CHAPTER 4

ULTRASTRUCTURAL STUDIES ON THE CYSTIC AND VACUOLAR FORMS OF BLASTOCYSTIS SP. FROM ANIMAL ISOLATES
4.1 Introduction

Many authors have reported on the ultrastructural descriptions of vacuolar forms (Mehlhorn., 1988; Zaman et al., 1997a) and cystic forms (Zaman et al., 1995b; Moe et al., 1996; Khalifa., 1999) of human isolates. Zaman et al., (1997b) observed two layers of surface coat surrounding the vacuolar forms. He further described that in some cells, the attachment of the fibrillar material of the surface coat to bacteria may cause cytoplasmic damage to the bacterial cell. In contrast, Cassidy et al., (1994) have suggested that the surface coat protects the parasite against osmotic shock. Most cystic forms are surrounded by an irregular fibrillar layer with a thickness ranging from 50-100 nm with some parasites showing as many as three mitochondria (Zaman et al., 1995a). Besides this, other studies have reported the presence of prominent rough endoplasmic reticulum and nucleus containing chromatoid-like structures in some cystic forms (Moe et al., 1996; Khalifa., 1999).

Only a few studies have described the ultrastructural features of *Blastocystis* sp. in pigs (Cassidy et al., 1994), monkeys (Cassidy et al., 1994; Stenzel et al., 1997), chickens (Cassidy et al., 1994; Stenzel et al., 1997; Lee at al., 1999), ducks and geese (Stenzel et al., 1994), cockroaches (Yoshikawa et al., 2007) and circus animals comprising of camel, llama and highland bull (Stenzel et al., 1993). This could possibly be due to the limited numbers of cysts usually found in the faecal material of animal hosts. Till to date, most descriptions on either cysts or vacuolar forms of *Blastocystis* sp. isolated from animal hosts have been based on a limited range of animals. There has been no comprehensive study thus far to compare and contrast the vacuolar and cystic forms isolated from a wide range of animals within the scope of one study. This would
provide a better basis to compare the morphology of these life cycle stages from a wider range of animal groups. Therefore the present study attempts to describe the distinct ultrastructural characteristics of cystic and vacuolar forms from a range of animals which includes peacock, orang utan, ostrich, pig, cow, goat and sheep. This will help in source tracking when cysts are detected in water sources especially when an unknown Blastocystis sp. is found in the future as well as provide insights into the morphological diversity of these life cycle stages.

4.2 Materials and methods

Figure 4.1: Schematic representation of the overall methodology emphasizing on the ultrastructural analysis of Blastocystis sp. isolated from animals.

4.2.1 Source of Blastocystis sp. cyst

Faecal samples of seven animal species which included ostrich, cow, goat and sheep were collected from Infotermak Sg.Siput farm located at Ipoh, while faecal
samples of orang utan and peacock were collected from Bukit Merah Conservation Centre and pig samples were collected from a private farm in Ipoh. The present study, focused only on the 7 out of 9 animals found positive for Blastocystis sp. shown in the previous study (Chapter 3). Due to the limited number of cysts obtained from the fecal samples of gours and deer despite several attempts, these two groups of animals were omitted from the present study. For the peacock, orang utan, ostrich, pigs and cows, sampling were carried out from faecal droppings from the ground housing the respective animals whereas rectal swabs were made directly on the smaller ruminant animals such as goats and sheep. The fresh faecal samples from animals were stored in stool containers and processed soon after collection.

4.2.2 Laboratory testing

4.2.2.1 In-vitro cultivation of Blastocystis sp. isolates

Blastocystis sp. was isolated from the faecal samples of various animals by in-vitro cultivation using Jones’ medium supplemented with 10% heat-inactivated horse serum at 37°C. Subsequently after isolation, the parasites were maintained in Jones’ medium by consecutive sub-cultures every 2 to 3 days for at least one month prior to ultrastructural analysis (Suresh et al., 1994).

4.2.2.2 Isolation of Blastocystis sp. cysts

Fresh faecal samples were dissolved in normal saline and sieved into 15ml Falcon culture tubes. The culture tubes were centrifuged at 3000rpm for 10min. The sediment were layered on a 5ml of Ficoll-Paque solution in a culture tube and
centrifuged at 3500rpm for 20 minutes using Beckman Coulter 365303 Spinchron DLX centrifuge machine. The cystic stages, banded between 3 and 4cm located between third and fourth layer in the culture tube (Figure 4.1). These were pipetted into clean 14ml culture tubes. The cysts were washed twice by adding up to 14ml with phosphate buffer solution (PBS). The culture tubes were capped tight, inverted a few times and centrifuged at 3000rpm for 10 minutes for each washing. The supernatant was then discarded completely and 1ml of PBS solution was added into the culture tube containing the sediment. The cysts were fixed in 4% glutaraldehyde fixative and stored at -20°C prior to ultrastructural analysis.

![Figure 4.2: Ficoll-Paque concentration of the feacal samples.](image)

4.2.3 Microscopy

4.2.3.1 Light microscopy
All the 14 isolates were examined for *Blastocystis* sp. by wet preparation under light microscopy at 40x magnification. The cells of each isolate were pooled together from day 3 cultures to make a final concentration of $1 \times 10^6$ cells/ml in 3 ml screw-capped tubes containing Jones’ medium supplemented with 10% horse serum. All cultures were kept in airtight screw-capped tubes and incubated at 37°C. Fifty parasites were randomly chosen from the day 3 culture tube for size measurement. All experiments were done in triplicates.

### 4.2.3.2 Transmission electron microscopy

The cysts were extracted from the fresh faecal samples of animals using Ficoll-paque concentration technique. Subsequently, the parasites were cultured in 3ml Jones’ medium supplemented with 10% horse serum at 37°C. Each set consisted of cysts obtained from isolation after Ficoll-paque concentration and day 3 culture were prepared in triplicate. The parasites of each isolate were pooled together after Ficoll-paque concentration to make a final concentration of $1 \times 10^6$ parasites/ml. Similarly, the contents of day 3 culture were pooled together to make a final concentration of $1 \times 10^6$ parasites/ml. All samples were washed three times using phosphate buffered saline (PBS) pH 7.4 before subjecting them for the transmission electron microscopy analysis. The samples were centrifuged at 3000rpm 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 ascending series of ethanols (30%, 50%, 70%, 80%, 90% and 100%) and embedded in epoxy resin. Semi-thin sections were stained with toluidine blue. Ultrathin sections were cut, contrasted with uranyl acetate and lead citrate and viewed using a transmission electron microscope (LEO Libra120) (Tan et al., 2006).
4.3 Results

4.3.1 Light microscopic observation

4.3.1.1 Vacuolar form

Spherical vacuolar form with various sizes were commonly seen in the faecal culture of all seven animal species comprising of ostrich, pig, goat, orangutan, cow, peacock and sheep (Figure 4.3 A-G). The size of vacuolar form isolated from ostrich, pig, goat, orangutan, cow, peacock and sheep ranged from 9 to 42.9 µm, 4 to 35 µm, 6 to 30 µm, 5 to 25 µm, 10 to 55 µm, 3 to 15 µm and 5 to 28 µm respectively. Vacuolar forms of Blastocystis sp. with the largest size range were seen in the isolate from the cow whilst the smallest size range was seen in the peacock isolate (Table 4.1).
Figure 4.3: Vacuolar form of *Blastocystis* sp. from faecal cultures of A.) ostrich, B.) pig, C.) goat, D.) orang utan, E.) cow, F.) peacock and G.) sheep seen under light
microscopy at 40x magnification. Various sizes of vacuolar forms (arrow) were observed.

**Table 4.1** Vacuolar forms (VF) of *Blastocystis* sp. from animal isolates (light microscopy)

<table>
<thead>
<tr>
<th>Animals</th>
<th>Ostrich</th>
<th>Pig</th>
<th>Goat</th>
<th>Orangutan</th>
<th>Cow</th>
<th>Peacock</th>
<th>Sheep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (Size range) (µm)</td>
<td>28.4 (9 - 42.9)</td>
<td>18.6 (4 – 35)</td>
<td>13.9 (6 – 30)</td>
<td>13.2 (5 – 25)</td>
<td>30.2 (10 – 55)</td>
<td>9 (3 – 15)</td>
<td>15.7 (5 – 28)</td>
</tr>
<tr>
<td>Shape</td>
<td>Spherical</td>
<td>Spherical</td>
<td>Spherical</td>
<td>Spherical</td>
<td>Spherical</td>
<td>Spherical</td>
<td>Spherical</td>
</tr>
</tbody>
</table>

### 4.3.1.2 Cystic form

Rounded cystic form were commonly seen in fresh faecal samples from all seven animal species comprising of ostrich, pig, goat, orang utan, cow, peacock and sheep (Figure 4.4 A-G). The mean diameter of cystic forms isolated from ostrich, pig, goat, orang utan, cow, peacock and sheep ranged from 3.5 to 6.8µm, 3.3 to 6.3µm, 2.5 to 5.5µm, 3.5 to 6.6µm, 4.5 to 7.1µm, 2.0 to 4.5µm and 3.0 to 5.8µm respectively. Cystic forms of *Blastocystis* sp. with the largest size range were seen in the faecal samples from the cow and smallest size range in the peacock isolate. Table 4.2 shows the description of cystic forms of *Blastocystis* sp. from animal isolates.
Figure 4.4: Cystic form of Blastocystis sp. from fresh faeces of A.) ostrich, B.) pig, C.) goat, D.) orang utan, E.) cow, F.) peacock and G.) sheep seen under light microscopy at 40x magnification. Various sizes of cystic forms (arrow) were observed.
Table 4.2: Cystic forms (CF) of *Blastocystis* sp. from animal isolates (light microscopy)

<table>
<thead>
<tr>
<th>Animals</th>
<th>Ostrich (µm)</th>
<th>Pig (µm)</th>
<th>Goat (µm)</th>
<th>Orangutan (µm)</th>
<th>Cow (µm)</th>
<th>Peacock (µm)</th>
<th>Sheep (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (Size range)</td>
<td>5.1 (3.5-6.8)</td>
<td>5 (3.3-6.3)</td>
<td>4.2 (2.5-5.5)</td>
<td>4.9 (3.5-6.6)</td>
<td>6 (4.5-7.1)</td>
<td>3.4 (2.0-4.5)</td>
<td>4.3 (3.0-5.8)</td>
</tr>
<tr>
<td>Shape</td>
<td>Spherical</td>
<td>Spherical</td>
<td>Spherical</td>
<td>Spherical</td>
<td>Spherical</td>
<td>Spherical</td>
<td>Spherical</td>
</tr>
</tbody>
</table>

4.3.2 Transmission electron microscope analysis

Cysts from the faecal samples of seven animals found positive for *Blastocystis* sp. was extracted from the faecal sample of the respective animals. The TEM figures shown are the representative of what were generally seen in each group of animals. Two animals were selected from each group of animals. TEM study showed distinct ultrastructural characteristics of cystic and vacuolar forms from the seven animals which includes the shape, the presence of prominent nucleus, mitochondrion-like-organelles (MLO), electron dense material and granules as well as the thickness of membrane.

4.3.2.1 Typical characteristics of vacuolar form of *Blastocystis* sp. from animal isolates based on transmission electron microscopic analysis

Vacuolar forms of *Blastocystis* sp. isolated from the ostrich culture were mostly irregular in shape with a high electron dense area observed in the central body. Prominent mitochondrion-like-organelle with cristae and nucleus were seen at the peripheral of cytoplasm with a thick fuzzy coat surrounding the parasite (Figure 4.5A).
Most of the vacuolar forms of *Blastocytis* sp. isolated from the pigs were spherical in shape with the high electron dense material in the central body and a prominent nucleus observed at one end of the peripheral cytoplasm in most of the cells (Figure 4.5B). *Blastocytis* sp. vacuolar forms isolated from the goat were spherical in shape with some showing central body filled with electron-dense granules. Prominent nucleus was observed in the cytoplasm (Figure 4.5C). Vacuolar forms of *Blastocytis* sp. isolated from the orang utan were mostly irregular in shape with less electron dense material in the central body. There was a prominent thick fuzzy coat surrounding the parasite with the presence of MLO showing prominent cristae (Figure 4.5D). Spherical vacuolar forms of *Blastocytis* sp. isolated from the cow show a thin fuzzy coat surrounding the parasite with high electron dense material observed within the central body (Figure 4.5E). Vacuolar forms from the peacock culture were mostly irregular in shape with numerous granules in the central vacuole. Thick and intact fuzzy coat was often seen surrounding the parasite (Figure 4.5F). Most of the vacuolar forms from the sheep isolate were irregular in shape with a prominent thick fuzzy coat surrounding the parasite. High electron dense material were seen to surrounding the central body containing the electron dense granules (Figure 4.5G). The membrane layer surrounding the vacuolar forms isolated from ostrich, pig, goat, orang utan, cow, peacock and sheep ranged from 235.48 to 345.22 nm, 227.99 to 258.22 nm, 214.89 to 232.71 nm, 110.45 to 142.50 nm, 77.18 to 119.33 nm, 118.72 to 167.99 nm and 104.84 to 157.47 nm respectively. The thickest membrane observed was seen in the parasites from the ostrich isolate whilst the thinnest in parasites from the cow isolate. Detailed description of vacuolar forms of animal isolates is shown (Table 4.3).
Figure 4.5A Irregular vacuolar form of *Blastocytis* sp. seen in the ostrich isolate based on TEM analysis. Note a thick fuzzy coat surrounding the parasite. A high electron dense area was observed in the central body (CB). Prominent mitochondrion-like-organelle (M) with cristae and nucleus (Nu) were seen at the peripheral of cytoplasm.

Figure 4.5B Spherical vacuolar form of *Blastocytis* sp. from the pig isolate based on TEM analysis. Central body (CB) contains high electron dense material with prominent nucleus (Nu) at one end of the peripheral cytoplasm.
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Figure 4.5C Spherical vacuolar form of *Blastocytis* sp. from the goat isolate based on TEM analysis. Central body (CB) is filled with electron-dense granules with prominent nucleus (Nu) at the end of the peripheral cytoplasm.

Figure 4.5D Irregular vacuolar form of *Blastocytis* sp. from the orang utan isolate based on TEM analysis. Central body (CB) contains less electron dense material with presence of MLO with cristae at one end of the peripheral cytoplasm. Thick fuzzy coat (FC) is seen surrounding the parasite.
Figure 4.5E Spherical vacuolar form of *Blastocystis* sp. from the cow isolate based on TEM analysis. Central body (CB) contains high electron dense material. Thin fuzzy coat (FC) is seen surrounding the parasite.

Figure 4.5F Irregular vacuolar form of *Blastocystis* sp. from the peacock isolate based on TEM analysis. Central body (CB) contains numerous granules and small vacuoles (V). Thick and intact fuzzy coat (FC) is surrounding the parasite.
Figure 4.5G Irregular vacuolar form of *Blastocystis* sp. from the sheep isolate based on TEM analysis. High electron dense area is seen surrounding the central body (CB) containing electron dense granules. Thick fuzzy coat (FC) is observed surrounding the parasite.
Table 4.3: Detailed description of vacuolar forms of animal isolates (Transmission electron microscopy analysis).

<table>
<thead>
<tr>
<th>Animals</th>
<th>Peacock</th>
<th>Pig</th>
<th>Cow</th>
<th>Sheep</th>
<th>Goat</th>
<th>Ostrich</th>
<th>Orang utan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane thickness mean (nm) (range)</td>
<td>139.49 (118.72-167.99)</td>
<td>236.45 (227.99-258.22)</td>
<td>96.42 (77.18-119.33)</td>
<td>134.70 (104.84-157.47)</td>
<td>218 (214.89-232.71)</td>
<td>302.47 (235.48-345.22)</td>
<td>131.37 (110.45-142.50)</td>
</tr>
<tr>
<td>Shape</td>
<td>Irregular</td>
<td>Spherical</td>
<td>Spherical</td>
<td>Irregular</td>
<td>Spherical</td>
<td>Irregular</td>
<td>Irregular</td>
</tr>
<tr>
<td>Presence of nucleus</td>
<td>Absent</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
<td>Present</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Presence of MLO</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Electron dense material</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>Less</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
4.3.2.2 Typical characteristics of cystic forms of *Blastocystis* sp. from animal isolates based on transmission electron microscopic analysis

Cystic forms from ostrich isolates were spherical in shape with high electron dense observed in the central body and numerous prominent small vacuoles were seen within the parasite (Figure 4.6A). Cystic forms of pig isolates were spherical in shape with a central body which is less dense. Prominent nucleus was observed at the peripheral cytoplasm with a thick fuzzy coat surrounding the cyst (Figure 4.6B). Cystic forms of goat isolates were spherical in shape with the central body containing less electron dense material with prominent small vacuoles (Figure 4.6C). Spherical cystic forms of orangutan isolates have been shown to be surrounded by a thick fuzzy coat surrounding the central body which contained high electron dense materials. Irregular and elongated form of MLO with prominent cristae was also seen in the cytoplasm (Figure 4.6D). Cysts from the fresh faeces of the cow were spherical in shape with high electron dense area and numerous granules observed in the central body (Figure 4.6E). Cystic forms from peacock isolates were spherical in shape with thick fuzzy coat surrounding the cyst. Less electron dense material were observed in the central body with more than two prominent nuclei seen in the cytoplasm (Figure 4.6F). Cystic forms from sheep isolates were spherical in shape with thick fuzzy coat surrounding the cyst. Prominent nucleus was observed at the end of the peripheral cytoplasm with less electron dense material in the central body. (Figure 4.6G). The cyst wall layer of the cystic forms isolated from ostrich, pig, goat, orangutan, cow, peacock and sheep ranged from 298.15 to 365.45 nm, 274.19 to 292.58 nm, 136.23 to 183.97 nm, 125.56 to 152.92 nm, 97.89 to 141.15 nm, 150.22 to 180.72 nm and 130.44 to 178.47 nm respectively. The thickest cyst wall was observed in the ostrich isolate and the thinnest in the cow isolate. Detailed description of cystic forms of animal isolates is shown in Table 4.4.
Figure 4.6A Cyst form from the ostrich isolate based on TEM analysis. Small vacuoles (V) were seen within the parasite. High electron dense area was observed in the central body (CB).

Figure 4.6B Cyst form from the pig isolate based on TEM analysis. Presence of thick and prominent fuzzy coat (FC) surrounding the parasite. Central body (CB) contains less electron dense material with presence of nucleus (Nu) at one end of the peripheral cytoplasm.
Figure 4.6C Cyst form from the goat isolate based on TEM analysis. Presence of thin fuzzy coat (FC) surrounding the parasite. Central body (CB) contains less electron dense material with presence of small vacuoles (V).

Figure 4.6D Spherical cystic forms of orangutan isolates are surrounded by a thick fuzzy coat (FC) and central body (CB) contains high electron dense materials based on TEM analysis. Irregular and elongated form of MLO (M) with prominent cristae was also seen in the cytoplasm.
Figure 4.6E Cyst form from the cow isolate based on TEM analysis. Central body (CB) contains high electron dense granules.

Figure 4.6F Cyst form from the peacock isolate based on TEM analysis. Central body (CB) contains less electron dense material with more than two prominent nuclei (Nu) at the end of the peripheral cytoplasm. Thick fuzzy coat (FC) is seen surrounding the parasite.
Figure 4.6G Cyst form from the sheep isolate based on TEM analysis. Central body (CB) contains less electron dense material with prominent nucleus (Nu) at the end of the peripheral cytoplasm. Thick fuzzy coat (FC) is seen surrounding the cyst.
Table 4.4: Detailed description of cystic forms of animal isolates (Transmission electron microscopy analysis).

<table>
<thead>
<tr>
<th>Animals</th>
<th>Peacock</th>
<th>Pig</th>
<th>Cow</th>
<th>Sheep</th>
<th>Goat</th>
<th>Ostrich</th>
<th>Orang utan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyst wall thickness mean (nm) (range)</td>
<td>165.82 (150.22-180.72)</td>
<td>283.28 (274.19-292.58)</td>
<td>115.22 (97.89-141.15)</td>
<td>148.93 (130.44-178.47)</td>
<td>161.59 (136.23-183.97)</td>
<td>328.98 (298.15-365.45)</td>
<td>136.98 (125.56-152.92)</td>
</tr>
<tr>
<td>Shape</td>
<td>Spherical</td>
<td>Spherical</td>
<td>Spherical</td>
<td>Spherical</td>
<td>Spherical</td>
<td>Spherical</td>
<td>Spherical</td>
</tr>
<tr>
<td>Presence of nucleus</td>
<td>Present</td>
<td>Present</td>
<td>Absent</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Presence of MLO</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Electron dense material</td>
<td>Less</td>
<td>Less</td>
<td>High</td>
<td>High</td>
<td>Less</td>
<td>Less</td>
<td>High</td>
</tr>
</tbody>
</table>
4.4 Discussion and conclusion

Based on the light microscopy, the diameter range of vacuolar forms from the animal isolates were comparatively smaller than human isolates which usually range from 2 to 200 μm (Stenzel & Boreham, 1996; Zierdt, 1991). The cystic forms of the animal isolates were comparatively larger than the human isolate which range from 3 to 6 μm (Zaman et al., 1995b). In the present study, ultrastructural findings on Blastocystis sp. of animal isolates concurred with the previous studies (Tan, 2004; Yoshikawa et al., 2007). Most of the Blastocystis sp. appeared to be rounded or slightly irregular in shape with a thick and compact surface coat seen to be surrounding the cell. One or more nuclei were seen in most of the cystic and vacuolar forms of the animal hosts.

Studies previously have shown that vacuolar forms of Blastocystis sp. isolated from the ostriches are consistently small with a diameter of 6-10μm (Stenzel et al., 1994). However, in the present study, the size range of the vacuolar forms ranged from 9 - 42.9 μm which is remarkably larger, the finding of which contradicts with the previous finding. This could be due to the culture conditions where it has been reported previously that culture medium is capable of altering the general appearance of the organism (Stenzel et al., 1991). In the present study, the cyst wall of ostrich cyst was seen to be the thickest. Similar findings were also reported by Stenzel et al., (1994) where they showed that the ostrich isolate had the thickest surface coat relative to the cell diameter when compared to the other avian hosts. These findings imply that greater resistances of the cystic forms isolated from non-human hosts do have the ability to cause infection in other animal species and human. Besides that, the cell organelles such as MLO (mitochondria-like organism) and nucleus were very prominent with high
electron dense material often seen occupying the central vacuole of the ostrich isolate in the present study.

Cystic forms of Blastocystis sp. from the fresh faeces of pig have been studied previously (Cassidy et al., 1994) however there was very little information on the morphology reported. They described only the surface thickness of the cyst based on transmission microscopy which ranged from 250 to 500nm. In the present study, the surface thickness of the cystic stage from pigs showed a thickness of 274.19 to 292.58nm. Both cystic and vacuolar forms isolated from the pig were mostly spherical in shape and the central body of most vacuolar forms showed high electron dense material occupying the central body of the vacuolar forms.

The present study is the first to describe the ultrastructure of Blastocystis sp. cyst isolated from orang utan which was shown to be mostly spherical and surrounded by a thick fuzzy coat with a high electron dense material occupying in the central body. A previous study highlighted that most cystic forms from monkeys were round, except some which appeared elongated and irregular in shape (Stenzel et al., 1996). In comparison, cysts from orang utan in the present study were smaller based on light microscopic observation than the cystic forms of monkeys which ranged from 12 to 15 µm in diameter (Stenzel et al., 1996). In contrast, the vacuolar forms from orang utan in the present study were comparatively larger than Blastocystis sp. from the monkey isolate which is usually small (approximately 6±10 µm in diameter). One very unique characteristic shown in the present study was the large, irregular and elongated form of MLO with prominent cristae in the cytoplasm than those seen in the vacuolar forms.
This finding concurred with the previous study seen in the monkey samples (Stenzel et al., 1996).

Despite previous studies that have reported on the prevalence of *Blastocystis* sp. in birds (Stenzel et al., 1994) such as peacock (Abe et al., 2002; Yoshikawa et al., 2004), there has been no study highlighting the morphological and ultrastructural features of *Blastocystis* sp. from the peacock isolate. In the present study, the smallest size range of *Blastocystis* sp was observed in the peacock isolate for both vacuolar and cystic forms. The vacuolar forms ranged from 3 to 15 µm compared to those isolated from other avian hosts which was previously reported to be from 3 to 120 µm (Lee & Stenzel., 1999; Stenzel et al., 1994). However, the cystic forms were similarly small with a range from 3 to 4 µm (Lee & Stenzel., 1999). In the present study, the central body of parasite isolated from the peacock contains numerous granules and small vacuoles in the vacuolar forms, a similar description seen in *Blastocystis* sp. isolated from the duck (Stenzel et al., 1994). Most cystic forms from the peacock showed multiple nuclei. A similar finding was reported in the domestic chicken (Lee & Stenzel 1999).

Several studies have reported the prevalence of *Blastocystis* sp. among the livestock animals such as cattle, goat and sheep (Quilez et al., 1995; Abe et al., 2002; Lee et al., 2012), however none have described the morphological and ultrastructural features of *Blastocystis* sp. isolated from these animals. In the present study, both vacuolar and cystic forms of *Blastocystis* sp. isolated from the cow showed the thinnest membrane when compared to the other animals in the study group. Meanwhile, cystic forms from sheep and goat isolates mostly showed less electron dense materials, however the cystic forms from the sheep isolate contain prominent nucleus with a thick fuzzy coat often surrounding the cells, while nucleus was not seen in most of the cysts from the goat and
were surrounded by a thin membrane. However, there has been no previous study carried out to describe the ultrastructural features of *Blastocystis* sp. isolated from the livestock animals such as cow, sheep and goat.

In conclusion, the present study is the first to show distinct ultrastructural characteristics of cystic and vacuolar forms from a range of animals. Most studies confine the same attempt within one or two animal groups but this will not give an opportunity to compare and appreciate the morphological diversity that exists between various animal groups. Based on the description and the size range of cystic and vacuolar forms from a range of animals, it was possible to develop a schematic diagram to demonstrate major differences in the morphology of the vacuolar (Figure 4.7) and cystic (Figure 4.8) forms especially from these seven animals described in the schematic representation below in order to trace the source of the cysts when seen in aquatic sources. A simple guideline is included below to assist researchers in using the diagram below for source tracking.

1.) Vacuolar forms:

   a) Samples should be subjected for both light and electron microscopy studies.
   b) The sizes should be noted and based on the size eg: if more than 30µm, then it is possible that the parasite could belong to pig, cow and ostrich.
   c) Based on TEM analysis, if the membrane thickness of the parasite is lesser than 200nm and the shape of the parasite is spherical, the parasite could belong to cow.
   d) If the parasite does not have a prominent nucleus, then it could belong to cow.
e) If the parasite had no prominent mitochondria-like organism (MLO), then it could belong to cow.

f) If the central vacuole contain high electron dense material, the parasite can be confirmed as cow isolate.

2.) Cystic forms:-

g) Samples should be subjected for both light and electron microscopy studies.

h) The sizes should be noted and based on the size eg: if lesser than 6µm, then it is possible that the cyst could belong to peacock, sheep and goat.

i) Based on TEM analysis, if cyst wall of the cyst is lesser than 200nm and the shape of the cyst is spherical, the cyst could belong to peacock, sheep or goat.

j) If the cyst does not have a prominent nucleus, the cyst could belong to goat.

k) If the cyst had no prominent mitochondria-like organism (MLO), the cyst could belong to goat.

l) If the central vacuole contain less electron dense material, the cyst can be confirmed as goat isolate.
Figure 4.7 Schematic representation of the major differences in the morphology of the vacuolar forms of *Blastocystis* sp.
Figure 4.8 Schematic representation of the major differences in the morphology of the cystic forms of Blastocystis sp.