Tropical Biomedicine 35(2): 501–510 (2018)

Impact of pH on the viability and morphology of *Blastocystis* isolates

Farah Haziqah, M.T.[1,2], Chandrawathani, P.[3], Douadi, B.[1], Suresh, K.[4], Wilson, J.J.[5], Mohd Khalid, M.K.N.[6], Rajamanikam, A.[4], Lewis, J.W.[7] and Mohd Zain, S.N.[1*]

[1]Institute of Biological Sciences, Faculty of Sciences, University of Malaya, 50603 Kuala Lumpur, Malaysia
[2]School of Biological Sciences, Universiti Sains Malaysia, 11800 Pulalu Pinang, Malaysia
[3]Disease Control Division, Ministry of Health, Level 3 Block E 10, Complex E, Precint 1, Federal Govenment Administrative Centre, 62590 Putrajaya, Malaysia
[4]Department of Parasitology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia
[5]International College Beijing, China Agricultural University, Beijing, 100083, P. R. China
[6]Molecular Diagnostics and Protein Unit, Specialised Diagnostics Centre, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia
[7]School of Biological Sciences, Royal Hollaway, University of London, Egham, Surrey, TW20 OEX, UK
[*]Corresponding author e-mail: nsheena@um.edu.my
Received 24 August 2017; received in revised form 27 November 2017; accepted 1 December 2017

Abstract. *Blastocystis* sp. is ubiquitous in avian, mammalian and human hosts and propagates in either neutral or slightly alkaline conditions within the host's gastro-intestinal tract. Of the few previous studies on this enteric protozoan parasite in feline and canine hosts, prevalence values have been shown to range between 0 to 70.8%. In view of the close association between humans, and canine and feline hosts as companion animals, faecal samples of 180 *Felis catus* and 82 *Canis lupus*, collected from Penang and Kuala Lumpur, Malaysia, were initially screened by *in vitro* cultivation followed by molecular characterization. No positive isolates were identified in culture but in 12 feline samples DNA barcoding detected a zoonotic subtype *Blastocystis* ST1 for the first time. Consequently, avian and human isolates, which had previously been successfully cultured, were used to investigate the impact of pH on the viability and morphology of *Blastocystis* sp. The use of Trypan blue showed that the number of viable cells increased when exposed to pH 4 and a significant increase in viability occurred in pH values of 5 to 7. Development of *Blastocystis* cells in both isolates was suppressed in media less than pH 5 followed by the disappearance of viable cells from avian isolates in more acidic media below pH 4. Morphologically at pH 4 cells from avian isolates were less rounded, and with wrinkled / shrunken surfaces, than the more normal rounded cells from human isolates. On the other hand, at values below pH 3, no viable cells in human isolates were visible. The present findings therefore confirm that gastro-intestinal pH is an important determinant of *Blastocystis* viability and consequently influences the epidemiology of infection within avian, mammalian and human hosts.

INTRODUCTION

The genus *Blastocystis* comprises a group of single-celled parasites belonging to the Stramenopiles which inhabit the gastro-intestinal tract of vertebrate and human hosts (Li *et al.*, 2007; Leelayoova *et al.*, 2008; Ithoi *et al.*, 2011) and especially domesticated mammals (Parkar *et al.*, 2010; Roberts *et al.*, 2013; Hemalatha *et al.*, 2014), rodents (Lavier, 1952; Chen *et al.*, 1997), birds (Belova and Kostenko, 1990; Belova, 1991; Belova, 1992; Chandrasekaran *et al.*, 2014), amphibians (Yoshikawa *et al.*, 2004), and reptiles (Teow *et al.*, 1991; Singh *et al.*, 1996). Human and non-human *Blastocystis* isolates are morphologically similar and can be vacuolar (Stenzel and Boreham, 1996), granular (Tan, 2004), cyst (Tan, 2008) or amoeboid (Tan and Suresh, 2006). Human transmission involves the cyst form and occurs via the faecal-oral route. *Blastocystis*

species have low host specificity with possible zoonotic subtypes (Parkar *et al.*, 2010).

Knowles and Das Gupta (1924) reported the presence of *Blastocystis* sp. for the first time in Australian domestic cats, with low prevalences of 11.7% recorded in cats from the USA (Ruaux and Stang, 2014) and 10.0% in Egypt (Maysa, 2010) compared with a high prevalence of 67.3% in cats from Australia (Duda *et al.*, 1998). Duda *et al.* (1998) also reported a high prevalence 0.8% in dogs using a culture-based study, whereas Wang *et al.* (2013) detected only 2.5% using molecular methods. Prevalences in dogs overall appear to be on low ranging from 1.3% in Cambodia (Wang *et al.*, 2013), to 3.1% in Egypt (Awadallah and Salem, 2015), 3.4% in France (Osman *et al.*, 2015), 9.7% in the USA (Ruaux and Stang, 2014), but with higher prevalences of 24.0% in India (Wang *et al.*, 2013), 28.0% in Iran (Daryani *et al.*, 2008) and 37.0% in Colombia (Ramírez *et al.*, 2014).

These relatively low prevalences and in some instances the absence of infection in domesticated hosts from Germany (König and Müller, 1997), Malaysia (Chuong *et al.*, 1996; Suresh *et al.*, 1996) and Japan (Abe *et al.*, 2002), suggested that companion animals are unnatural reservoir hosts. Sutton (2004) suggested that the low gastric pH in the alimentary tract, together with high concentrations of enzyme secretions, provides an unsuitable environment for *Blastocystis* to reproduce. Therefore, the objectives of the present study are to: (1) to review all reported cases of *Blastocystis* infections worldwide; (2) to study the prevalence of subtypes of *Blastocystis* in local stray populations of cats and dogs and (3) to determine the impact of low pH in the stomach of these hosts, on the morphology and viability of *Blastocystis* isolates.

MATERIALS AND METHODS

Collection of faecal samples and *in vitro* cultivation

Fresh faecal samples of 180 cats (*Felis catus*) and 82 dogs (*Canis lupus*) were collected from several animal shelters located in Perak, Penang Island, and in the Federal Territory of Kuala Lumpur in which the animals were individually housed in cages. Faecal samples were stored in stool containers and processed within 12 hours after collection. All feline and canine hosts were handled according to protocols approved by the Institutional Animal Care and Use Committee (IACUC), University Malaya (Case No.: ISB/31/01/2013/SNMZ (R)).

Up to 2g of each faecal sample was inoculated into a sterile screw-top vial containing 3 ml of Jones medium supplemented with 10% heat-activated horse serum (Jones, 1946; Suresh and Smith, 2004) and incubated at 37ºC for 48 to 72 hours.

DNA barcoding

Genomic DNA from *Blastocystis* was extracted using the Qiagen stool extraction kit according to the manufacturer's instructions. All isolates were subjected to DNA barcoding as described by Mohd Zain *et al.* (2017). From results of the Blast analysis, subtypes were assigned against databases at www.pubmlst.org/blastocystis and the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST). *Blastocystis* subtypes were reported in ST format which is the consensus terminology reported by Stensvold *et al.* (2007b).

Viability experiments

Avian *Blastocystis* isolates were obtained from a village chicken *Gallus gallus* (A3) and a peafowl *Pavo cristatus* (M2) whereas the three human *Blastocystis* isolates (H1, H2 and H3) were obtained from patients at the University of Malaya Medical Centre (UMMC). Human ethical approval was obtained in accordance with University Malaya Medical Centre research policy (Reference No.: 2054-12181). Six media (Jones medium supplemented with 10% horse serum) were prepared with descending values of from pH 6, 5, 4, 3, 2, and 1 and adjusted by adding 01M HCl. A control medium with pH 7 were also prepared.

Approximately 1 x 10^6 cells of day 3 cultures of avian isolates A3 and M2 and 1 x

10^5 cells of human isolates (H1, H2 and H3) were inoculated into 3 ml of each of the six media plus the control medium. Culture tubes were prepared in triplicate for each medium and subsequently incubated at 37°C. After 24 hours, cells were counted in 0.5% Tryphan blue solution using a haemocytometer chamber (Improved Neubauer, Hausser Scientific) and evaluated for indicators of viability such as distinct size, shape and staining properties.

RESULTS

Prevalence of *Blastocystis*

Of thirteen previous studies on *Blastocystis* infections in cats and dogs worldwide (Table 1) up to seven zoonotic genotypes from both feline and canine hosts were identified including ST1, ST2, ST3, ST4, ST5, ST6 and ST10 (Wang *et al.*, 2013; Ruaux and Stang, 2014; Ramírez *et al.*, 2014; Osman *et al.*, 2015). More detailed morphological characteristics of *Blastocystis* sp. were described by Duda *et al.* (1998) in 70.8% infected dogs from Australia, whereby irregularly-shaped cells appeared to be vacuolar, with a thin outer rim of cytoplasm and barely discernable organelles surrounding a central vacuole. Duda *et al.* (1998) also showed by transmission electron microscopy that canine isolates of *Blastocystis* sp. appeared to be morphologically similar to cultured isolates of *B.hominis*. In short term cultures the cellular surface coat measured up to 3 µm thick and often with adhering bacteria. The nuclei were round or slightly elongate, measuring between 1- 2 µm, with the majority of nuclei contained within a crescentric band of opaque material.

In the present study *in vitro* cultures of fresh faecal samples from 180 cats (*Felis catus*) and 82 dogs (*Canis lupus*) tested negative for *Blastocystis* sp. On the other hand, DNA barcoding successfully detected 20% (n=12/60) positivity for *Blastocystis* sp. in cat faecal samples with sequences assigned to ST1 (allele 4). There were additional five faint PCR bands might indicate an extremely low infection.

Viability of *Blastocystis*

Figure 1 showed that the number of viable cells of *Blastocystis* increase when cultured in a less acidic medium. Cultured isolates of avian and human origin in media ranging from pH 5 to 7 show cells which are round and vacuolar. When exposed to a medium of pH 4, avian isolates exhibit cells which become wrinkled and shrunken although cells from human *Blastocystis* isolates remain unaffected. In pH values of less than 4 both avian and human isolates become suppressed and no viable cells were observed (Figure 2).

DISCUSSION

Global prevalences of *Blastocystis* infections in companion animals vary widely e.g. a high prevalence of *Blastocystis* was reported from an Australian culture-based study on dogs (Duda *et al.*, 1998), whereas more recent studies from the same location reported a marked lower prevalence using molecular methods (Wang *et al.*, 2013). Such variation appears to reflect the sensitivity of different screening methods (Stensvold *et al.*, 2007a) e.g. light microscopy not only appears to have the lowest sensitivity but is also prone to false positives compared with PCR-based methodology (Roberts *et al.*, 2011). This observation is largely based on *Blastocystis* sp. being pleomorphic with extensive variation in morphology (Wang *et al.*, 2013). Xenic *in vitro* cultures appeared to be highly sensitive compared with concentration or direct smear/light microscopic techniques (Leelayoova *et al.*, 2002). *Blastocystis* was also successfully cultured from two dog samples in an inspissated egg slant medium over a short growth period but these cultures did not materialize from cat faeces (Duda *et al.*, 1998). In the present study, xenic cultures of *Blastocystis* took place in the Jones' medium supplemented with 10% horse serum (Leelayoova *et al.*, 2002; Suresh and Smith, 2004; Stensvold *et al.*, 2007) but the cultivation of isolates did not occur in this medium. Instead a time-consuming DNA barcoding method, which sequenced a species-specific DNA fragment, successfully

Table 1. The prevalence (%) and zoonotic genotypes of *Blastocystis* sp. in canine and feline hosts worldwide

Host	Reference/Origin	Prevalence (%)	Morphological characteristics			Genotypes
			Light microscope	SEM	TEM	
Canine	Suresh *et al.*, 1996/Malaysia	– 0: Domestic dog	–	–	–	–
	Chuong *et al.* (1996)/Malaysia	– 0: Domestic dog	–	–	–	–
	König and Müller (1997)/Germany	– 0: Domestic dog	–	–	–	–
	Duda *et al.* (1998)/Australia	– 51/72 (70.8): Domestic dog	– The cells were irregular in shape. – Size: 3 to 10 µm in diameter (average 4.5 µm). – The cells appeared as the vacuolar form: a thin outer rim of cytoplasm, containing barely discernible organelles, surrounded a central vacuole of varying morphology.	–	– *Blastocystis* sp. from canine isolates appeared morphologically similar to cultured isolates of *B. hominis*. – In the shorter-term cultures, the cells had a thick surface coat up to 0.3 µm, often with adherent bacteria. – The nuclei were rounded or slightly elongated and were approximately 1 to 2 µm in diameter. – The nuclei commonly contained a crescentic band of electron opaque material and, sometimes, an additional 'spot' of electron opaque material.	–
	Abe *et al.* (2002)/Japan	– 0/54 (0): Pet dog	–	–	–	–
	Daryani *et al.* (2008)/Iran	– 14/50 (28.0): Domestic dog	–	–	–	–
	Wang *et al.* (2013)/Australia	– 2/80 (2.5): Pet and pound dog (Australia)	–	–	–	ST1
		– 1/80 (1.3): Semi-domesticated dog (Cambodia)	–	–	–	ST2
		– 19/80 (24.0): Stray dog (India)	–	–	–	ST 1, ST4, ST5 and ST6
	Ruaux and Stang (2014)/USA	– 10/103 (9.7): Shelter-resident canines	–	–	–	ST1 and ST10
	Ramírez *et al.* (2014)/Colombia	– 15/40 (37.0)	–	–	–	ST2
	Awadallah and Salem (2015)/Egypt	– 4/130 (3.07)	–	–	–	–
	Osman *et al.* (2015)/France	– 4/116 (3.4)	–	–	–	ST2 and ST10
Feline	Knowles and Gupta (1924) /India	Not mentioned	–	–	–	–
	Duda *et al.* (1998)/Australia	– 35/52 (67.3)	–	–	–	–
	Maysa (2010)/Egypt	– 5/50 (10.0)	–	–	–	–
	Ruaux and Stang (2014)/USA	– 12/103 (11.65): shelter-resident felines	–	–	–	ST1, ST3 and ST10

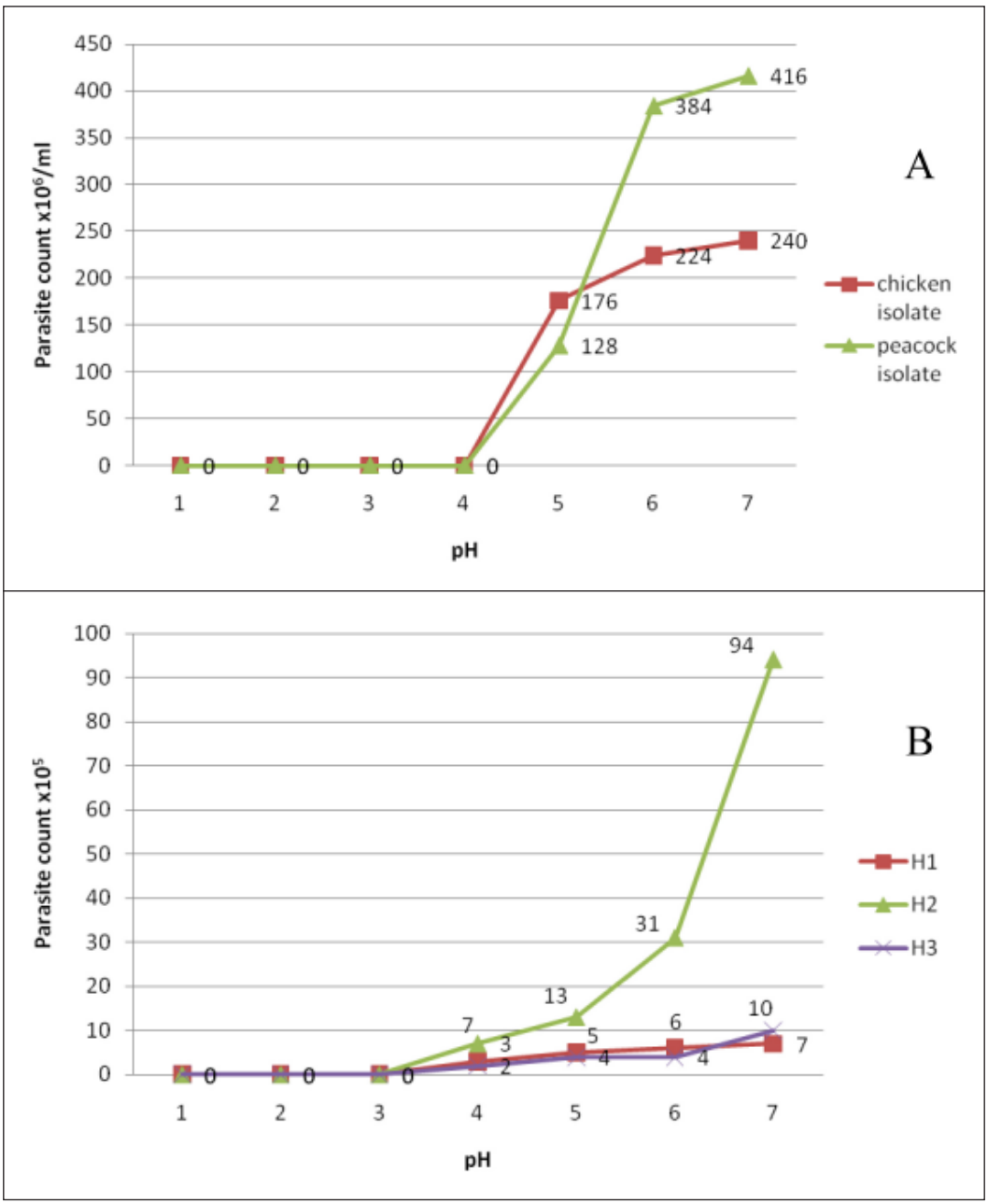

Figure 1. Viable cell counts in A) avian isolates i.e. chicken (triangles) peacock (squares) and B) human isolates i.e., H1 (squares), H2 (triangles) and H3 (cross shape) subjected to pH changes from pH 1 to 7 over a 24-hour incubation period.

detected the presence of *Blastocystis* but only in cat samples. This method, which was the more sensitive than light microscopy and cultivation (Stensvold *et al.*, 2007a), proved to be a most useful proxy for intra-subtype diversity.

Overall, the present results confirm the presence of low prevalences of *Blastocystis* especially in feline hosts. This suggests that both canine and feline hosts are not suitable reservoirs of infection for potentially zoonotic subtypes (ST1 – ST6 and ST10), which were

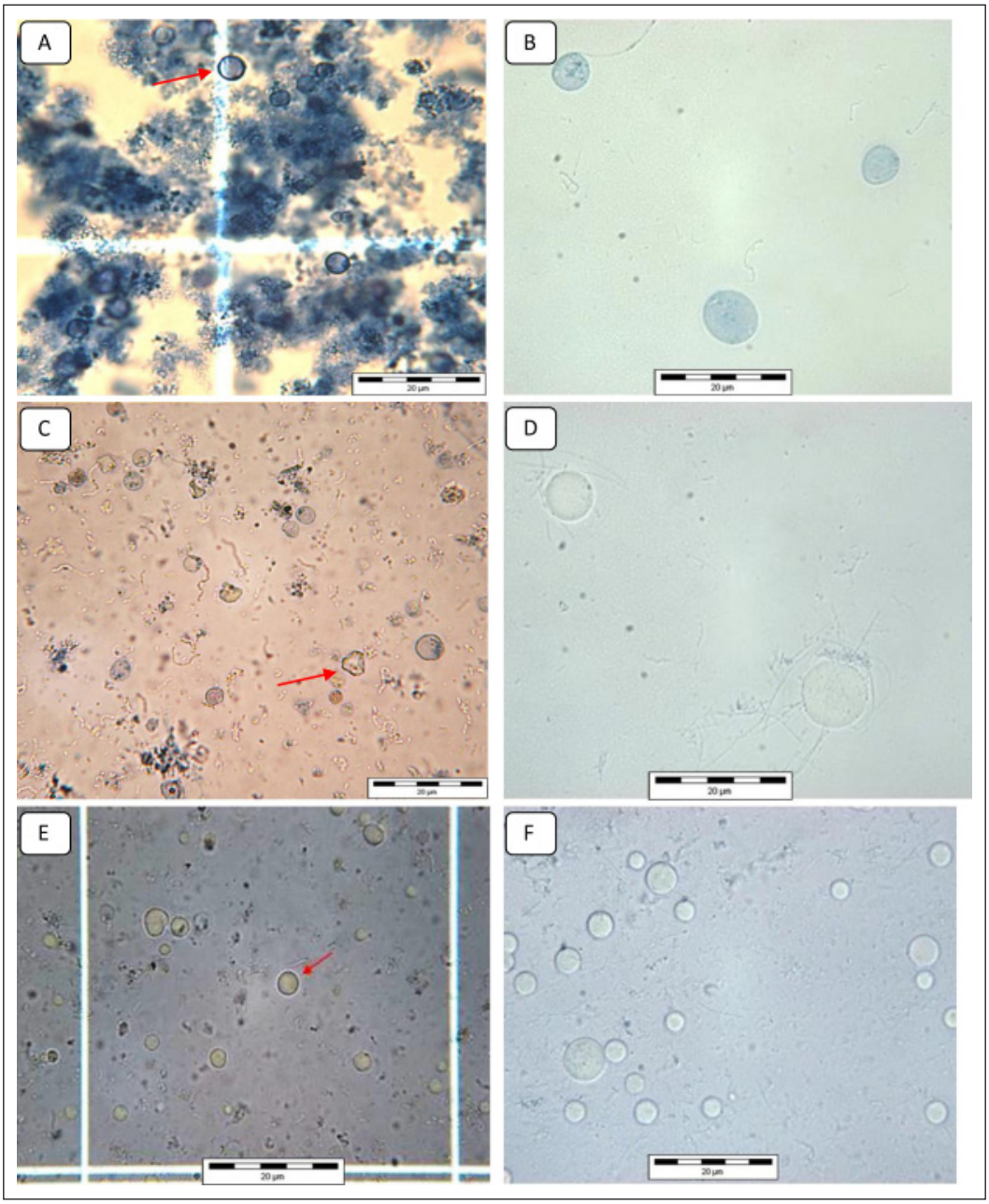

Figure 2. *Blastocystis* cultures of A) avian and B) human isolates in a pH range of 1 - 4 with rounded or oval- shaped non-viable cells; C) avian isolates in pH4 with non-viable vacuolar and wrinkled/ shrunken cells and D) human isolates in pH 4 with smaller viable vacuolar cells; typical rounded vacuolar cells in E) avian and F) human isolates within a pH range of 5 – 7.

possibly transient or opportunistically infected through coprophagia of other host faeces or drinking contaminated water. Nevertheless, *Blastocystis* ST1 DNA was recovered from cat samples. The template for PCR amplification was likely to be DNA derived from non-viable *Blastocystis* cells damaged due to high gastric acid levels within the alimentary tract, as no positive isolates were successfully cultured in growth media.

However, ST1 is the most common human subtype in Thailand, Libya, Nigeria and Peninsular Malaysia (Alfellani *et al.*, 2013; Leelayoova *et al.*, 2008; Nithyamathi *et al.*, 2016) and is associated with human irritable bowel symptoms (Alfellani *et al.*, 2013). This subtype, which has been isolated from farm animals (Tan, 2008) predominantly occurs in dogs from Australia, Cambodia and India (Wang *et al.*, 2013) also in a cat from USA (KJ872776) (Ruaux and Stang, 2014).

Physiologically, *Blastocystis* requires a neutral pH for optimum development and growth (Stenzel and Boreham, 1996) and the present study supports the view that pH values between 5 and 7 facilitate optimal growth of avian isolates. On the other hand, human isolates appear to survive over a wider pH range of 4 to 7 with smaller viable cells being observed at pH 4. This suggests that human isolates are more tolerant of less extreme acidic conditions especially under low pH levels in the human digestive tract (Kararli, 1995).

Growth of *Blastocystis* isolates from both feline and canine hosts was not observed *in vitro* using the Jones' medium suggesting that viable cells or cysts were destroyed under the extreme acidic (< pH 4) conditions similar to those in the gastrointestinal tract. The low pH in the stomach in both hosts is attributable to gastric juice, produced by parietal/oxyntic cells, comprising concentrated hydrochloric acid up to 0.1 M, together with potassium and sodium chlorides. Gastric juice is involved in protein digestion and denaturation together with the activation of pepsin, but the inactivation of ingested microflora such as bacteria (Persson, 2008). On the other hand, gastric acid secretion in the avian gastrointestinal tract, unlike that in mammals, occurs only in the proventriculus where protein denaturation and activation of pepsinogen to pepsin takes place. According to Rynsburger (2009) in ageing broiler chickens, there is both a linear decline in pH from 5.20–3.37 in the proventriculus and also 3.49–3.27 in the gizzard, with a diet of oilseed, soybean and wheat also having an impact on pH.

CONCLUSION

Malaysian cats and dogs were clear of *Blastocystis* infections, despite molecular evidence to show that cats were opportunistically infected with the zoonotic subtype ST1. This could be attributed to the extreme acidity in the gastrointestinal tract that creates an unsuitable environment for the growth of *Blastocystis*. The viability of *Blastocystis* isolates in culture is pH-dependent with growth being suppressed at low pH whilst the yield of viable cells improves as pH rises to neutral.

Acknowledgements. The authors wish to extend their grateful thanks to the Ministry of Education Malaysia (ER011-2012A) and the University of Malaya (RG110/11SUS and PG003-2013B) for funding this study. A special thanks to Parasitology and Haematology staff at the Veterinary Research Institute for helpful guidance and also to the undergraduate students for assistance in collecting samples for this study.

REFERENCES

Abe, N., Nagoshi, M., Takami, K., Sawano, Y., & Yoshikawa, H. (2002). A survey of *Blastocystis* sp. in livestock, pets, and zoo animals in Japan. *Veterinary Parasitology* **106**: 203-212.

Alfellani, M.A., Stensvold, C.R., Vidal-Lapiedra, A., Onuoha, E.S., Fagbenro-Beyioku, A.F. & Clark, C.G. (2013). Variable geographic distribution of *Blastocystis* subtypes and its potential implications. *Acta Tropica* **126**(1): 11-18.

Awadallah, M.A.I. & Salem, L.M.A. (2015). Zoonotic enteric parasites transmitted from dogs in Egypt with special concern to *Toxocara canis* infection. *Veterinary World* **8**(8): 946-957.

Belova, L.M. (1991). *Blastocystis anatis* sp. nov. (Rhizopoda, Lobosea) from domestic ducks. *Zoologicheskii Zhurnal* **70**: 5-10.

Belova, L.M. (1992a). *Blastocystis anseri* (Protista: Rhizopoda) from domestic geese. *Parazitologiia* **26**: 80-82.

Belova, L.M. & Kostenko, L.A. (1990). *Blastocystis galli* sp. n. (Protista: Rhizopoda) from the intestine of domestic hens. *Parazitologiia* **24**: 164-168.

Brosey, B.P.R., Hill, C. & Scott, K.C. (2000). Gastrointestinal volatile fatty acid concentrations and pH in cats. *American Journal of Veterinary Research* **61**: 359-361.

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.

Chen, X.Q., Singh, M., Ho, L.C., Moe, K.T., Tan, S.W. & Yap, E.H. (1997). A survey of *Blastocystis* in rodents. *Laboratory Animal Science* **47**: 91-94.

Chuong, L.S., Suresh, K., Mak, J.W., Init, I. & Kathijah, O. (1996). Prevalence of *Blastocystis* in animals from domesticated surroundings. *Southeast Asian Journal of Tropical Medicine and Public Health* **27**: 850-852.

Duda, A., Stenzel, D.J. & Boreham, P.F.L. (1998). Detection of *Blastocystis* sp. in domestic dogs and cats. *Veterinary Parasitology* **76**: 9-17.

Daryani, A., Ettehad, G.H., Sharif, M., Ghorbani, L. & Ziaei, H. (2008). Prevalence of intestinal parasites in vegetables consumed in Ardabil, Iran. *Food Control* **19**(8): 790-794.

Hemalatha, C., Chandrawathani, P., Suresh Kumar, G., Premaalatha, B., Geethamalar, S., Lily Rozita, M.H., Farah Haziqah, M.T., Sabapathy, D. & Ramlan, M. (2014). The diagnosis of *Blastocystis* sp. from animals – An emerging zoonosis. *Malaysian Journal of Veterinary Research* **5**(2): 15-21.

Ithoi, I., Azman, J., Mak, J.W., Wan Yusuff, W.S. & Rohela, M. (2011). Occurrence of *Blastocystis* in Water of two rivers from recreational areas in Malaysia. *Journal of Parasitology Research* **2011**: 1-8.

Knowles, R. & Das Gupta, B.M. (1924). On the nature of *Blastocystis hominis*. *Indian Journal of Medical Research* **12**: 31-38.

König, G. & Müller, H.E. (1997). *Blastocystis hominis* in animals: incidence of four serogroups. Zen. Bakt. Parasit. *Infektion und Hygiene* **286**: 435-440.

Kararli, T.T. (1995). Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. *Biopharmaceutics & Drug Disposition* **16**: 351-380.

Lavier, G. 1952. Observation sur les *Blastocystis*. *Annales de Parasitologie Humaine et Comparee (Paris)* **27**: 339-356.

Li, L.H., Zhou, X.N., Du, Z.W., Wang, X.Z., Wang, L.B., Jiang, J.Y., Yoshikawa, H., Steinmann, P., Utzinger, J., Wu, Z., Chen, J.X., Chen, S.H. & Zhang, L. (2007). Molecular epidemiology of human *Blastocystis* in a village in Yunnan province, China. *Parasitology International* **56**: 281-286.

Leelayoova, S., Siripattanapipong, S., Thathaisong, U., Naaglor, T., Taamasri, P., Piyaraj, P. & Mungthin, M. (2008). Drinking water: a possible source of *Blastocystis* spp. subtype 1 infection in schoolchildren of a rural community in central Thailand. *American Journal of Tropical Medicine and Hygiene* **79**(3): 401-406.

Leelayoova, S., Taamasri, P., Rangsin, R., Naaglor, T., Thathaisong, U. & Mungthin, M. (2002). *In vitro* cultivation: a sensitive method for detecting *Blastocystis hominis*. *Annals of Tropical Medicine Parasitology* **96**: 803-807.

Maysa, A.I.A. (2010). Endoparasites of zoonotic importance. *Global Veterinary* **5**(6): 348-355.

Mohd Zain, S.N., Farah Haziqah, M.T., Who, P.Y., Fazly Ann, Z., Vickneshwaran, M., Mohd Khalid, M.K.N., Arutchelvan, R. & Suresh, K. (2017). Morphological and molecular detection of *Blastocystis* in wildlife from Tioman Island, Malaysia. *Tropical Biomedicine* **34** (1): 249-255.

Nagel, R., Cuttell, L., Stensvold, C.R., Mills, P.C., Bielefeldt-Ohmann, H. & Traub, R.J. (2012). *Blastocystis* subtypes in symptomatic and asymptomatic family members and pets and response to therapy. *Journal of International Medicine* **42**(11): 1187-1195.

Nithyamathi, K., Chandramathi, S. & Kumar, S. (2016). Predominane of *Blastocystis* sp. infection among school children in Peninsular Malaysia. *Plos One* **11**(2): 1-14.

Osman, M., Bories, J., El Safadi, D., Poirel, M.T., Nausicaa Gantois, N., Benamrouz-Vanneste, S., Laurence Delhaes, L., Hugonnard, M., Certad, G., Lionel Zenner, L. & Viscogliosi, E. (2015). Prevalence and genetic diversity of the intestinal parasites *Blastocystis* sp. and *Cryptosporidium* spp. in household dogs in France and evaluation of zoonotic transmission risk. *Veterinary Parasitology* **214**(1-2): 167-170.

Parkar, U., Traub, R.J., Vitali, S., Elliot, A., Levecke, B., Robertson, I., Geurden, T., Steele, J., Drake, B. & Thompson, R.C.A. (2010). Molecular characterization of *Blastocystis* isolates from zoo animals and their animal-keepers. *Veterinary Parasitology* **169**: 8-17.

Persson, P. B. (1963). Chapter 2: The relation between the rate of gastric secretion and the acidity during steady state conditions in the intact stomach of man and dog. Acta Physiologica Schandinavica, 58(s202) (pp. 26–39). Retrieved from http://onlinelibrary.wiley.com/doi/10.1111/j.1748-1716.1963.tb02674.x/pdf.

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 Evolution* **22**: 223-228.

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.

Ruaux, C.G. & Stang, B.V. (2014). Prevalence of *Blastocystis* in shelter-resident and client-owned companion animals in the US Pacific Northwest. *Plos One* **9**(9): 1-5.

Rynsburger, J.M. (2009). Physiological and nutritional factors affecting protein digestion in broiler chickens. Unpublished master's thesis for Master of Science degree. University of Saskatchewan, Saskatoon, Canada.

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

Singh, M., Ho, L.C., Yap, A.L., Ng, G.C., Tan, S.W., Moe, K.T. & Yap, E.H. (1996). Axenic culture of reptilian *Blastocystis* isolates in monophasic medium and speciation by karyotypic typing. *Parasitology Research* **82**: 165-169.

Sjaastad, O.V., Sand, O. & Hove, K. (2010). Physiology of domestic animals. 2nd edition. Scandinavian Veterinary Press.

Stenzel, D.J. & Boreham, P.F.L. (1996). *Blastocystis hominis* revisited. *Clinical Microbiology Reviews* **9**: 563-584.

Stensvold, C.R., Arendrup, M.C., Jespersgaard, C., Molbak, K. & Nielsen, H.V. (2007a). Detecting *Blastocystis* using parasitologic and DNA-based methods: a comparative study. *Diagnostic Microbiology and Infectious Disease* **59**: 303-330.

Stensvold, C.R., Suresh, G.K., Tan, K.S., Thompson, R.C., Traub, R.J., Viscogliosi, E., Yoshikawa, H. & Clark, C.G. (2007b). Terminology for *Blastocystis* subtypes – a consensus. *Trends Parasitology* **23**(3): 93-96.

Suresh, K., Khairul Anuar, A., Init, I. & Saminathan, R. (1996). Prevalence of *Blastocystis* in laboratory-bred animals. *Tropical Biomedicine* **13**: 89-91.

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

Sutton, S.C. (2004). Companion animal physiology and dosage form performance. *Advanced Drug Delivery Reviews* **56**: 1383-1398.

Tan, K.S.W. (2004). *Blastocystis* in humans and animals: new insights using modern methodologies. *Veterinary Parasitology* **126**: 121-144.

Tan, K.S.W. 2008. New Insights on classification, identification, and clinical relevance of *Blastocystis* spp. *Clinical Microbiology Reviews* **21**(4): 639-665.

Tan, T.C. & Suresh, K.G. (2006). Amoeboid form of *Blastocystis hominis* – a detailed ultrastructural insight. *Parasitology Research* **104**: 737-742.

Teow, W.L., Zaman, V., Ng, G.C., Chan, Y.C., Yap, E.H., Howe, J., Gopalakrishnakone, P. & Singh, M. (1991). A *Blastocystis* species from the sea-snake, *Lapemis hardwickii* (Serpentes, Hydrophiidae). *International Journal of Parasitology* **21**: 723-726.

Wang, W., Cuttell, L., Bielefeldt-Ohmann, H., Inpankaew, T., Owen, H. & Traub, R.J. (2013). Diversity of *Blastocystis* subtypes in dogs in different geographical settings. *Parasites and Vectors* **6**: 215.

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

Zhang, X., Zhang, S., Qiao, J., Wu, X., Zhao, L., Liu, Y. & Fan, X. (2012). Ultrastructural insights into morphology and reproductive mode of *Blastocystis hominis*. *Parasitology Research* **110**: 1165-1172.