DETECTION OF *BLASTOCYSTIS* SP. AMONG CHICKEN FROM SELECTED FARMS IN KELANTAN

By

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ABSTRACT

DETECTION OF *BLASTOCYSTIS* SP. AMONG CHICKEN FROM SELECTED FARMS IN KELANTAN

An abstract of the research paper presented to the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, in partial requirement on the course DVT 55204- Research Project.

ABSTRACT: Chickens are reservoirs for *Blastocystis* sp., a zoonotic protozoan that infects humans via the fecal-oral route. However, studies on *Blastocystis* sp. in poultry are limited, particularly in Malaysia. Therefore, this study aims to detect *Blastocystis* sp. among chickens in Kelantan. The detection of *Blastocystis* sp. infection was conducted on free-range and cage-reared chickens from selected farms in Kelantan. A total of 30 cloacal swab samples were collected, which were then cultured in Jones medium supplemented with 10% horse serum and incubated at 37°C for 24 hours. Fecal smear examinations were performed on all samples. Positive results were fixed with methanol and stained with 10% Giemsa to observe the detailed morphology of the protozoan at 100x magnification using light microscopy. *Blastocystis* sp. was identified and isolated from the selected poultry farms in Kelantan which detected in 33.3% (10/30) of the samples, with 58.3% (7/12) of free-range chickens and 16.6% (3/18) of cage-reared chickens testing positive. The most common form observed was the vacuolar form, characterized by large, spherically shaped cells containing a central body resembling a large vacuole, which was the predominant cell type seen in the samples. A statistically significant association was found between *Blastocystis* sp. infection and the collection area (extensive vs. intensive) and biosecurity measures. However, no statistically significant association was observed between the health status of the animals, deworming, and vaccination. To achieve more accurate and precise identification down to the subtype and species level, more advanced molecular identification techniques should be employed.

Keywords: *Blastocystis* sp., Zoonotic, Zoonotic protozoan, Poultry parasites

ABSTRAK

PENGESANAN *BLASTOCYSTIS* SP. ANTARA AYAM DARI LADANG TERPILIH DI KELANTAN

Abstrak daripada kertas penyelidikan yang dibentangkan kepada Fakulti Perubatan Veterinar, Universiti Malaysia Kelantan, sebagai keperluan sebahagian daripada kursus DVT 55204 – Projek Penyelidikan.

ABSTRAK: Ayam adalah takungan untuk Blastocystis sp., protozoa zoonosis yang menjangkiti manusia melalui laluan fecal-oral. Walau bagaimanapun, kajian terhadap Blastocystis sp. dalam ayam adalah terhad, terutamanya di Malaysia. Oleh itu, kajian ini bertujuan untuk mengesan Blastocystis sp. antara ayam di Kelantan. Pengesanan Blastocystis sp. jangkitan telah dijalankan ke atas ayam ternakan bebas dan sangkar dari ladang terpilih di Kelantan. Sebanyak 30 sampel swab kloaka telah dikumpul, yang kemudiannya dikultur dalam medium Jones ditambah dengan 10% serum kuda dan diinkubasi pada suhu 37°C selama 24 jam. Pemeriksaan smear najis dilakukan ke atas semua sampel. Keputusan positif telah ditetapkan dengan metanol dan diwarnakan dengan 10% Giemsa untuk melihat morfologi terperinci protozoa pada pembesaran 100x menggunakan mikroskop cahaya. Blastocystis sp. telah dikenalpasti dan diasingkan daripada ladang ayam terpilih di Kelantan yang dikesan dalam 33.3% (10/30) sampel, dengan 58.3% (7/12) ayam ternakan bebas dan 16.6% (3/18) dalam sangkar. ayam ujian positif. Bentuk yang paling biasa diperhatikan ialah bentuk vakuolar, dicirikan oleh sel-sel besar, berbentuk sfera yang mengandungi badan pusat yang menyerupai vakuol besar, yang merupakan jenis sel utama yang dilihat dalam sampel. Perkaitan yang signifikan secara statistik didapati antara Blastocystis sp. jangkitan dan kawasan pengumpulan (luas vs intensif) dan langkah biosekuriti. Walau bagaimanapun, tiada perkaitan yang signifikan secara statistik diperhatikan antara status kesihatan haiwan, deworming, dan vaksinasi. Untuk mencapai pengecaman yang lebih tepat dan tepat sehingga ke peringkat subjenis dan spesies, teknik pengecaman molekul yang lebih maju harus digunakan.

Kata kunci: *Blastocystis* sp., Zoonotic, Protoza Zoonotik, Parasit Unggas

CERTIFICATION

This is to certify that we have read this research paper entitled **'Detection of *Blastocystis* sp. Among Chicken from Selected Farms in Kelantan'** by Nur Irdina binti Fiesal and in our opinion, it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course DVT - Research Project

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DEDICATIONS

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LIST OF ABBREVIATIONS

SPSS Statistical Package for the Social Sciences

PCR Polymerase Chain Reaction

DNA Deoxyribonucleic acid

ND Newcastle Disease

LIST OF SYMBOLS

% Percentage

°C Degree Celsius

x times

mL Milliliters

CHAPTER 1: INTRODUCTION

1.1 Background

Blastocystis sp. is an anaerobic protozoan that inhabits the intestinal tract of various animals and humans, causing infections that may be symptomatic or asymptomatic (Shao,2007). *Blastocystis hominis* infection was traditionally used to refer specifically to infections in humans. In contrast, for animal infections, the isolated protozoan was typically identified only at the genus level as *Blastocystis* sp. (Haziqah *et al*., 2014). There were at least 17 subtypes of *Blastocystis* (Thompson,2018). To date, six subtypes were identified in birds including ST1, ST2, ST4, ST6, ST7 and ST8. Among the six subtypes, ST6 and ST7 were the predominant subtypes in avian hosts (Zuhair Mohammed Abed, et al., 2024).

Blastocystis sp. is a polymorphic organism and the four common forms: vacuolar, granular, amoeboid and cysts forms (Haziqah *et al.*, 2014). Among all the forms, the fecal cyst is the only environmentally resistant transmissible form (Tan,2013). Fecal-oral transmission is the most accepted pathway and transmission involves only the cyst form of the parasite (Chandrasekaran *et al.*, 2014).

The clinical signs and symptoms of *Blastocystis* sp. infections are non-specific and associated with gastrointestinal symptoms such as diarrhoea, abdominal pain, vomiting, anorexia and flatulence. However, more pathogenic symptoms such as intestinal inflammation, altered bowel habits, lethargy, chronic diarrhea and death were observed in animals with severe infection (Chandrasekaran *et al*., 2014). *Blastocystis* sp. is considered a zoonotic and it is believed that animals such as chickens constitute large reservoirs for human infection via the fecal-oral route (Greige *et al*. ,2018).

1.2 Problem Statement

Several studies on *Blastocystis* sp. have been conducted in Malaysia. However, most of the previous studies focused on *Blastocystis* infection in humans. There are limited studies on *Blastocystis* isolated from poultry in Malaysia. The first report on detection of *Blastocystis* infection among chickens was conducted in Perak and Selangor (Haziqah *et al*., 2014). The detection of *Blastocystis* sp. isolated from chickens in Kelantan has never been studied up to this date. This study aims to detect *Blastocystis* sp. in poultry from selected farms in Kelantan.

1.3 Research Questions

1.3.1 Is *Blastocystis* sp. infections present among chickens on the selected farms in Kelantan?

1.3.2 What is the most common form of *Blastocystis* sp. observed among chickens on the selected farms in Kelantan?

1.3.3 Are the collecting area, biosecurity measures, and flock health status correlated with *Blastocystis* sp. infection in chickens?

1.4 Hypothesis

1.4.1 There is presence of *Blastocystis* sp. which can identify among chickens from a selected farm in Kelantan.

1.4.2 The most common form of *Blastocystis* sp. observed in chickens from selected farm in Kelantan is vacuolar form.

1.4.3 There are correlations between *Blastocystis* sp. infection in chickens and collection area, biosecurity measure and flock health status.

1.5 Objectives

1.5.1 To determine the occurrence of *Blastocystis* sp. in chickens from selected farms in Kelantan.

1.5.2 To determine the common form of *Blastocystis* sp. seen among chickens in selected farms in Kelantan.

1.5.3 To identify the correlation between the occurrence of *Blastocystis* with the collection area, the management such as biosecurity, and the health status of the flock.

CHAPTER 2: LITERATURE REVIEW

2.1 Overview of Blastocystis

Blastocystis sp. is a common protozoan parasite frequently identified in the digestive tract of humans and various animal hosts, including poultry (Liu et al., 2022). *Blastocystis* exhibits multiple life cycle forms, including vacuolar, amoeboid, granular, and cystic (Tan, 2013). The primary route of *Blastocystis* transmission is fecal-oral, and humans can become infected through the consumption of food and water contaminated with *Blastocystis* cysts (Zuhair Mohammed Abed et al., 2024). Its zoonotic potential suggests that animals may serve as reservoirs for transmission (Rauff-Adedotun et al., 2020). The effects of *Blastocystis* infection in humans can vary widely. In symptomatic individuals, the infection typically causes mild to moderate gastrointestinal issues such as diarrhea, bloating, flatulence, and nausea, often resembling irritable bowel syndrome. In some cases, *Blastocystis* infection can lead to more severe symptoms, including persistent or bloody diarrhea (Koch, 2012).

2.2 Blastocystis in Chicken

In the study by Buitrago et al., (2017), *Blastocystis* sp. was found in 30% of chicken samples from commercial farms. Blastocystis infection has often been associated with gastrointestinal distress, clinical signs shown such as diarrhea, weight loss and poor growth rate being observed (Kaczmarek,2019). Some researchers believe that *Blastocystis* sp. may act as a co-infection agent, exacerbating the severity of other intestinal diseases like Coccidiosis and Salmonella infection (Stensvold,2012). Additionally, *Blastocystis* sp. is often found in the feces of infected chicken, suggesting that manure management could be a critical factor in limiting transmission. Studies have shown that fecal contamination of feed and water source is a significant vector for *Blastocystis* transmission in poultry (Sogawa, H., et al. ,2020). *Blastocystis* is a zoonotic parasite, and chickens are believed to serve as significant reservoirs for human infection via the fecal-oral route (Naguib et al, 2022). Therefore, *Blastocystis* infection was surveyed in free-range and cage-reared chickens consisting of broilers, layers and chicken kept for recreation purposes (Haziqah *et al.,* 2014). The results indicated that the most free-range chicken tested positive for *Blastocystis* sp. The vacuolated form was the most common cellular form of *Blastocystis* identified in culture (Haziqah *et al.,* 2014).

2.3 Diagnosis of Blastocystis

For diagnosis of *Blastocystis* sp., the fecal samples collected using cloacal swabs in pea-sized amounts. Each specimen is subsequently cultivated for *Blastocystis* sp. in 3 mL of sterile Jones' medium supplemented with 10% heat-inactivated horse serum. The cultures are incubated at 37°C for 24 hours. (Haziqah *et al*., 2014).

A drop of sediments of cultures are taken using a sterile pipette, placed on the glass slide and examined under light microscopy at 100× magnification for the detection of *Blastocystis* sp. The positive fecal smears are fixed with methanol and stained with 10% Giemsa to observe the detailed morphology of the protozoan. (Lee,1999) The stained forms of *Blastocystis* sp. are analyzed under light microscopy at 100× magnification based on the shape and size of the microorganism (Haziqah et al., 2014).

2.4 Subtypes of Blastocystis

Blastocystis exhibits significant genetic diversity, with several subtypes identified some of which are zoonotic (Shirley,2009). Studies have shown that chickens can harbor a variety of Blastocystis subtypes, including ST1, ST2, ST4, ST6, ST7 and ST8. Among the six subtypes, ST6 and ST7 were predominantly in the avian hosts (Zuhair Mohammed Abed, *et al*., 2024). Subtype analysis using the sequenced-tagged site (STS) primers based on PCR amplification using the DNA barcoding primer. These subtypes in chickens highlight the potential for cross-species transmission (Hassan,2015).

2.5 Prevention of Blastocystis

To prevent *Blastocystis* sp. transmission, which occurs via fecal-oral route, proper management of environmental conditions is essential. Preventative measures include maintaining clean floors, utensils, and equipment; regularly removing feces and bedding; and ensuring that food and water containers are kept clean. These practices help reduce the risk of infection (Bishop L. et al.,2015). Proper sanitation practices may serve as effective inhibitors of environmental contamination, thereby preventing infections in poultry (Falkowski *et al*.,2022). Besides, *Blastocystis* sp. can be

carried by wild and domestic birds that migrate to the poultry house, it is best to have proper enclosed housing to protect the transmission of the *Blastocystis* in chicken.

However, introduction of benzimidazole drugs such as fenbendazole has been used broadly in a wide range of species for the control of parasitic helminths (Saemi Soudkolaei *et al.*, 2021). Fenbendazole is one of the safest drugs used in food animals in terms of food residues. It is essential in the poultry industry where benzimidazoles are veterinary drugs extensively used for treatment and prevention of parasitic infestations. For human prevention of Blastocystis infection, it is essential to thoroughly wash and cook food, as poultry feces, often used as fertilizer for crops cultivated for human consumption, can serve as a source of contamination (CDC,2024)

CHAPTER 3: RESEARCH METHODOLOGY

3.1 Study Area

This research study was conducted in selected farms covering four districts in Kelantan.

3.2 Study Design

The study design selected for this study is cross-sectional study.

3.3 Source Population

The source population in this study is chicken species in selected poultry farms in Kelantan.

3.4 Study Population

The study population was selected using simple random sampling, with 6 chickens chosen from the populations of 5 different farms. A total of 30 chickens from various species were collected from the selected farms in Kelantan.

3.5 Selection Criteria

3.5.1 Inclusion criteria

The inclusion criteria of the sample would be chickens presented as free range and barn-reared chicken in the selected farm in Kelantan.

3.5.2 Exclusion criteria.

The exclusion criteria of the sample would be chickens that had dewormed within the last 6 months of duration.

3.6 Material and Equipment

Fecal samples, approximately the size of a pea, were collected from the cloacal swabs of the chickens using sterile swabs. The swabs were then inoculated into 3 mL of sterile Jones medium supplemented with 10% heat-activated horse serum and properly labeled. Each sample was incubated at 37°C for 24 hours.

After incubation, a drop of sediment from the cultures was taken using a sterile plastic pipette and examined at 100× magnification using light microscopy for the detection of *Blastocystis* sp. If no organisms were observed in the cultures, the samples were considered negative.

Positive fecal smears were fixed with methanol and stained with 10% Giemsa to observe the detailed morphology of the protozoan at 100× magnification using light microscopy. The forms of *Blastocystis* sp. observed under the microscope after staining were analyzed based on the shape and size of the microorganisms.

Statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS) 30.0 software package. Fisher's exact test was carried out to determine whether infections were associated with collecting area, biosecurity, and health status of the flock.

3.7 Data Collection

Table 1.0 Number of samples collected from local poultry farms in Kelantan.

Poultry Farm	No. of Samples
Farm 1: Pusat Pemulihan Orang Kurang Upaya, Kampung Aur Telong Bachok, Kelantan	6
Farm 2: Lot 2318 Kg Gelong Machang Sering 16150 Kota Bharu, Kelantan	6
Farm 3: 630 Kg Perupok, 172020 Pasir Mas Kelantan	6
Farm 4: Lot 1982, Kg Perupok, 16250 Wakaf Bharu, Kelantan	6
Farm 5: 419 Lorong Pak Da Daud Kampung Bunut Payong	6

A total of 30 cloacal swab samples were collected from domestic chickens across five local poultry farms, covering four districts in Kelantan, as shown in table 1.0. Six samples were collected from each farm. The farms included: (1) Pusat Pemulihan Orang Kurang Upaya, Kampung Aur Telong, Bachok, which reared village chickens intensively; (2) Lot 2318, Kg Gelong, Machang Sering 16150, Kota Bharu, which reared village chickens extensively; (3) 630 Kg Perupok, 172020, Pasir Mas, which reared Lohmann Brown chickens intensively; (4) Lot 1982, Kg Perupok, 16250, Wakaf Bharu, which reared Lohmann Brown chickens extensively; and (5) 419 Lorong Pak Da Daud, Kampung Bunut Payong, which reared Ayam Serama chickens intensively.

CHAPTER 4: FINDINGS AND DISCUSSION

4.1 Occurence of Blastocystis

Table 2.0 Occurrence of *Blastocystis* sp. in Domestic Chickens by Farm Location

Farms	Farm 1 (PROKA)	Farm 2 (Kota Bharu)	Farm 3 (Pasir Mas)	Farm 4 (Wakaf Bharu)	Farm 5 (Bunut Payong)	Percentage
Positive Blastocystis	0	3	2	4	1	10 (33.3%)
Negative Blastocystis	6	3	4	2	5	20 (66.6%)

Table 2.1 Occurrence of *Blastocystis* sp. in Domestic Chicken by Rearing System

Study animals	No of fecal samples	No. of chicken infected
Cage-reared chickens		
Village Chicken	6	0
Ayam Serama	6	1 (16.7%)
Lohmann Brown	6	2 (33.3%)
Free-range chicken		
Village Chicken	6	3 (50%)
Lohmann Brown	6	4 (66.7%)
Total	30	10 (33.3%)

A total of 33.3% (10/30) of chicken fecal samples screened were positive for *Blastocystis* sp. and 66.6% (20/30) screened were negative for *Blastocystis* sp. as shown in table 2.0. Based on the result, chicken that reared extensively showed a high number in *Blastocystis* sp. infection compared to reared intensively as shown in table 2.1 None of the birds showed any clinical signs of infection such as diarrhea, weight loss and poor growth rate.

Table 3.0 Correlation between collection area and *Blastocystis* sp. infection.

	Extensive	Intensive	Total
Positive Blastocystis	7 (23.3%)	3 (10%)	10 (33.3%)
Negative Blastocystis	5 (16.6%)	15 (50%)	20 (66.6%)
Total	12 (40%)	18 (60%)	30

For collection area of the chicken based on the data taken as shown in table 3.0, a total of 40% (12/30) samples were collected from extensive chicken farm that consists of 23.3% (7/30) positive samples and 16.6% (5/30) negative samples. Meanwhile, a total of 60% (18/30) samples were collected from intensive chicken farms that consists of 10% (3/30) positive samples and 50% (15/30) negative samples. Based on the *Fisher's exact test result*, for the collecting area, it showed there was a statistically significant association between collecting area mainly focused on extensive and intensive and *Blastocystis* infection ($\chi 2 = 6.3$, [df] = 1, P = 0.02) recorded in this study.

Table 4.0 Correlation between biosecurity and *Blastocystis* sp. infection

	Poor	Moderate	Good	Total
Positive Blastocystis	9 (30%)	1 (3.33%)	0	10 (33.3%)
Negative Blastocystis	9 (30%)	5 (16.6%)	6 (20%)	20 (66.6%)
Total	18 (60%)	6 (20%)	6 (20%)	**30**

Besides, for biosecurity measures, based on the data taken as shown in table 4.0, a total of 60% (18/30) samples were collected from poor biosecurity farm that consists of 30% (9/30) positive samples and 30% (9/30) negative samples. Meanwhile, a total of 20% (6/30) samples were collected from moderate biosecurity farm that consists of 3.33% (1/30) positive samples and 16.66% (5/30) negative samples. Next, a total of 20% (6/30) samples were collected from good biosecurity farm that consists of only negative samples 20% (6/30) and no positive samples. However, the results from the Fisher's exact test revealed that there was a statistically significant

association between biosecurity and *Blastocystis* infection ($\chi 2 = 6$, [df] = 1, P = 0.025) recorded in this study.

Table 5.0 Correlation between flock health status (vaccination) and *Blastocystis* sp. infection

	Vaccinated	Not vaccinated	Total
Positive	6 (20%)	4 (13.3%)	10 (33.3%)
Negative	12 (40%)	8 (26.6%)	20 (66.6%)
Total	18 (60%)	12 (40%)	30

Next for flock health statis of vaccination, based on the data taken as shown in table 5.0, a total of 60% (18/30) samples were collected from vaccinated chicken that consists of 20% (6/30) positive samples and 12% (9/30) negative samples. Meanwhile, a total of 40% (12/30) samples were collected from unvaccinated chicken that consists of 13.3% (4/30) positive samples and 26.6% (8/30) negative samples. However, the results from the Fisher's exact test revealed that there was a statistically significant association between biosecurity and *Blastocystis* infection ($\chi 2 = 3$, [df] = 1, P = 0.14) recorded in this study.

Table 6.0 The flock health status of deworming and *Blastocystis* sp. infection

	Dewormed	Not dewormed	Total
Positive	0	10 (33.3%)	10 (33.3%)
Negative	0	20 (66.6%)	20 (66.6%)
Total	0	30 (100%)	30

Next, for the flock health status of deworming, based on the data taken as shown in table 6.0, a total of 0 % samples were collected from dewormed chicken. Meanwhile, a total of 100% (12/30) samples were collected from non-dewormed chicken that consists of 33.3% (10/30) positive samples and 66.6% (20/30) negative samples. However, Fisher's exact test cannot be run in this correlation due to none of animal were dewormed.

4.2 Blastocystis Identification

Table 7.0 Form of Blastocystis

Type of Form	Vacuolar	Granular	Amoeboid	Cyst
Total	8 (26.6%)	2 (6.6%)	0	0

Results indicate that there was an occurrence of natural infection of *Blastocystis sp.* in the poultry sampled. It was found that 33.3% (10 out of 30) chicken fecal samples were positive for *Blastocystis sp*. In this study, granular form was 6.6% (2/10) and the vacuolar form 26.6% (8/30) were found in the culture as shown in table 7.0. The vacuolar was the common *Blastocystis* cell form.

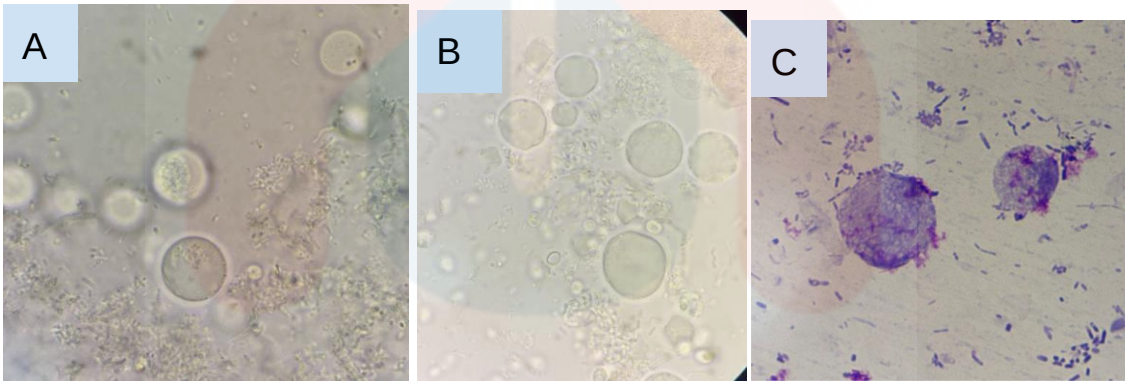

Figure 1.0: Vacuolated form from the culture (under 100x magnification). Picture A and B appear as large, spherical cells containing a central structure resembling a large vacuole that occupies approximately 90% of the cell. Picture C highlights the presence of purplish-colored nucleus and granules at the peripheral membrane of the cytoplasm.

Figure 1.0 illustrates the morphology of the vacuolar form from the culture, observed under 100x magnification. Picture A and B show the organisms as observed by direct smear observation, where they usually appear as huge, spherical cells with a thin coating of cytoplasm at the periphery and a central structure that resembles a giant vacuole that takes up about 90% of the cell. The measurements of Blastocystis sp.'s vacuolar shape varied significantly. Picture C presents the morphology of the vacuolar form with Giemsa staining, emphasizing the presence of granules at the cytoplasm's peripheral membrane and a purplish-coloured nucleus.

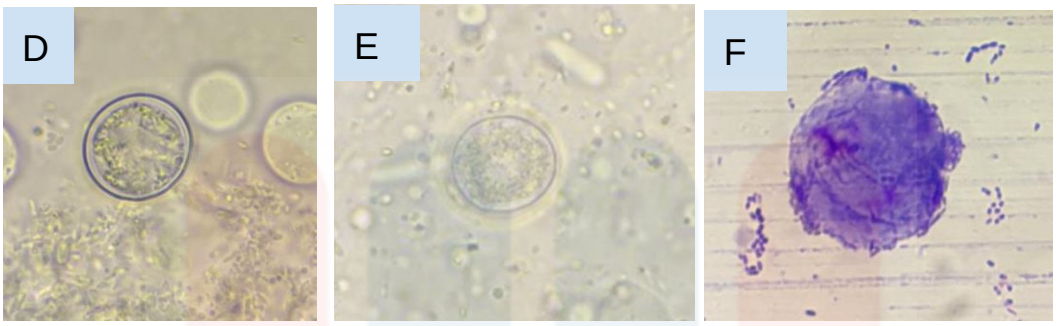

Figure 2.0: Granular form with clumps of granules in the central vacuoles (under 100x magnification). Picture D and E presence of granules packed inside the cell. Picture F indicates entire cell appears dark purple, reflecting its dense granule content

Figure 2.0 illustrates the morphology and microscopy of the granular form, also observed under 100x magnification. Similar to the vacuolar form, the granular forms were distinguished by the large number of granules present in both the thin peripheral cytoplasm and the core vacuole. The granules packed inside the cell are visible under direct smear examination in Pictures D and E. Picture F shows the granular form's shape using Giemsa staining, which makes the entire cell appear dark purple, indicating that it contains a lot of granules.

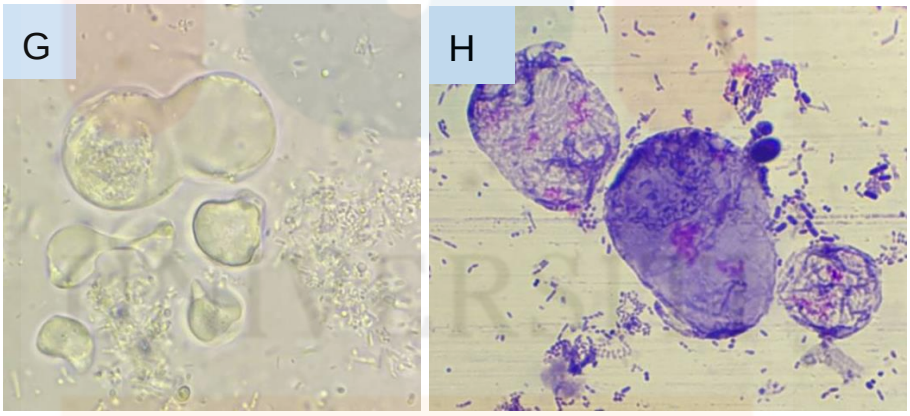

Figure 3.0: A typical binary fission. Picture H showed partition of the cytoplasm of the mother cell and results in two daughter cells with an equal size and shape. Picture H clearly visualized the splitting of the cell into two separate parts under Giemsa stain.

Figure 3 depicted the morphology and microscopy of binary fission, the most prevalent reproductive type. The mother cell's cytoplasm is divided, as seen in Picture G, producing two daughter cells that have the same size and form. Giemsa staining of the cell was used in Picture H, making it easy to see how the cell divided into two distinct sections.

4.3 Discussion

In this study, Blastocystis were isolated and confirmed with microscopic examination. Blastocystis is a polymorphic organism, so the vacuolar form was the predominant cell type seen in the in vitro cultures. Two reproductive modes for *Blastocystis* sp. have been suggested such as binary fission and budding or plasmotomy (Tan and Stenzel, 2003). However, this study only observed the binary fission mode which is characterized by the partition of the cytoplasm of the mother cell and results in two daughter cells with an equal size and shape. In the study by Haziqah et al., (2014) also stated the most common reproductive form was binary division.

There was a correlation between the occurrence of *Blastocystis* sp. with factors such as the collection area and biosecurity measures but no correlation in the health status of the flock on the selected chicken farms. Regarding the collection area, most of the samples were taken from rural areas where chickens are typically reared as backyard or free-range poultry. *Blastocystis* was detected with 58.3% (7/12) of free-range chickens and 16.6% (3/18) of cage-reared chickens testing positive. The overall prevalence of *Blastocystis* sp. was moderately higher in the free-range than barn-reared chickens. Free-range chickens were likely to be more prone to *Blastocystis* sp., owing to their scavenging habits (Haziqah *et al*., 2014). This practice may expose the chickens to a wider variety of environmental contaminants, including fecal matter from infected animals, thereby increasing the risk of transmission. According to Mokhtar (2018), the scavenging habits of chicken, particularly those reared in rural areas where they are allowed to free-range for feeding, increase the likelihood of acquiring infections from the environment.

Secondly, most of the farmers did not priorities or practice good biosecurity measures due to lack of knowledge. Proper hygiene and sanitation are crucial to prevent contamination of chicken from the environment via fecal-oral transmission (Falkowski *et al*.,2022). The poultry facility lacks a proper bedding system, using soil, which increases the chickens' exposure to potential contamination. Additionally, the farm does not have an effective pest control system or adequate housing, which allows wild birds and insects to enter the poultry house and potentially transmit *Blastocystis* sp. to the chickens (De Waard, J. H., *et al*.,2007). Despite being reared in an intensive barn system designed to minimize contamination, *Blastocystis* sp. was still present. The source of infection in birds remains unknown; however, one plausible explanation is contamination of the water and food provided (Hublin,2021). The feeding system on the farm typically uses bell feeders and drinkers, where the chickens share food and water. The study by Lee (1999) noted that extremely high hygiene standards were maintained, with floors and equipment regularly washed to remove chicken excreta. Feeding areas were kept clean and free of debris, and appropriate, constant temperatures were maintained within the buildings. These practices were intended to inhibit environmental contamination and the subsequent transmission of fecal organisms.

Lastly, the health status factor in this study shows no significant association with *Blastocystis* sp. The history indicates that the chickens have not been dewormed or vaccinated. The absence of both factors may contribute to infection, albeit indirectly.

Regarding the health status of vaccination, 60% (18/30) of the positive samples were from vaccinated chickens, while 20% (6/30) were from negative samples, and 12% (9/30) of the negative samples came from vaccinated chickens. Meanwhile, 40% (12/30) of the positive samples

were from unvaccinated chickens, with 13.3% (4/30) from positive samples and 26.6% (8/30) from negative samples. Vaccinated chickens were still infected with this parasite. In the study by Papini (2010), it was mentioned that there is a significant difference in the humoral immune response after ND vaccination. The synthesis of immunoglobulins is reduced in animals severely infected by parasites, owing to an absolute loss of protein. The low antibody response may result from pre-existing parasitism. Birds exposed to immunosuppressive factors usually show reduced competence in both humoral and cellular immunity, which can lead to helminth infections (Alfellani,2013). This statement is supported by a study by Spradbrow (1988), which reported that ND vaccination failures in village chickens might be explained by immune suppression caused by parasitism.

Regarding deworming and health status, none of the chickens in this study were dewormed. The use of dewormers is restricted in the free-range system due to the risk of accumulation of residues in the animals' muscles, leading to potential issues for consumers and the development of resistant nematodes. In the European Union, only anthelmintics such as flubendazole and fenbendazole are approved to treat parasitic infections (Maloney,2021). The lack of deworming can result in a higher burden of intestinal worms, weakening the immune system and making chickens more vulnerable to protozoal infections (Michaud,2006).

The information is vital to free range poultry farmers and veterinary health care workers so as to enable proper care and protection in practice when handling the animals and samples. The zoonotic implications of this disease make it important for further research to elucidate the transmission cycle from animals to humans (Ali *et al.*,2013).).

CONCLUSION

In summary, *Blastocystis sp.* were identified and isolated in the fecal samples that were obtained from the chosen poultry farm in Kelantan. A total of 10 (33.3%) chicken faecal samples screened were positive and 66.6% (20/30) screened were negative for *Blastocystis* sp. Furthermore, the most common form observed was the vacuolar form, with large, spherically shaped cells containing a central body resembling a large vacuole. This was the predominant cell type seen in the samples. The most common reproductive form was binary division, in which the cytoplasm of the mother cell partitions, resulting in two daughter cells. There was a statistically significant association between *Blastocystis* sp. infection and collection area (extensive and intensive) as well as biosecurity measures. However, no statistically significant association was found between the healthy status of the animal, deworming, and vaccination. To achieve more accurate and precise identification down to the subtype and species level, more sophisticated molecular identification techniques should be used

APPENDIX

Figure 4.0: Cloaca sampling using sterile cotton swab

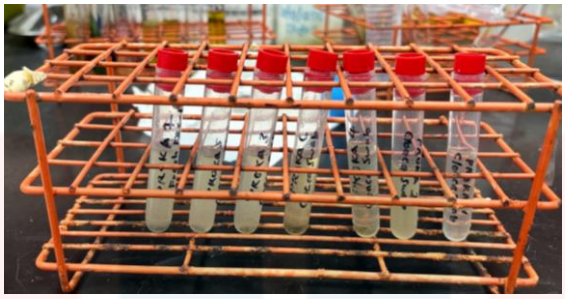

Figure 5.0: Cultured in Jones medium supplemented with 10% horse serum and incubated at 37°C for 24 hours

Figure 6.0: Preparation of direct fecal smear

Figure 7.0: Positive results were fixed with methanol and stained with 10% Giemsa

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