SHORT COMMUNICATION

MORPHOLOGICAL CHARACTERISTICS OF *BLASTOCYSTIS* SP. ISOLATED FROM PEAFOWL FROM A ZOOLOGICAL GARDEN IN PERAK, MALAYSIA

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ABSTRACT. Four peafowls (*Pavo cristatus*) from a private mini zoological garden in Perak were examined for *Blastocystis* by using *in vitro* cultivation. Positive cultures were then processed for light microscopy by Giemsa staining, scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Isolates of *Blastocystis* sp. obtained from peafowls were examined by light and electron microscopy to further understand the morphology of *Blastocystis*. Vacuolar forms were the most observed forms with the diameter ranging from 4 to 10 µm. Electron micrographs revealed smooth surface coats and tiny, unevenly distributed electron-dense particles in the large central vacuole. This is the first report on the occurrence and morphological characteristics of *Blastocystis* isolated from peafowls in Malaysia.

Keywords: Blastocystis, electron microscopy, in vitro culture, peafowl

INTRODUCTION

Blastocystis is an intestinal protist commonly found in humans and animals worldwide (Tan, 2008, Skotarczak, 2018). The name "*Blastocystis*" was proposed by Alexeieff in 1911, when he described the organism as a harmless gastrointestinal saprophytic yeast (Zierdt, 1991). *Blastocystis* has thereafter been described as the cyst form of a flagellate, an amoeba, and a sporozoan. However, *Blastocystis* has currently been identified as a protist belonging to the Stramenopiles branch of eukaryotes (Silberman *et al*., 1996, Ahmed *et al*., 2019).

In literature, several forms of this organism have been described: vacuolar, multivacuolar, avacuolar, granular, amoeboid, and cyst forms (Yamada & Yoshikawa, 2012, Padukone et al., 2018). Isolates of Blastocystis from different hosts are morphologically very similar (Beghini et al., 2017), thus differentiating between these isolates based on light microscopy has been impossible. Ultrastructural examination of *Blastocystis* sp. isolates from different hosts are needed to assemble consistent features for isolates of each host group thereby providing an information base for comparison and ultimately contributing to the biology of *Blastocystis*.

There are a lot of ultrastructural studies on *Blastocystis* sp. isolated from both symptomatic and asymptomatic human hosts (Dunn *et al*., 1989, Zhang *et al*., 2012, Ragavan *et al*., 2014, and Ahmed *et al*., 2019). Stenzel *et al*. (1994) have documented the morphology of *Blastocystis* sp. isolated from chickens, ducks, geese, and ostriches using transmission electron microscopy (TEM). Ultrastructure has also been described for *Blastocystis* sp. from chickens (Lee & Stenzel, 1999, Farah Haziqah *et al*., 2018) and ostriches (Chandrasekaran *et al*., 2014). This study uses light microscopy, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) to observe *Blastocystis* sp. from peafowl (*Pavo cristatus*).

MATERIALS AND METHODS

Study Animal

A total of four individual peafowls (*Pavo cristatus*) from a private mini zoological garden in Perak were screened for protozoan parasites. Sampling activity was conducted early in the morning in which fresh faecal samples were gathered from the ground instantly right after defecation. The samples were then put in screw cap collection bottles without adding up any type of preservative at all.

Screening of *Blastocystis* sp.

The collected samples were then cultured using Jones' medium supplemented with 10 % horse serum following the method of Siti Alawiyah *et al.* (2021). After 24 hours, the positive cultures were smeared and fixed in methanol and then stained with 10 % Giemsa solution to observe the detailed morphology at 400x and 1000x magnification using light microscopy.

Electron microscopy technique

Day-3 *Blastocystis* positive culture samples were fixed with 2.5 % glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.3). After that the samples were transported to be processed at the Electron Microscopy Unit in Institute of Medical Research, Kuala Lumpur.

For SEM, the contents of the samples were washed three times using phosphate buffered saline (PBS) pH 7.4. Then, the samples were centrifuged at 3000 rpm for 5 minutes. The pellet cells were fixed with 2.5 % glutaraldehyde and post-fixed with 1 % osmium tetroxide. The specimens were mounted on polycarbonate membrane (Nuclipore Agar Scientific, USA) and dehydrated in increasing concentration of ethanol (30 %, 50 %, 70 %, 80 %, 90 %, and 100 %). The specimens were critical–points dried with carbon dioxide coated with gold and examined with SEM (FEI-Quanta 200 FESEM, USA) (Ragavan *et al.,* 2014).

For TEM, the sample contents were washed three times using phosphate buffered saline (PBS) pH 7.4. The samples were then centrifuged at 3000 rpm for 5 minutes. The pelleted cells were re-suspended overnight in 2.5 % glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.3 at 4 °C, washed thoroughly with cacodylate buffer and post fixed for 30 min in 1 % osmium tetroxide in cacodylate buffer. The fixed cells were dehydrated for 5 minutes in an ascending series of ethanol (30 %, 50 %, 70 %, 80 %, 90 %, and 100 %) and embedded in epoxy resin. Semithin sections were stained with toluidine blue. Ultrathin sections were cut using an ultramicrotome, contrasted with uranyl acetate and lead citrate and later viewed using TEM (LEO Libra120) (Tan & Suresh, 2006).

RESULTS AND DISCUSSION

All four faecal samples of peafowl (*Pavo cristatus*) from a private mini zoological garden in Perak were positive (100 %) for *Blastocystis*. Among these samples, one was positive (25 %) for *Trichomonas* sp. All the animal hosts were asymptomatic in which none presented any gastrointestinal symptoms such as bloating or diarrhoea.

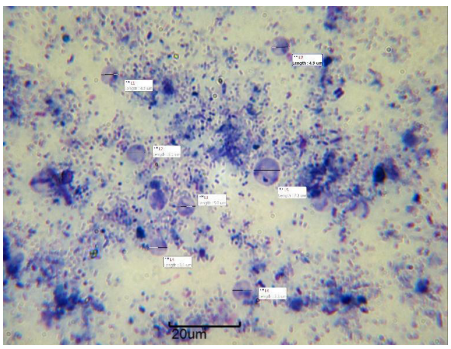

Figure 1. Giemsa-stained permanent smear of *Blastocystis* vacuolar forms from *in vitro* culture of peafowl faecal samples. The scale bar is 20 µm

Blastocystis is rather difficult to be identified by using wet mount preparation because of variation in size and shape. Based on the study of Sreekumar *et al.* (2014), permanent smears, particularly Giemsa stained, appear to be the appropriate procedure for light microscopic diagnosis of *Blastocystis* sp. Vacuolar form (Figure 1) was commonly seen in the culture medium of peafowl samples with one large central vacuole that occupies the cell space, limiting the cytoplasm and other components to a fine peripheral rim (Tan, 2008). The isolates from peafowl were morphologically similar to chicken isolates with the exception of variation in size. The size of the *Blastocystis* vacuolar forms encountered in peafowl sample was varied from 4 to 10 µm which is quite small compared to the size vacuolar form isolated from free-range chickens and barn-reared chickens that measure approximately 10 µm to 100 µm in diameter with the average diameter of cells between 20 to 30 µm (Farah Haziqah *et al.,* 2018). Variability of *Blastocystis* size could be influenced by several factors such as the host diet, as the composition of chicken feed ingredients was high in protein (Babiker *et al.,* 2009).

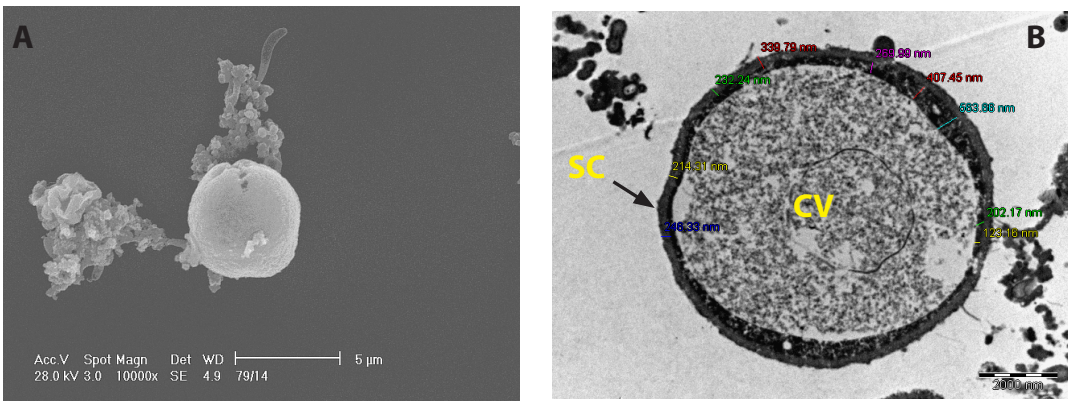

Figure 2. *Blastocystis* sp. isolated from peafowl **(A)** Surface structure of vacuolar form from *in vitro* culture **(B)** Transmission electron micrograph with peripheral thin rim of cytoplasm and large central vacuole. CV; central vacuole, SC; surface coat.

Ultrastructural variations within and between hosts have been reported by several authors in which differences in the surface morphology between *Blastocystis* isolates from fresh faecal samples of humans, monkeys, pigs, and chickens had been previously reported by Cassidy *et al*. (1994). Besides, variation in the structure and thickness of surface coat and in the appearance of central vacuole contents of *Blastocystis* cells from chicken were also described by Lee and Stenzel (1999). Some variation in the nucleus between *Blastocystis* isolates of cockroaches have also been found (Yoshikawa *et al*., 2007).

The ultrastructural report on poultry was previously reported by Stenzel *et al.* (1994) on *Blastocystis* isolated from chicken, duck, goose, and ostrich faecal samples whereas Farah Haziqah *et al.* (2018) reported on *Blastocystis* isolated from free-range chicken and barn-reared chicken. None was reported on the ultrastructure of *Blastocystis* isolated from peafowl. In this study, transmission electron micrographs found *Blastocystis* sp. isolated from peacock showed cells spherical in shape and containing a large central vacuole with tiny electron-dense particles, which were unevenly distributed with the average measurement of 230.96 µm. However, vacuolar cells from a laboratory culture from the free-range chicken isolate possessed a completely electron-lucent central vacuole whereas the isolates from barn-reared chickens showed an electron-opaque and fully distended central vacuole (Farah Haziqah *et al.,* 2018). Based on the study of Zierdt and Williams (1974), the electron dense material were granules in which probably acts as a form of energy storage for cell growth. As supported by Chandrasekaran *et al.* (2014), the fine granular or flocculent content observed distributed within the central body were lipid contents as indicated by the reaction with Sudan Black B staining. Meanwhile, scanning electron micrographs showed that the surface from the cultured form of peafowl *Blastocystis* isolates appeared similar with the surface of village chicken isolates (Farah Haziqah *et al.,* 2018). The cell surface was generally round in shape and had a smooth surface coat.

Most recently, Maloney *et al.* (2020) reported on two novel subtypes namely, ST27 and ST28 which were identified in two Indian peafowls from Uberlândia and Belo Horizonte, Brazil. From that study, data indicate that Indian peafowl may be the main host to these unique subtypes of *Blastocystis*. Therefore, further molecular examination needs to be undertaken to ascertain the subtypes of peafowl in Malaysia.

CONCLUSION

This present study is the first study that provides new insights into the morphological and ultrastructural characteristics of *Blastocystis* isolated from peafowl in Malaysia. It is suggested that there is insignificant morphological variation represented in the peafowl and chicken isolates, yet they might compose of genetically distinct subtypes. Therefore, these uncertain conclusions require morphological studies in larger numbers of peafowl, and confirmation by molecular techniques because birds may significantly contribute to the transmission of zoonotic *Blastocystis* subtypes as well as enzootic subtypes.

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