss mice. The mice were sacrificed 10 days post-inoculation, and their intestines were harvested for histopathological examination. The intestinal tissues were processed, sectioned, and stained with hematoxylin-eosin (H&E) for microscopic evaluation. The study aimed to determine the pathogenic potential of *Blastocystis* from insect vectors and its impact on the intestinal tissue of Swiss mice, providing insights into the parasite's zoonotic potential and the risks associated with insect-borne transmission.

A. Specimen and Sampling

A hundred selected flies and cockroaches were washed one by one in sterile saline by manual shaking for 3 minutes, and the washings were collected for examination. The washing was centrifuged, and the sediment was resuspended in 1 ml of Ringer's solution. A drop of the sediment was spread on a slide for staining and microscopic viewing to detect *Blastocystis*. The insects were then decontaminated with 70% alcohol, dried, and washed with normal saline. The intestinal contents were exposed by crushing the samples, macerated in 1 ml of Ringer's solution, and examined microscopically for *Blastocystis* by staining methods [34]. The parasite identification was further confirmed using molecular testing.

The tubes of collected specimens per insect vector group were combined into one final tube, which contained bacteria and debris. To render it suitable for inoculation to experimental mice, the *Blastocystis* suspension was concentrated and purified through centrifugation and washing with Ringer's solution. The final sediment was resuspended in Ringer's solution and checked microscopically for intact and viable organisms. The purified suspension was then subcultured in plated media to detect and exclude bacteria, and the remaining suspension was cultured in Ringer's Saline Serum (RSS) broth to keep the *Blastocystis* cysts viable. After confirming the absence of bacteria, the final suspension was purified and resuspended in 2 ml of Ringer's solution, ready for animal inoculation. This suspension was used to inoculate ten Swiss mice grouped according to the insect source of *Blastocystis* [34].

B. Experimental Protocol

Thirty Swiss mice, 10-12 weeks old, were prepared and housed in partitioned cages to prevent unnecessary contact between groups. They were fed a normal diet and given potable water ad libitum, and maintained in an animal laboratory at 25°C with a 12-hour light/dark cycle. Prior to the experiment, the mice were pre-screened by fecalysis to ensure they were free of *Blastocystis* and other intestinal parasitic infections. The mice were grouped into three: negative control, *Periplaneta*, and *Musca* groups, with each group consisting of ten mice infected with *Blastocystis* isolated from their corresponding insect vector source. The negative control group

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remained uninfected throughout the study. To infect the mice, *Blastocystis* from RSS broth cultures was purified and concentrated through differential centrifugation, and the inoculum was standardized to contain 10,000 cysts per 100 uL using a hemocytometer. The inoculum was administered orally through a feeding tube attached to a tuberculin syringe, with each mouse receiving 100 uL of the purified *Blastocystis* suspension. The control mice were caged separately to prevent infection via the fecal-oral route, and did not receive any parasite inoculum.

C. Data Gathering Procedure

After 10 days post-inoculation, the Swiss mice were sacrificed and dissected to remove the intestines, and preserved in 10% formalin for histopathologic studies. The entire length of the intestines, including both small and large intestines, were submitted. Tissue samples were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin-eosin (H&E) for examination under a light microscope by a Veterinary Pathologist. The cellular changes in the mice intestines were observed to detect any signs of *Blastocystis* pathogenicity, allowing for the assessment of the parasite's impact on the intestinal tissue.

Pathological Grading of Intestinal Villi Erosion

Mild Erosion - the damage is localized and superficial. The overall architecture of the intestinal wall remains intact. Villi Structure: the tips of the villi may appear slightly rounded or blunted rather than sharp and slender. Epithelium: there is minor loss of the brush border or a few shedding epithelial cells at the apex of the villus. Lamina propia: minimal infiltration of inflammatory cells.

Moderate Erosion - represents a significant loss of surface area and a breakdown of the mucosal barrier. Villi Structure: villi are noticeably shortened (atrophy) and may begin to fuse together. Epithelium: significant denudation (stripping) of the epithelial layer. Lamina propia: naked where the protective cell layer has sloughed off. Moderate congestion of blood vessels and a more pronounced presence of inflammatory cells.

Severe Erosion - characterized by near-total destruction of the absorptive surface and deep tissue involvement. Villi Structure: complete loss or flat villi. The intestinal lining may look smooth or cratered under the microscope because the projections are gone. Epithelium: extensive ulceration. The erosion may extend beyond the villi into the crypts (the pits at the base of the villi). Lamina propia: fully exposed or hemorrhagic.

RESULTS AND DISCUSSION

This study demonstrated that *Periplaneta americana* and *Musca domestica* carry *Blastocystis* parasites on their external surfaces and intestines. To investigate the effects of *Blastocystis* in Swiss mice after inoculation, the mice were euthanized for the histopathologic study of its intestines on day 10. The tissue sections were stained using Hematoxylin and Eosin, and microscopically evaluated by a Veterinary Pathologist in the Department of Science and Technology.

Table I. The pathogenic effect of *Blastocystis* in the intestines of Swiss mice

Code	Histopathology Result
Swiss mice infected with *Blastocystis* from *Periplaneta americana*	
PA-01	Moderate to severe erosion of villi
PA-02	Moderate to severe erosion of villi
PA-03	Severe erosion of villi
PA-04	Mild to moderate erosion of villi
PA-05	Severe erosion of villi
PA-06	Moderate to severe erosion of villi
PA-07	Moderate erosion of villi

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PA-08	Mild to moderate erosion of villi
PA-09	Severe erosion of villi
PA-10	Moderate to severe erosion of villi
Swiss mice infected with *Blastocystis* from *Musca domestica*	
MD-01	Severe erosion of villi
MD-02	Moderate to severe erosion of villi
MD-03	Moderate erosion of villi
MD-04	Severe erosion of villi
MD-05	Moderate to severe erosion of villi
MD-06	Severe erosion of villi
MD-07	Moderate to severe erosion of villi
MD-08	Severe erosion of villi
MD-09	Mild to moderate erosion of villi
MD-10	Moderate erosion of villi
Swiss mice Negative Control group	
C-01	Mild erosion of villi
C-02	Mild erosion of villi
C-03	No erosion of villi
C-04	No erosion of villi
C-05	No erosion of villi
C-06	Mild erosion of villi
C-07	Mild erosion of villi
C-08	Mild erosion of villi
C-09	Mild erosion of villi
C-10	No erosion of villi

Table I reflects the pathogenic effect of *Blastocystis* in the intestines of mice. *Blastocystis* infection resulted in the erosion of the intestinal villi of mice, both in the small and large intestines. The villi erosion was graded by the Veterinary Pathologist as to the severity. Majority of the infected mice showed villi erosion from moderate to severe, whereas, the negative mice control showed results from no villi erosion to an equivocal mild villi erosion. There was no difference between the effects of *Blastocystis* in Swiss mice when sourced from either *Periplaneta americana* or *Musca domestica*.

In *Periplaneta americana* – sourced inoculum (PA group), severe villi erosion occurred in three mice (PA-03, PA-05, and PA-09), moderate to severe in five samples (PA-01, PA-02, PA-06, PA-07, and PA-10), and only mild to moderate in two samples (PA-04 and PA-08). The villi of the intestines were damaged and almost obliterated as seen in tissue sections. These findings strongly show the infectivity and pathogenicity of *Blastocystis* in majority of the infected mice.

In *Musca domestica* - sourced inoculum (MD group), severe villi erosion was observed in four (MD-01, MD-04, MD-06, and MD-08), moderate to severe in five (MD-02, MD-03, MD-05, MD-07, and MD-10), and mild to moderate in only one (MD-09) of the mice subjects. The intestines of the control mice, on the other hand, showed no villi erosion to mild erosion in majority of the samples.

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The photomicrographs (Plate A) show the extent of damage caused by the parasite to the intestines of Swiss mice. The severity of the infection is best exemplified in its effect to the fingerlike projections known as the intestinal villi. The intestinal villus core is the lamina propia. The lamina propria is a layer of loose connective tissue beneath the intestinal epithelium that provides structural support and contains blood vessels, lymphatics, nerves, and a large population of immune cells. The erosion of the villi caused the thinning and wearing down of the intestinal mucosa.

A show the normal cross section of the mice small intestine with an undisturbed brush border, unexposed lamina propia, deep crypts reaching the glands which abut the outer serosa layer.

B show a slightly eroded intestinal villi tip, the usually rounded ends of the villi is blunted, with some cells detached and freely floating the lumen. The lamina propia is slightly affected depending on the erosion on the borders. The glands are unremarkable with an intact serosa.

C show mild to moderate erosion of the intestinal villi. The finger-like projections have fragmented tips exposing the lamina propia and the inner crypts. The thickness of the mucosa begins to wear thin. This picture shows a damage slightly more severe than the mild villi erosion. No *Blastocystis* form of any type can be seen invading the cells or floating in the lumen.

D show a severely affected large intestine cross section with a lamina propia cut in half and eliminating the upper portion of the villi. The inner crypts are widely exposed and the remaining mucosal layer is thinner.

E the tissue section reported an almost total denudation of the intestinal villi as the most severe effect of *Blastocystis* infection in the Swiss mice intestines, both the small and the large intestines were proven to be affected. Fragments of the fingerlike villi are seen detached and floating in the lumen. There are no crypts seen, but a flattened, very thin layer of intestinal glands remaining. No *Blastocystis* was observed deep in the layers, nor signs of inflammation or tissue invasion.

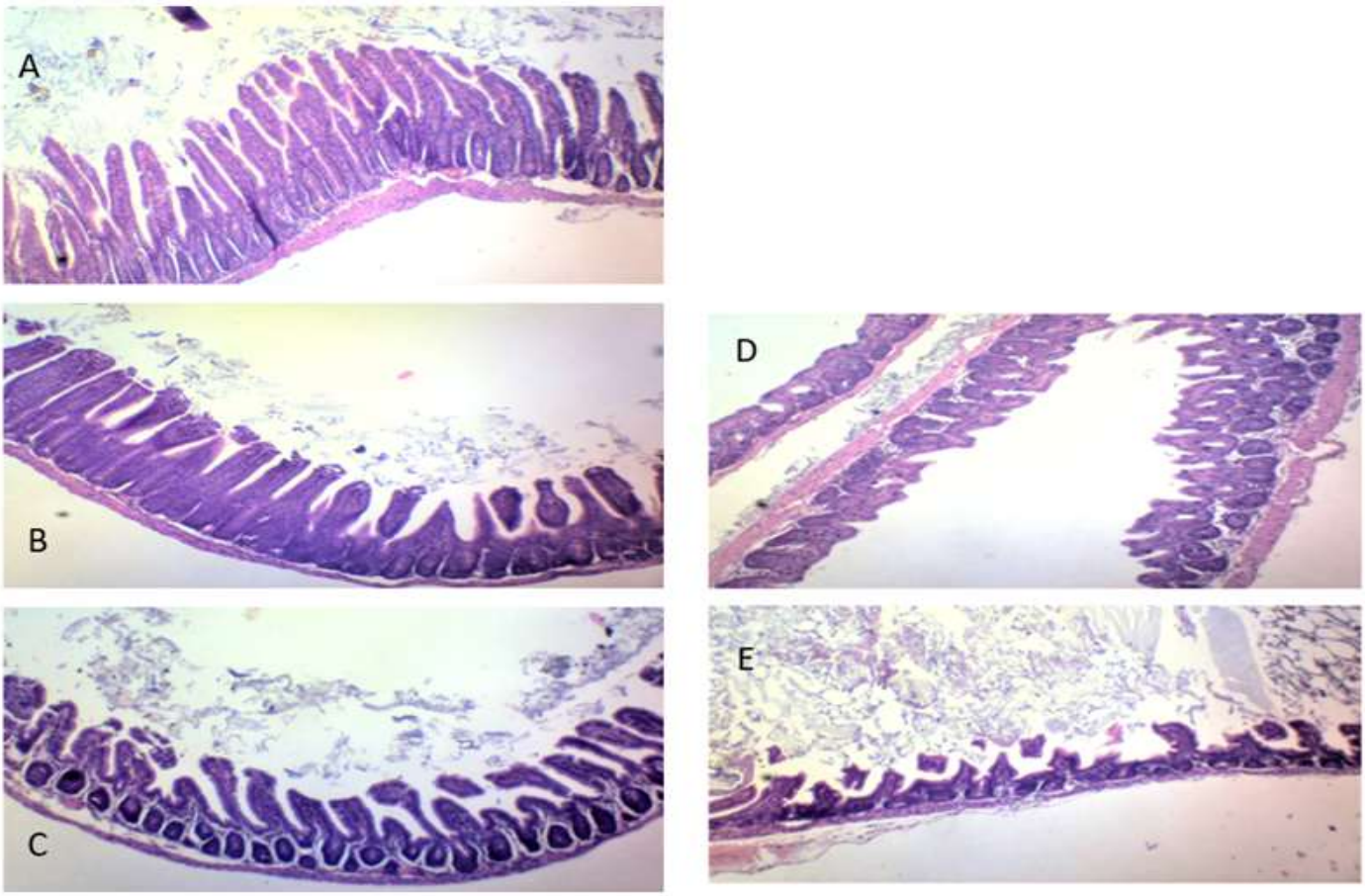

Plate A. Composite pictures showing the severity of *Blastocystis* pathogenic effect in the intestines of Swiss Mice.

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Blastocystis is an enteric protozoan that colonizes the cecum and colon. Following oral intubation, the cysts must excyst and transform into vacuolar or amoeboid forms. Colonization typically stabilizes within 3–5 days. By day 10, the parasite has had sufficient time to multiply and interact with the intestinal epithelial barrier. Studies often show that fecal shedding and intestinal burden peak between day 7 and day 14. A 10-day sacrifice captures the height of this burden [35]. The intensity of infection and the resulting mucosal response in rodent models are most pronounced during the first two weeks, making the 10-day mark a standard for acute phase analysis [36]. In research specifically targeting the effect of *Blastocystis* on the intestinal mucosa, a 10-to-12-day window was utilized to demonstrate significant increases in pro-inflammatory cytokines and visible villi blunting [37]. This short-term window was decided to identify the graded severity of mucosal damage before natural recovery began [12]. Beyond this period, immune clearance and repair may take place with the risk of "false negatives" due to healing.

The presence of mild villi erosion in negative controls is usually attributed to three scientifically documented factors: post mortem autolysis, physiological turnover, and technical artifacts. Intestinal mucosa in rodents is highly susceptible to rapid post-mortem autolysis, which can be mistaken for antemortem erosion [38]. The constant turnover of enterocytes (every 2–5 days in mice) results in a natural "shedding" appearance at the villus apex that can be mistaken for mild pathological erosion [39]. Villus blunting is a frequent artifact in sections where the plane of the cut does not pass directly through the longitudinal axis of the villus [40].

The findings of this study are both supported and contradicted by other histopathological studies on *Blastocystis* infection in mice. Pavanelli et al (2015), and Elwakil & Hewedi (2010) reported invasive behavior of the parasite, with infiltration of the muscular layer, lamina propria, submucosa, and muscle layers, indicating a more aggressive pathogenic mechanism [10,12]. In contrast, Ajjampur et al. (2009) agrees with the current study's findings, demonstrating the luminal nature of the parasite in colon loop experiments [41].

While some researchers see *Blastocystis* as a commensal luminal resident, others provide evidence of it breaching the epithelial barrier. This divergence can be analyzed through three critical lenses: strain virulence, infection dosage, and histopathologic sectioning. First, *Blastocystis* is not a single entity but a complex of at least 22 subtypes. Utilization of highly virulent strains (often associated with ST1 or ST3) that possess specific cysteine proteases may degrade the mucus layer and tight junction proteins. While subtyping offers epidemiological depth, the primary objective of this study was to characterize the microscopic enteropathy induced by *Blastocystis*. The consistency of the histopathological damage across different vector sources demonstrates a common pathogenic phenotype that transcends the need for molecular classification or subtyping in an acute infection model. Second, in high infection dosage models, heavy colonization leads to an overwhelming inflammatory response that physically compromises the intestinal wall, allowing for passive or active translocation of the parasite. In mice which maintained a robust mucosal barrier, the *Blastocystis* would remain trapped in the luminal stream or the outer mucus layer, unable to penetrate the lamina propria. And third, this study relied on standard transverse sections, and the points of invasion which are often focal and scattered might have been missed, leading to a purely luminal observation.

The study on intestinal *Blastocystis* rely heavily on qualitative data that doesn't always translate well to non-parametric ranking in Kruskal-Wallis. The scoring is measured through descriptive histopathology (e.g., "mild villi erosion" or "severe villi erosion") which are discrete categories. Despite the visual tissue changes which are subjectively scored, it captures the nuance of host-parasite interaction. It is best to prioritize the magnitude of the effect over the probability that the damage happened by chance (using the p value).

CONCLUSION

This study demonstrates that *Blastocystis spp*. isolated from synanthropic insects —specifically *Periplaneta americana* and *Musca domestica* —possess significant pathogenic potential, as evidenced by the acute intestinal enteropathy observed in Swiss mice. The 10-day infection model successfully captured a clear progression of mucosal damage, ranging from moderate to severe villi erosion, apical fragmentation, and the exposure of the lamina propria.

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The findings are particularly noteworthy as they show that the severity of intestinal disruption remains consistent regardless of the parasite's vector of origin. This suggests that *Blastocystis* maintains its virulence while being mechanically transported by common household pests. Consequently, this research validates the role of cockroaches and flies not merely as casual environmental residents, but as critical mechanical vectors capable of transmitting pathogenic strains of *Blastocystis* to mammalian hosts.

Ultimately, the clear histopathological evidence of tissue degradation provided here confirms that *Blastocystis* is a significant contributor to intestinal morbidity.

To complement the conclusion and provide a roadmap for where this research goes next, here are strategic recommendations:

1. Longitudinal Pathogenesis Studies. The current 10-day window successfully captured acute damage. A longitudinal study is recommended to observe the transition from acute villi erosion to potential chronic infection or tissue repair. This would clarify whether the mouse immune system can naturally resolve the "moderate to severe" erosion over time.
2. Integrated Vector Control and Public Health Policy. Given that both cockroaches and flies were confirmed as viable mechanical vectors for pathogenic *Blastocystis*, public health strategies should prioritize integrated pest management in food preparation areas and hospitals. Health education programs should emphasize that these insects are not just nuisances but active transporters of enteric pathogens.

Disclosure statement

The researchers have no conflict of interest to disclose.

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