



RESEARCH PAPER

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Correlation on the presence of *Blastocystis hominis* and tuberculosis from a provincial jail in the Philippines

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Abstract

Blastocystis hominis is a single-celled intestinal parasite that belongs to the phylum Stramenopila. Many aspects of its nature and pathogenic potential are still poorly understood, despite its medical significance. This study presents a pilot investigation in the Philippines aimed at detecting *Blastocystis hominis* in a provincial jail, focusing on its correlation with Pulmonary Tuberculosis (PTB) among Persons Deprived of Liberty (PDL), and its implications on treatment progression across different phases. Fecal samples from participants were gathered and subjected to analysis through multiple detection methods, including in vitro cultivation using Modified Locke Egg Medium. Following microscopic examination, four instances of *Blastocystis hominis* were detected out of 67 samples analyzed. Statistical analyses using Pearson's r Correlation and Pearson chi-square test in SPSS software revealed a significant correlation between *Blastocystis hominis* and tuberculosis treatment phase ($p < 0.05$). Specifically, inmates who underwent Directly Observed Therapy Short-Course showed notable correlation. The presence of *Blastocystis hominis* among individuals with a history of PTB signifies an ongoing health concern, especially pronounced among inmates who have undergone DOTS. Thus, this correlation underscores the increased susceptibility of individuals with a history of PTB to parasitic infections such as *Blastocystis hominis*. This study marks the first documented report of detecting *Blastocystis hominis* among tuberculosis-afflicted inmates in a provincial jail in the Philippines.

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Introduction

Blastocystis hominis is a unicellular enteric parasite classified under the phylum Stramenopila that can invade humans and a broad variety of non-human animals (CDC, 2019). Common symptoms associated with *Blastocystis* include diarrhea, abdominal pain, bloating, and other gastrointestinal issues. Interestingly, respiratory symptoms have also been linked to patients positive for *Blastocystis hominis*, notably in cases of tuberculosis. Key areas of interest include its morphological structures, pathogenicity in humans, correlation with other diseases, impact on immunocompromised individuals, and effectiveness of treatment stages (Castañeda *et al.*, 2020). For this reason, this parasite remains contentious among researchers and its pathogenicity still necessitates further investigation.

In Russia, *Blastocystis hominis* was substantially more prevalent in patients with pulmonary tuberculosis (TB) and those with HIV infection (Davis *et al.*, 2014). In addition, *Blastocystis* was found to be the most prevalent parasite among tuberculosis patients in Iran (Taghipour *et al.*, 2019). At the same time, residents of Tarlac, also known as *Tarlaqueños*, are more susceptible to contracting *Blastocystis hominis*, given that their primary economy revolves around agriculture. Established in 1990 through collaborative efforts of the late former President Corazon C. Aquino and former Tarlac Governor Mariano UN Ocampo III, the Tarlac Provincial Jail was created to ensure the fundamental needs and security of all detainees within Tarlac Province. With this, the researchers endeavor to further investigate the presence of *Blastocystis hominis* from respiratory patients at Tarlac Provincial Jail, with the goal of contributing novel insights into its pathogenicity.

Given the growing body of research linking *Blastocystis hominis* with tuberculosis, the study intends to examine the presence of this parasite in inmates diagnosed with tuberculosis. Furthermore, the researchers aim to explore the potential impact of the protozoan on different stages of tuberculosis

treatment. Moreover, numerous studies suggest conducting more studies about the given parasite to ascertain its mode of transmission so that preventive measures against *Blastocystis hominis* infections can be formulated (Mayo Clinic, 2022). For this reason, this study represents a pilot investigation aimed to determine the correlation of *Blastocystis hominis* and Tuberculosis at Tarlac Provincial Jail, Philippines, and contribute new insights regarding its pathogenicity.

Materials and Methods

The study focuses on the correlation between *Blastocystis hominis* and Tuberculosis among the patients from Tarlac Provincial Jail. In this study, the presence of *Blastocystis hominis* on patients with tuberculosis was determined through in vitro cultivation with the use of Modified Locke Egg Medium, followed by microscopic examination.

Stool sample collection

The sampling location, Tarlac, is more susceptible to contracting parasitic infection, given that their primary economy revolves around agriculture. For the sample collection, the stool samples used in the study were obtained from Tarlac Provincial Jail inmates who had completed and met the specific criteria outlined by the researchers. Subsequently, a total of 67 stool samples were collected in 15 mL containers, each labeled with a unique identification code (e.g., F1, H1, J1).

The samples were stored in a cool transport container with ice, supplemented with 1 kg of rock salt, and transported to Far Eastern University Manila for sample processing.

In vitro cultivation

For the sample inoculation into the prepared Modified Locke Egg (LE) Medium, an *in vitro* cultivation technique was used. A quantity of 50 milligrams of stool samples from 67 participants were subjected to culturing in LE Medium (8g NaCl, 0.2g KCl, 0.2g CaCl₂, 0.1g MgCl₂, 0.1g of Na₂PO₄·2H₂O, 0.1g NaHCO₃) with addition of four hen eggs and

supplemented with 10% horse serum. Sample processing was done by mixing the stool sample in the liquid component of the slant using an applicator stick. It was then incubated at 37°C for duration of 3-7 days and was subjected to microscopic examination (Belleza *et al.*, 2015).

Microscopic examination

For the microscopic examination, the researchers utilized the direct wet-mount technique to prepare two smears for each colony, one unstained and one stained. A drop of colony is placed into a clean glass slide followed by a drop of Lugol's iodine. Both unstained and stained smears were then observed under low (x10) and high (x40) power objectives to identify the different morphological forms of *Blastocystis hominis* such as vacuolar, granular, and amoeboid (Hegazy *et al.*, 2021). The researchers confirmed the presence of *Blastocystis hominis* in the samples with the guidance of three Registered Medical Technologists.

Samples yielding negative results during the microscopic examination were subsequently sub cultured to sustain microbial growth. Conversely, samples exhibiting the morphological characteristics of *Blastocystis hominis* during the microscopic examination were identified as positive. Upon the detection of *Blastocystis* sp. forms, a portion of the culture was preserved at -20°C for subsequent molecular analyses.

Results and discussion

Demographic profile

The research encompassed a group of 67 incarcerated individuals diagnosed with Tuberculosis (TB), aged between 18 and 60 years. Based on the findings shown in Fig. 1, a substantial male majority constitutes 98.5% of the participants, with a significant concentration within the 35–44 age group. Moreover, over half of the respondents (52.24%) indicated Tarlac as their permanent place of residence.

Demographic profile of the participants shows a higher proportion of male inmates, consistent with

broader demographic trends in jails. Moreover, the concentration of participants aged 35-44 may indicate a potential age-related susceptibility of inmates to PTB infection or diagnosis, as well as susceptibility to other infectious diseases due to exposure factors and limited healthcare access. Lastly, the significant number of participants permanently residing in Tarlac, a rural community, indicates that due to its high population density, leading to more close contact and increased transmission of TB, especially in confined spaces like jail. This pilot study provides preliminary findings on the prevalence of tuberculosis (TB) among incarcerated inmates carried out in Tarlac. While limited in scope, the results of this research emphasize the necessity of further investigation and focused interventions for populations including the ones in jail, highlighting the significance of community involvement and feasibility studies in combating tuberculosis in this setting.

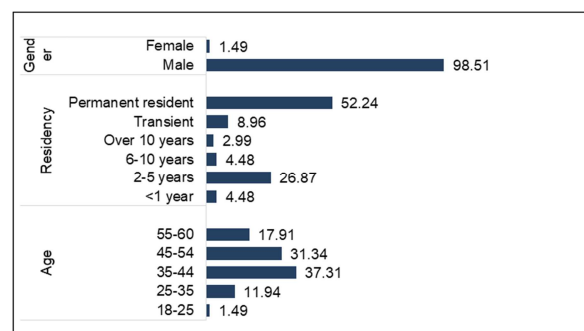


Fig. 1. Graph showing the inmates' demographic profile

Exposure factors

In terms of exposure factors illustrated in Fig. 2, most inmates reported consuming root crops and tap water, resulting in 95.03% and 52.24%, respectively. Additionally, 91.04% of inmates shared public latrines, accommodating 20–27 individuals per cell. Inmates consistently adhered to hygiene practices, notably hand washing and meal preparation, with a mean score of 3.46-4.00; however, they have limited access to hygiene products such as soap. The confluence of exposure factors amplifies the risk of both tuberculosis and parasitic infections spreading rapidly among the inmate population, given that this creates an

environment conducive to the transmission of infectious diseases (Montoya-Barthelemy *et al.*, 2022). Shared living spaces, limited restroom facilities, engaging in soil-related activities, and consuming root crops and tap water exacerbate the challenges of maintaining hygiene practices. Also, inmates struggle to access essential hygiene products such as soap, further hindering their ability to prevent infection spread through proper hand washing.

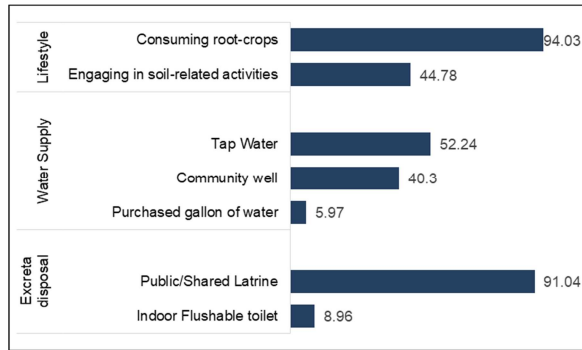


Fig. 2. Graph showing exposure factors to acquiring infectious diseases

Health assessment and clinical status

Among 67 total participants, various symptoms were reported: 17.91% were experiencing nausea; 11.94% experienced loss of appetite; 10.45% were experiencing fever; 7.46% were experiencing weight loss; 4.48% were experiencing abdominal pain; 2.99% were experiencing diarrhea; and 0.00% were experiencing blood in stools in the past 14 days (Fig. 3). Notably, nausea was the most prevalent symptom, followed by loss of appetite. Highlighting their clinical status, 20.90% had been previously diagnosed with parasitic infection, with 17.91% recently receiving treatment. All 67 inmates have been diagnosed with Tuberculosis. People who have completed the treatment for tuberculosis remain at considerable risk for recurring infection (Cudahy *et al.*, 2020). Among them, 100% have undergone Directly Observed Treatment Short Course (DOTS). Since 5.97% of the inmates are currently undergoing DOTS and 2.99% recently initiated this treatment course, they pose a risk of causing a relapsing infection. Thus, individuals with successful DOTS advised to undergo the treatment course repeatedly.

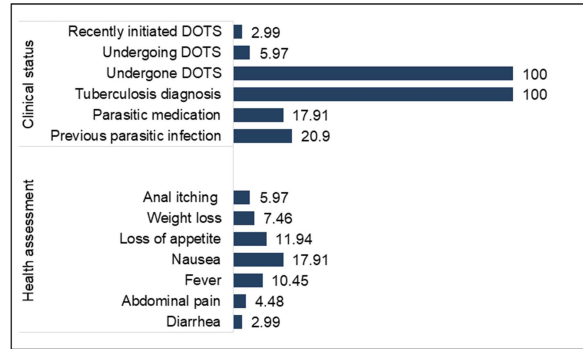


Fig. 3. Graph showing the health assessment and clinical status of the inmates

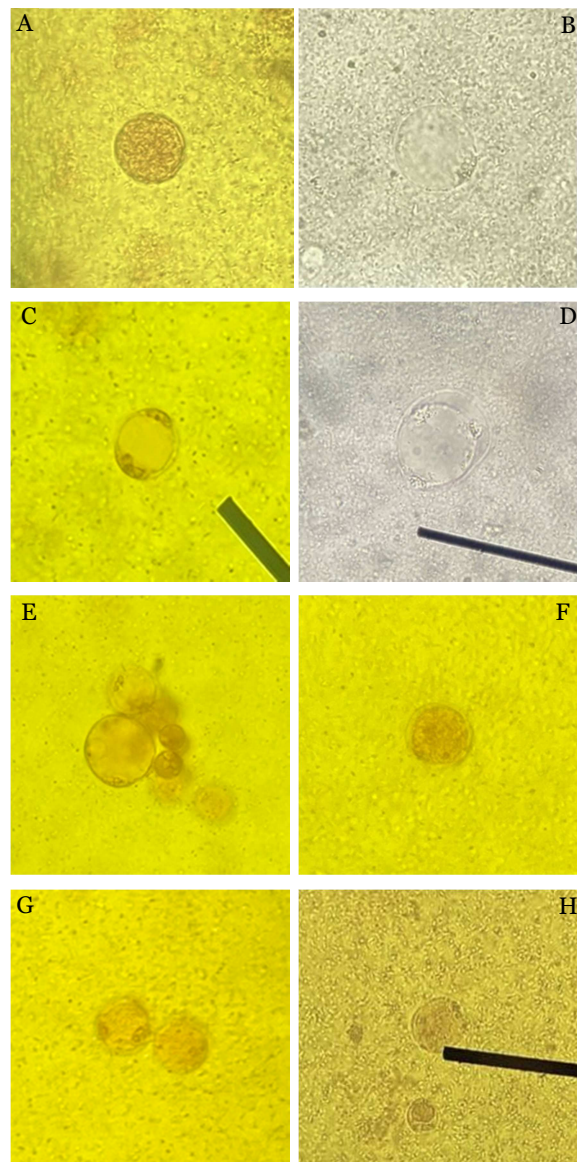


Fig. 4A-H. Different forms of parasite

Colony growth & microscopic findings

Following incubation of 37°C in a span of 3-7 days, the samples were observed under the light microscope. This

is consistent with the description of Belleza *et al.* (2015) as they have reported similar characteristics in their study. After 48 hours, the appearance of the solution in the tubes was different—turbid, hazy, and clear. In those that were turbid, there were small, colony structures formed where the slant meets the butt of the Locke Egg (LE) medium. The cultures with no presence of the parasite on the last of incubation after being subcultured were reported as negative.

The samples with the presence of the *Blastocystis hominis* under unstained and stained with Lugol's iodine microscopic examinations were considered positive. One of the positive samples had the presence of the parasite after the second subculture in a span of 72 hours, while the rest of the three were observed on the seventh day of incubation. Most of the observed *Blastocystis hominis* forms under the microscope were stained with Lugol's iodine, and the rest were unstained. Based on the illustrations of CDC (2019), the detected *Blastocystis hominis* were identified as vacuolar and granular forms. Figs 4A to 4B as well as Figs 4C to 4D show stained and unstained granular and vacuolar forms, respectively. Figs 4E to 4H depict the vacuolar form of the parasite.

Table 1. Prevalence of *Blastocystis hominis* among inmates

Inmates	Frequency	%infected
Undergone DOTS	4	100%
Currently on DOTS	0	0.00%
Recently initiated DOTS	0	0.00%

Blastocystis hominis prevalence among inmates

The pathologic potential of *Blastocystis hominis* has become a public health concern, especially to those who are living in poor conditions, such as in jail. Among the inmates, those who completed the Directly Observed Treatment Short course (DOTS) had the most isolated *Blastocystis hominis* in their stool samples. The majority of the demographic distribution of patients that results positive with stool microscopy is adult men aged 35-44 followed by men aged 55-60. Table 1 shows the prevalence of *Blastocystis hominis* in individuals who underwent, currently receiving, and recently

initiated Directly Observed Therapy Short course (DOTS).

Blastocystis hominis infection is considered significant due to its zoonotic transmission with recurrent symptoms (Beyhan *et al.*, 2015). Moreover, this microorganism that inhabits the intestines can last for weeks, months, or even years inside the body (CDC, 2020). The demographic profile, exposure factors, and health assessment of the four individuals who tested positive for *Blastocystis hominis* provide valuable insights, influencing their infection with the parasite. Eminently, half of the incarcerated individuals who tested positive are permanent residents of Tarlac. Furthermore, three out of the four positive cases have a medical history of diagnosed parasitic infections and exhibit clinical symptoms such as abdominal pain, nausea, and fever. In terms of exposure factors, all individuals partake in soil-related activities and frequently consume root crops and tap water. The fact that 4 out of 67 (5.97%) of the individuals with previous Tuberculosis infection tested positive for *Blastocystis hominis* suggests an ongoing health concern in the population.

Blastocystis hominis prevalence across treatment phases

Among the three treatment phases, inmates who underwent DOTS yielded the presence of *Blastocystis hominis*. Statistical analysis using SPSS software employed Pearson's r correlation and Pearson's Chi-square test. Pearson's r correlation showed the correlation between the presence of *Blastocystis hominis* and the treatment phase of tuberculosis ($p = 0.000$, $\alpha = 0.05$). Moreover, the calculated Pearson's r statistic of 0.0824 represents a highly positive correlation between the presence of *Blastocystis hominis* and the treatment stage of respondents. On the other hand, Pearson's chi-square test revealed a χ^2 value of 67.000. Notably, five cells, or 83.30% of the cell's dataset, are below 5, surpassing the 20.00% threshold, which violates the Pearson Chi-Square Test.

Thus, it necessitates the utilization of the likelihood ratio, which yields 30.303. The resulting p-value of 0.000, derived from the likelihood ratio at $\alpha = 0.05$, underscores a strong correlation between having *Blastocystis hominis* and the treatment phase of tuberculosis. With this, null hypothesis is rejected.

The analysis of different treatment phases such as completed, undergoing, and initiated treatment reveals varying degrees of correlation to *Blastocystis hominis* infection. Notably, participants who completed the course of treatment with Directly Observed Treatment Short-Course (DOTS) have the strongest association of *Blastocystis hominis*. This finding suggests that post-TB individuals are more susceptible to infections. Tuberculosis and its treatment itself leave permanent damage to the tissues of individuals that give them an elevated risk for future infections (Nightingale *et al.*, 2023). Conversely, individuals who are currently undergoing and recently initiated the treatment exhibit much lower correlation with the parasite. This observation highlights the importance of sustained surveillance and necessity for treatments to efficiently handle *Blastocystis hominis* infection throughout the course of treatment.

Conclusion

The study demonstrates a correlation between the occurrence of *Blastocystis hominis* and Tuberculosis. The presence of individuals who previously had Tuberculosis and test positive for *Blastocystis hominis* indicates a persistent health issue within the population. This holds significant implications for public health, especially for individuals residing in substandard conditions within jails. The demographic profile, exposure factors, and health assessment of individuals testing positive for *Blastocystis hominis* offer significant correlations, predisposing them to infectious diseases. Subsequently, it is observed that inmates who already completed the Directly Observed Treatment Short-Course (DOTS) showed the strongest association with acquiring *Blastocystis hominis*. Having a history of Tuberculosis makes individuals much more susceptible to parasitic infections such as *Blastocystis hominis*. Thus, this pilot study represents the first detection of *Blastocystis hominis* among tuberculosis patients in the

Philippines, specifically among inmates in a provincial jail, marking the first documented instance of its presence in this population.

Recommendations

To address the correlation between *Blastocystis hominis* and Tuberculosis, the following actions are recommended:

1. Hygiene Practices: Enhance hygiene in healthcare facilities by ensuring access to soap, promoting proper handwashing, and maintaining cleanliness to reduce the transmission of infections.
2. Health Education: Implement education programs targeting patients and healthcare workers on hygiene, sanitation, and infection prevention, including risks from contaminated water.
3. Routine Screening: Establish screening protocols for *Blastocystis hominis* and Tuberculosis, particularly for patients with a history of Tuberculosis, to enable timely diagnosis and treatment.
4. Environmental Management: Ensure proper waste disposal, ventilation, and cleanliness in healthcare facilities to create a healthier environment.
5. Monitoring and Surveillance: Develop systems to track infection rates, monitor patient health, and evaluate infection control measures regularly.
6. Further Research: Investigate the transmission dynamics, risk factors, and interventions related to *Blastocystis hominis* and its interaction with Tuberculosis.

Implementing these measures could significantly reduce the prevalence of *Blastocystis hominis* and tuberculosis in healthcare settings, leading to improved health outcomes.

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