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Short Communication

Current status of *Blastocystis* in cockroaches

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Abstract. There are few reports on *Blastocystis* spp. infections in invertebrate hosts namely, cockroaches. Due to their close proximity to humans especially to their dwellings prompted this study as these organisms could possibly play a role in human transmission. A total of 151 cockroaches consisted predominantly of nymph and adult stages were captured from several types of dwellings in the state of Perak and Selangor, Malaysia. Approximately half (40.4%) of the cockroach intestinal contents screened were positive and were found associated to two main factors, host-stage and types of dwellings. The granular and vacuolated forms were the most common cell form found in the *in vitro* cultures and were morphologically similar to *B. hominis*. However, the surface coat observed was thick with an electron lucent area observed in the central vacuole. The isolates grew in room temperature but optimal growth was observed at a 24°C similar to the reptilian *Blastocystis* with a high number of cells were recovered. Using the DNA barcoding method, two isolates were identified as ST3 (allele 56), one isolate was consider as the new subtype with close relation to allele 114.

Cockroaches are common house pest throughout the tropics. These arthropods are notorious mechanical vectors to many diseases and have worldwide distribution, infesting many types of dwelling. It is believed that cockroaches may be a reservoir to a range of pathogens such as *Staphylococcus* and *Streptococcus* and *Blastocystis*. There are only few reports on the isolation of *Blastocystis* from cockroaches. The occurrence of *Blastocystis* in the American cockroach (*Periplaneta americana*) was reported in Singapore with high infection in cockroaches caught from sewage tanks (Zaman *et al.*, 1993). However, low occurrence of *Blastocystis* was reported in cockroaches from urban human dwellings in Malaysia (Suresh *et al.*, 1997). After a gap of almost a decade, Yoshikawa *et al.* (2007) studied the ultrastructure by electron microscope and the SSU rRNA genes were sequenced. The present study is the first to screen *Blastocystis* in cockroaches recovered from various types of dwellings as well as isolate subtype characterization using a vital new tool; DNA barcoding methods.

Cockroaches were captured from urban dwellings and structures namely, drainage system, residential homes and grocery stores from two urban locations; Ipoh, Perak and Puchong, Selangor. The cockroaches were trapped using sticky traps and/or barehanded technique. Each specimen was transferred

into a sterile container, transported to the laboratory and anaesthetised at 4°C for 10 min. The host-stage for each specimen was noted prior to dissection. Dissection was carried out under sterile conditions in which the sides of the abdomen were cut on either side of the anus and the complete gut were removed posteriorly. The contents were then removed and stored in a container prior to the *in vitro* culture method using Jones' medium supplemented with 10% heat-activated horse serum (Suresh & Smith, 2004). The contents of the culture were examined after 24 hours incubation at both room temperature and 37°C. The positive isolates were stained with 10% Giemsa stain and the ultrastructure studied using transmission electron microscopy as previously described (Chandrasekaran *et al.*, 2014).

Genomic DNA was then extracted with a Qiagen stool extraction kit following the manufacturer's instructions. A *Blastocystis*-specific primer, BhRDr (GAGCTTTTTAAC TGCAACAACG; Scicluna *et al.*, 2006) was paired with eukaryote-specific primer, RD5 (ATCTGGTTGATCCTGCCAGT; Clark, 1997) and used, in a single step PCR reaction, to amplify a 600 bp region of 18S rRNA. The PCR was performed in a 25 µl volume containing 1.0 mM of dNTPs, 0.5 mM of each primer, 1 x PCR buffer, 2.5 mM MgCl, 1 U Taq DNA Polymerase (recombinant) (FERMENTAS, USA) and 5 µl of genomic DNA. PCR conditions consisted of an initial denaturing step of 94°C for 1 minute, followed by 30 cycles of 94°C for 1 minute, 59°C for 1 minute and 72°C for 1 minute, followed by a final elongation step of 72°C at 2 minutes (Thermo Cycler Bio-Rad, USA). PCR products were visualized on a 1.5% agarose gel prior to purification and cycle sequencing by a local commercial company. Sequencing data were checked using Seq Scanner 2 software (Applied Biosystems) for quality and subsequently were edited to remove low quality bases and primer sequences using BioEdit software (http://www.mbio.ncsu.edu/bioedit/bioedit.html). Next, the edited sequences were queried against the *Blastocystis* 18S rRNA database (http://www.publmst.org/blastocystis) (Roberts *et al.*, 2013).

Statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS) 21.0 software package. Chi-square analysis carried out to determine whether infections were associated to either extrinsic or intrinsic factors. A probability value of less than 0.05 was considered statistically significant.

This study records moderate infection (40.4%, n=115) in these invertebrates compared to previous studies. Statistical analyses found significant association between infection with host-stage ($\chi 2$ = 21.877, [df] = 1, P = 0.000), with higher infections in nymphs 58.2% (46/79) compared to adults 20.8% (15/72). Typically, nymphs are similar to adults, except for the absence of the wings, genitalia and also body colouring. Nymphs also differed in their feeding behaviour. According to Richter & Barwolf (1994), nymphs of *P. americana* took larger meals during the first three days post-moult. This behaviour suggests that nymphs were highly exposed to infection while foraging for food.

Blastocystis infections were also found associated to types of dwellings. High infections were noted for cockroaches captured from grocery stores compared to residential homes and drainage system. The abundance of starchy foods, sweet substances and meat product attracted the arthopods to come out from hiding such as sewers and toilets to forage in these dwellings. Zaman *et al.* (1993) reported infection incidences up to 80% in cockroaches and attributed the infections to type of habitats i.e. sewage tanks.

The *in vitro* cultures of cockroach isolates contained mostly vacuolar forms ranging in size from 7 to 14 µm (Fig. 1A). On occasion, granular forms were also observed measuring between 9 to 20 µm in which the granules were prominent and refractile (Fig. 1B). It was also noted that the isolates grew at both 25°C and 37°C. However, cell growth was optimal at room temperature with higher number of cells recovered compared to isolates incubated at 37°C. As previously

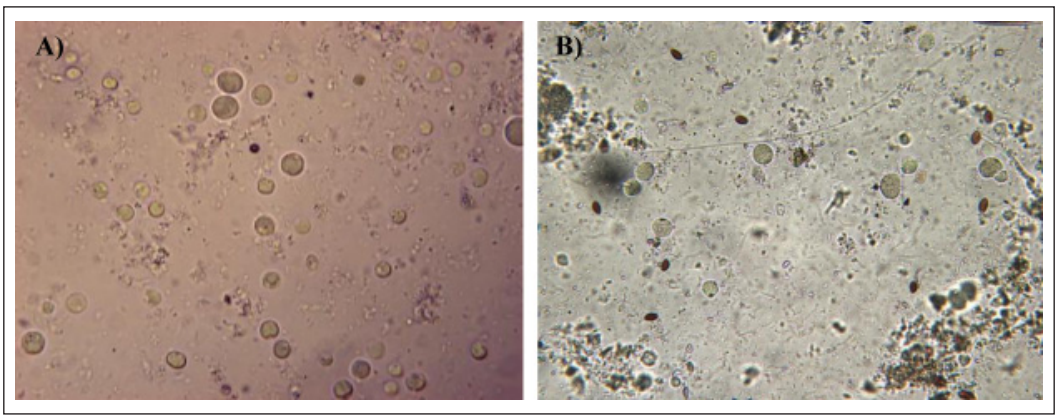

Figure 1. *Blastocystis* isolated from cockroach. A) Vacuolar form and B) Granular form.

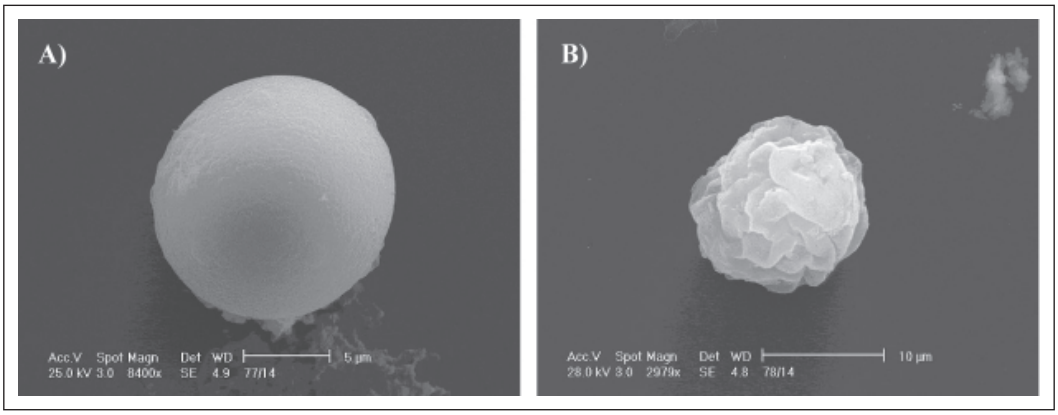

Figure 2. Surface structure of *Blastocystis* from cockroach. (a) Smooth surface (b) Coarse and folded surface.

described, some *Blastocystis* organisms in poikilothermal animals may have originated from homoiothermal hosts (Yoshikawa *et al.*, 2004).

Scanning electron images showed that majority of the cells possessed a smooth surface (Fig. 2A) similar to asymptomatic human isolates whereas, some cells showed coarse and folded surface (Fig. 2B) as seen in IBS isolates (Ragavan *et al.*, 2014). Meanwhile, transmission electron micrograph showed high electron dense material was observed in the central vacuole (Fig. 3A) indicating high lipid storage. According to Zierdt & Williams (1974), the highly electron dense material were granules to form the granular form and suggested that the central body probably acts as a form of energy storage for cell growth. Besides, cockroach isolates showed thicker cell membrane measuring between 214.31 to 248.03 nm (Fig. 3B). It is high likely that this surface could be sticky and may influence in the adherence of *Blastocystis*. In human, the thicker surface coat shown by the ultrastructural study in IBS isolates could influence cytopathic effect of *Blastocystis* towards the intestinal lining of the gut (Ragavan *et al.*, 2014).

Yoshikawa *et al.* (2007) demonstrated that SSU rRNA sequences of four *Blastocystis* isolates from cockroaches grouped together to record a new clade that branched early within the *Blastocystis* lineage. While amphibian and reptilian isolates were separately located at different positions with

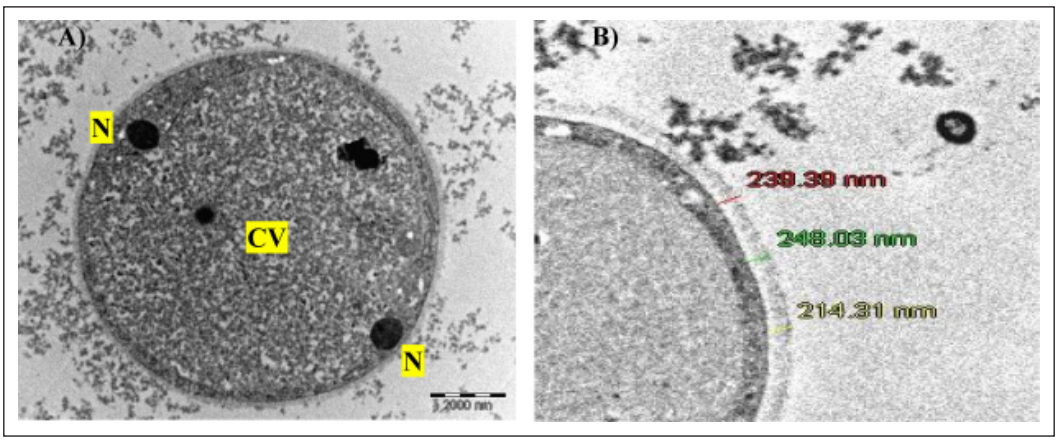

Figure 3. Electron micrograph of *Blastocystis* from cockroach. (a) Central vacuole with high electron dense materials. (b) Measurement of the surface coat. *Abbreviations: CV*, central vacuole; *N*, Nucleus.

an amphibian/reptilian clade (clade VIII) emerging immediately after the divergence of the cockroach clade (clade X) (Yoshikawa *et al.*, 2007). However, using the DNA barcoding method, two isolates were identified as ST3 which was never reported prior to this study in this species. ST3 is the most common subtype in humans exclusively in patients with IBS (Alfellani *et al.*, 2013; Ramírez *et al.*, 2014) and in other animal hosts such as non-human primates (Pertrášová *et al.*, 2011), giraffe (Alfellani *et al.*, 2013), goats (Tan *et al.*, 2013). With representation among humans, non-primates, insects and artiodactyls, ST3 clearly has a very wide host range. In addition, this study also records one isolate to be most likely a new subtype closely related to allele 114.

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REFERENCES

Alfellani, M.A., Jacob, A.S., Perea, N.O., Krecek, R.C., Taner-Mulla, D., Verweij, J.J., Levecke, B., Tannich, E., Clark, C.G. & Stensvold, C.R. (2013). Diversity and distribution of *Blastocystis* sp. subtypes in non-human primates. *Parasitology* **140**(8): 966-971.

Chandrasekaran, H., Govind, S.K., Panchadcharam, C., Bathmanaban, P., Raman, K. & Thergarajan, G. (2014). High lipid storage in vacoular forms of subtype 6 *Blastocystis* sp. in ostrich. *Parasites and Vectors* **7**: 469-475.

Clark, C.G. (1997). Extensive genetic diversity in *Blastocystis hominis*. *Molecular Biochemical Parasitology* **87**: 79-83.

Petrášová, J., Uzlíková, M., Kostka, M., Petřzelková, K.J., Huffman, M.A. & Modrý, D. (2011). Diversity and host specicity of *Blastocystis* in syntopic primates on Rubondo Island, Tanzania. *International Journal of Parasitology* **41**: 1113-1120.

Ragavan, N.D., Suresh, K.G., Tan, T.C. & Mahadeva, S. (2014). Phenotypic variation in *Blastocystis* sp. ST3. *Parasites and Vectors* **7**: 404-413.

Ramírez, J.D., Sánchez, L.V., Bautista, D.C., Corredor, A.F., Flórez, A.C. & Stensvold, C.R. (2014). *Blastocystis* subtypes detected in humans and animals from Colombia. *Infection, Genetics and Evolution* **22**: 223-228.

Richter, K. & Barwolf, D. (1994). Behavioral-changes are related to molt regulation in the cockroach, *Periplaneta americana*. *Physiological entomology* **19**(2): 133-138.

Roberts, T., Stark, D., Harkness, J. & Ellis, J. (2013). Subtype distribution of *Blastocystis* isolates from a variety of animals from New South Wales, Australia. *Veterinary Parasitology* **196**: 85-89.

Scicluna, S.M., Tawari, B. & Clark, G. (2006). DNA barcoding of *Blastocystis*. *Protist* **157**: 77-85.

Suresh, K., Mark, J.W., Chuong, L.S., Ragunathan, T. & Init, I. (1997). Sac-like pouches in *Blastocystis* from the house lizards (*Cosymbotus platyurus*). *Parasitology Research* **83**: 523-525.

Suresh, K. & Smith, H. (2004). Comparison of methods for detecting *Blastocystis hominis*. *European Journal of Clinical Microbiology and Infection Diseases* **23**: 509-511.

Tan, T.C., Tan, P.C., Sharma, R., Sugnaseelan, S. & Suresh, K.G. (2013). Genetic diversity of caprine *Blastocystis* from Peninsular Malaysia. *Parasitology Research* **112**(1): 85-89.

Yoshikawa, H., Morimoto, K., Nagashima, M. & Miyamoto, N. (2004). A survey of *Blastocystis* infection in anuran and urodele amphibians. *Veterinary Parasitology* **122**: 91-102.

Yoshikawa, H., Wu, Z., Howe, J., Hashimoto, T., Ng, G.C. & Tan, K.S.W. (2007). Ultrastructural and phylogenetic studies on *Blastocystis* isolates from cockroaches. *Journal of Eukaryotic Microbiology* **54**(1): 33-37.

Zaman, V., Ng, G.C., Suresh, K., Yap, E.H. & Singh, S. (1993). Isolation of *Blastocystis* from the cockroach (Dictyoptera: Blattidae). *Parasitology Research* **79**: 73-74.

Zierdt, C.H. & William, R.L. (1974). *Blastocystis hominis*: Axenic cultivation. Experimental *Parasitology* **36**: 233-243.