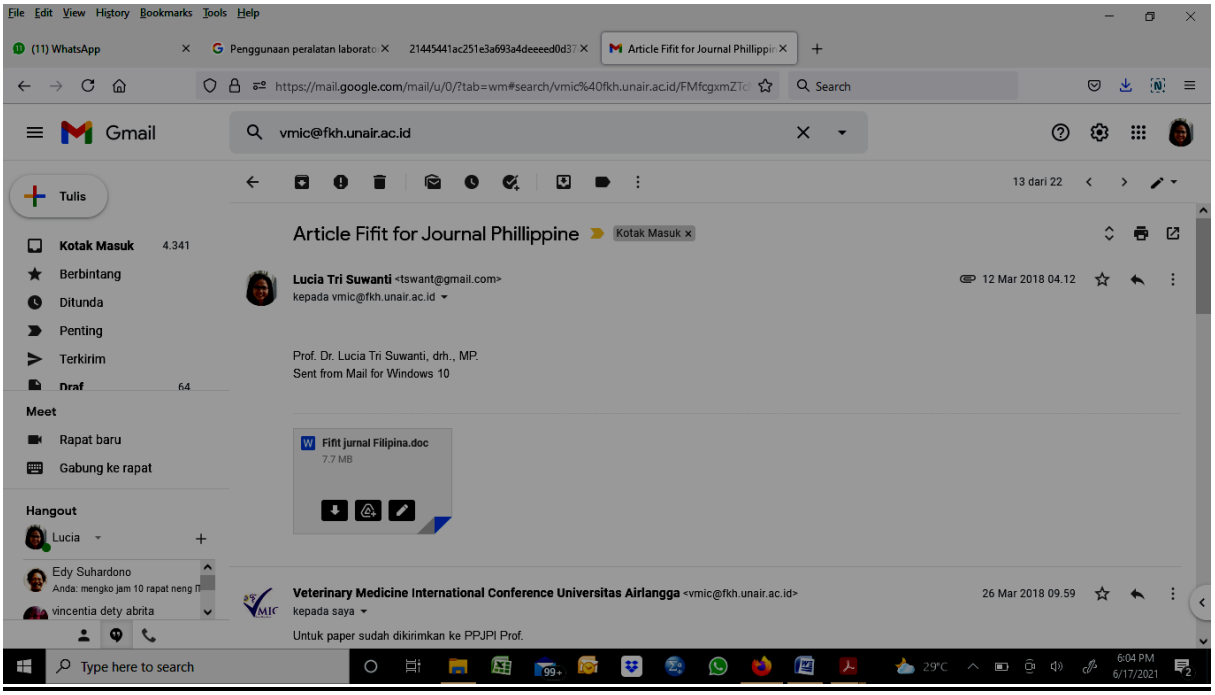
Bukti Corresponding Author
Submit Artikel :

Morphological Detection of the Intestinal Parasite Blastocystis sp. in Fresh and Cultured Feces of Pet Sugar Glider (Petaurus breviceps) in Surabaya, Indonesia.



ORIGINALE ARTICLE

Morphological Detection of the Intestinal Parasite Blastocystis sp. in Fresh and Cultured Feces of Pet Sugar Glider (Petaurus breviceps) in Surabaya, Indonesia.

Fifit Natalia, DVM, M.Si[1], Prof. Dr. Lucia Tri Suwanti, DVM, M.P.[2,4], Dr. Endang Suprihati, DVM, M.S.[2], Dr. Kusnoto, DVM, M.Si[2], Prof. Dr. Setiawan Koesdarto, DVM, M.Sc[2], Prof. Dr. Pudji Srianto, DVM, M.Kes[3]

[1]Student, Master Program of Medicine Science and Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Airlangga; [2]Departement of Parasitology, Faculty of Veterinary Medicine, Universitas Airlangga; [3]Departement of Reproduction, Faculty of Veterinary Medicine, Universitas Airlangga; [4] Researcher of Institute of Tropical Disease, Faculty of Veterinary Medicine, Universitas Airlangga

ABSTRACT

Many deadly suger glider diseases (*Petaurus breviceps*) are still underdiagnose. This study aims to identify the presence of *Blastocystis* sp in sugar glider. One hundred fresh stool of sugar glider which was taken from its colonies that have a history of died or sick. The fresh stool sample was directly stained using iodine, methylene blue, and giemsa and were cultured on simple medium and RPMI 1640. The result showed that The morphology of *Blastocystis* sp in sugar glider (*Petaurus breviceps*) was vacuolar, granular, cyst, and amuboid. Vacuolar form was predominat found in the fresh stool sample with size 0.38 – 2.95 µm (average of 1.46 µm). The prevalence of *Blastocystis* sp in sugar glider in Surabaya, respectively, was 94% and 100% by using iodine staining and Methylene blue, Giemsa staining and in culture medium. On the simple culture medium, *Blastocystis sp.* on the stool of sugar glider lived for 6 days and for 5 days on RPMI 1640 Medium, which both peak of development were on the third day.

Key words : *Blastocystis* sp, Culture Medium, Protozoan, Staining, Sugar glider,

INTRODUCTION

Recently, the taxonomy of *Blastocystis sp.* was rather controversial among researchers. The distribution of *Blastocystis sp.* was widespread throughout the world. *Blastocystis* sp was the most commonly found in protozoan isolation carried out in parasitological surveys. The prevalence of disease caused by *Blastocystis sp* in developing countries was higher than in developed countries, which were directly related to hygiene standards, garbage disposal, animal contacts, and food or beverage contamination. *Blastocystis sp* is an intestinal parasite generally found in human and animal faecal samples. The parasite lives in the digestive tract of humans, livestock, birds, rodents, reptiles, dogs, pigs, cats, and other animals (Duda *et al.,* 1997; Yoshikawa *et al.,* 2004*;* Yoshikawa *et al.,* 2016). *Blastocystis* sp in humans are classified into nine subtypes (STs); eight of which are also found in animals. The eight subtypes are

***FOR CORRESPONDENCE :**
(tswant@gmail.com)

considered to be zoonotic transmission. (Alfellani *et al.*, 2013; Yoshikawa *et al*., 2016)

Study about the disease of sugar glider in Indonesia has not conducted yet. The major issue for veterinarians is the cases of sudden death in sugar glider. In the preliminary study, it was found the presence of *Blastocystis* sp in the faecal sample of sugar glider of the sudden death case by wet mount method. However, such result is in need to be enforced. From the report of veterinarian practitioners, *Blastocystis* sp by wet mount examination was not detected. This suggested that there were constraints faced by the practitioners in diagnosing diseases in sugar glider, especially *Blastocystis* sp. This was due to the limited amount of information and studies on *Blastocystis* sp, including the morphology of *Blastocystis* sp. The practitioners found it difficult to understand *Blastocystis* sp by only wet mount examination. Therefore, to ensure the diagnosis, staining and culture method were considered to be necessary. This study was conducted to identify *Blastocystis* sp in sugar glider by staining and culture method.

There were several methods of staining and culture methods of *Blastocystis* sp that have been done by previous researchers. Common stain used by the researchers were iodine lugol, iodine, giemsa, trichrome, acid-fast, iron haematoxylin, the modification of Ziehl Neelsen (Leelayoova *et al*., 2002; Stensvold *et al*., 2007; Tan, 2008; Zhang *et al.,* 2012; Prasetyo, 2015). Meanwhile, the culture used were Jone' medium, Dulbecco's medium, RPMI 164 medium, 199 medium, formol ethyl acetate concentration technique, Boeck and Drbohlav medium (Sakhsirisampant *et al.,* 2003; Tan, 2008; Zhang *et al.,* 2012). The culture method in vitro can be used to identify *Blastocystis* sp for diagnosis purposes in terms of both clinical and field studies. By using culture method, identification of diseases becomes more accurate and was most likely to find various formation of *Blastocystis* sp, such as the formation of vacuolar, cyst, granular, and amuboid (Dogruman *et al.,* 2010; Zhang *et al.,* 2012).

MATERIALS AND METHODS

The study was conducted from August to October 2017. One hundred sugar glider's faecal samples were obtained from the breeder and lover who has sugar glider in Surabaya, with criteria, there was once a sugar glider which died or sick. Prior to the sampling, the sugar gliders were observed for clinical signs. Fecal samples are fresh feces that are just released during observation of clinical symptoms. The feces were collected and stored in plastic pot steril containing 2.5% potassium bichromate. The feces samples were examined wet mount and were stained using iodine, Methylene blue, and Giemsa. The morphplogy of *Blastocystis* sp was observed and measured under a light microscope connected to an optilab® camera. The culture medium used the simple culture medium modified from Mohammed *et al*. (2015) (500 mL ringer solution, 0.5 gm yeast extract, 5 gm peptone, 20 mL boiled rice water and oxytetracyclin 50-100 mg) and RPMI 1640 medium (10.4 g RPMI in 1000 mL double-distilled water) (Zhang *et al*., 2012). The development of protozoan in the culture medium was observed daily.

RESULTS AND DISCUSSION

The result showed that the vacuolar, granular, cyst, and amuboid form of *Blastocystis* sp were found form both in fresh samples and in cultured samples. The Vocuolar was the most dominant form. The diameter of *Blastocystis* sp in sugar glider were 0.38 – 2.95 μm (average of 1.46 μm). It was smaller in terms of size compared to *Blastocystis* sp found in dogs, cats, and

humans (Stenzel *et al.,* 1996; Duda *et al.,* 1997).

Protozoan *Blastocystis* sp was found in both sugar gliders with and without clinical symptoms. Sugar gliders with clinical symptoms were characterized by appetite lost, diarrhea, constipation, urticarial, and the presence of gas accumulation in the digestive tract. These results were similar to human studies. Some researchers have found *Blastocystis* in both symptomatic and non-symptomatic human patients and clinical symptoms also vary from skin disorders (itching) dan intestinal symptoms (nausea, diarrhea, flatulence and irritable bowel syndrome syndrome (Ramirez et al., 2017; Khademvatan et al., 2018). Based on the researcher's experience, if sugar gliders show symptoms of illness as already mentioned, often have sudden death cases. These research also found that the number of *Blastocystis* sp in sugar gliders with clinical symptoms were higher than without any clinical symptoms (the data was not shown).

The observation of *Blastocystis* sp in iodine staining was obtained in 94 (94%) out of 100 samples, methylene blue staining was obtained in 100 (100%) of 100 samples, and giemsa staining was obtained in 100 (100%) of 100 samples. It means the prevalence of *Blastocystis* sp found in sugar gliders in Surabaya was high, which reached 100% by using Methylene blue and Giemsa and 94% by using iodine staining. According to Zhang *et al.* (2012), the method of permanent staining with Methylen blue and Giemsa gave more positive result compared with wet mount smears of specimens stained with iodine.

High prevalence of *Blastocystis* sp in sugar glider was also supported with the growth of *Blastocystis* sp in cultured feces. All of feces (100%) positively contained *Blastocystis* sp in cultured feces. The development of *Blastocystis* sp in medium was observed for 7 days (Table 1). *Blastocystis* sp growth in both culture media, Simple Medium and RPMI 1640 Medium. The growth of *Blastocystis* in RPMI 1640 medium was faster, but death was also faster than growth in Simple Medium. In the Simple Medium, the growth of *Balstocystis* was seen on the second day and could live up to sixth day, while the growth already visible on the first day and survive until the fifth day, the sixth day was dead on RPMI medium. The peak of growth of *Blastocystis* on both mediums was on the third day**.** The simple medium constituted a new culture medium which is affordable, fast and easy to do without adding either human or horse serum (Mohammed *et al*., 2015).

The high prevalence of *Blastocystis* sp on the sugar glider raises the question of protozoa is actually a commensal protozoan or a pathogen. According to Review Parija and Padukone (2016), although *Blatocystis* sp has been identified 100 years ago but the understanding of the taxonomy, biology and pathogenity of *Blastocystis* sp is not yet fully clear, but, in the recent decades, many researchers have focused their research on the pathogenicity of *Blastocystis* sp.

In this research method mentioned that faecal samples were obtained from the breeder and lover who has sugar glider, with criteria, there was once a sugar glider which died or sick. The likelihood of death or illness of the previous sugar glider is due to *Blastocystis* sp and its cysts contaminate the environment and become a source of contagion to the living sugar glider. Transmission of *Blastocystis* infection occurs via fecal oral and the infective stage is the cyst (Yoshikawa et al., 2004). *Blastocystis* cysts in some animals contaminate water and water as a source of transmission (Lee et al., 2012).

Several studies have shown that Blastocystis infection has the potential to be a zoonotic parasite with the discovery of the same subtype in both animals and human (Osman et al., 2016). There are 17 subtypes of Blastocystis in mammals and birds, 9 subtypes (ST1-9) can infect humans (Cian et al., 2017). Further research is needed on the subtype of *Blastocystis* in the sugar glider and to know the zoonotic potential

CONCLUSIONS

The morphology of *Blastocystis* sp in sugar glider (*Petaurus breviceps*) was vacuolar, granular, cyst, and amuboid. The vacuolar form was predominat found in the fresh stool sample with size 0.38 – 2.95 µm (average of 1.46 µm).

The prevalence of *Blastocystis* sp in sugar glider in Surabaya, respectively, was 94% and 100% by using iodine staining and Methylene blue, Giemsa staining and in culture medium. The peak of growth of *Blastocystis* in mediums was on the third day.

ACKNOWLEDGMENTS

The researchers would like to thank the breeders of sugar gliders and those who pet the animal with the help in sample collecting. We would also like to express our gratitude to the Department of Parasitology of the Faculty of Veterinary Medicine of Universitas Airlangga and the Dean of the Faculty of Veterinary Medicine of Universitas Airlangga who have provided the facilities to conduct the study.

REFERENCES

Alfellani MA, Stensvold CR, Lapiedra AV, Onuoha ESU, Beyioku AFF, and Clark CG. 2013. Variable Geographic Distribution of *Blastocystis* Subtype and Its Potential Implication. *Acta Tropica* 126 : 11-18.

Cian A, El Safadi D, Osman M, Moriniere R, Gantois N, Benamrouz-Vanneste S, Delgado-Viscogliosi P, Guyot K, Li L-L, Monchy S, Noel C, Poirier P, Nourrisson C, Wawrzyniak I, Delbac F, Bosc S, Chabe M, Petit T, Certad G, and Viscoglios E. 2017. Molecular Epidemiology of *Blastocystis sp*. in Various Animal Groups fromTwo French Zoos and Evaluation of Potential Zoonotic Risk. *PLoS ONE* 12 (1): e0169659. doi:10.1371/journal.pone.0169659

Dogruman AF, Zahide S, and Kenneth B. 2010. Comparison of Methods of Detection of *Blastocystis hominis* in Routinely Submitted Stool Samples and Also in IBS/IBD Patients in Ankara. *PloS ONE* 5 (11):15484.

Duda A, Stenzel DJ, and Boreham PFL. 1998. Detection *Blastocystis sp.* in Domestic Dogs and Cats. *Veterinary Parasitology* 76 : 9-17.

Khademvatan S, Masjedizadeh R, Yousefi-Razin E, Mahbodfar H, Rahim F, Yousefi E, and Foroutan M. 2017. PCR-based molecular characterization of *Blastocystis hominis* subtypes in southwest of Iran. *Journal of Infection and Public Health* 11(1):43-47.

Lee L, Chye TT, Karmacharya BM, and Govind SK. 2012. *Blastocystis* sp.: waterborne zoonotic organism, a possibility? *Parasites & Vectors* 5:130-134

Leelayoova S, Taamasri P, Rangsin R, Naaglor T, Thathaisong U, and Mungthin M. 2002. Invitro Cultivation : a Sensitive Method for Detecting *Blastocystis hominis. Annals of Tropical Medicine and Parasitology* 96 : 803-807.

Mohammed ST, Sulaiman NM, and Kamal SB. 2015. Preparation Simplified Culture for Culturing Blastocystis Hominis Parasite. *Journal of Biology, Agriculture and Healthcare*, 5(20): 112-115.

Osman M, Bories J, Safadi DE, Poirel MT, Gantois N, Vanneste SB, Delhaes L, Hugonnard M, Certad G, Zenner L, and 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 :167-170.

Parija SC and Padukone S. 2016. Blastocystis: Pathogen or passenger? An evaluation of 101 years of research. *Tropical Parasitology* 6(2):163-164

Prasetyo RH. 2015. *Infeksi Parasit Usus Oportunistik*. Airlangga University Press. Surabaya. 35–38.

Ramírez JD, Flórez C, Olivera M, Bernal MC, Giraldo JC (2017) *Blastocystis* subtyping and its association with intestinal parasites in children from different geographical regions of Colombia. *PLoS ONE* 12(2): e0172586

Sakhsirisampant W, Nuchprayoon S, Wiwanitkit V, Yenthakam S, and Ampavasiri A. 2003. Intestinal Parasitic Infestations among Children in an Orphanage in Pathum Thani province. *Journal of the Medical Association of Thailand* 86(Suppl. 2)**:** S263–S270.

Stensvold CR, Arendrup MC, Jespersgaard C, Mølbak K, and Nielsen HV. 2007. Detecting *Blastocystis* Using Parasitologic and DNA-Based Methods: a Comparative Study. *Diagnostic Microbiology and Infectious Disease* 59**:**303–307.

Stenzel DJ and Boreham PFL. 1996. *Blastocystis hominis* Revisited. *Clinical Microbiology Reviews* 9. 563-584.

Tan KS. 2008. New Insights on Classification, Identification, and Clinical Relevance of *Blastocystis* spp. *Clinical Microbiology Reviews* 21. 639–665.

Yoshikawa H, Yoshida K, Nakajima A, Yamanari K, Iwatani S, and Kimata I. 2004. Fecal-Oral Transmission of The Cyst Form of *Blastocystis hominis* in Rats. *Parasitology Research* 94 : 391-396.

Yoshikawa H, Tokoro M, Nagamoto T, Arayama S, Asih PBS, Rozi IE, and Syafruddin D. 2016. Molecular Survey of Blastocystis sp. from Humans and Associated Animals in an Indonesian Community with Poor Hygiene. *Parasitol International* 65(6 Pt B):780-784.

Zhang X, Qiao J, Wu X, Da R, Zhao L, and Wei Z. 2012. In Vitro Culture of *Blastocystis hominis* in Three Liquid Media and Its Usefulness in the Diagnosis of Blastocystosis. *International Journal of Infectious Diseases* 16 : e23-e28.

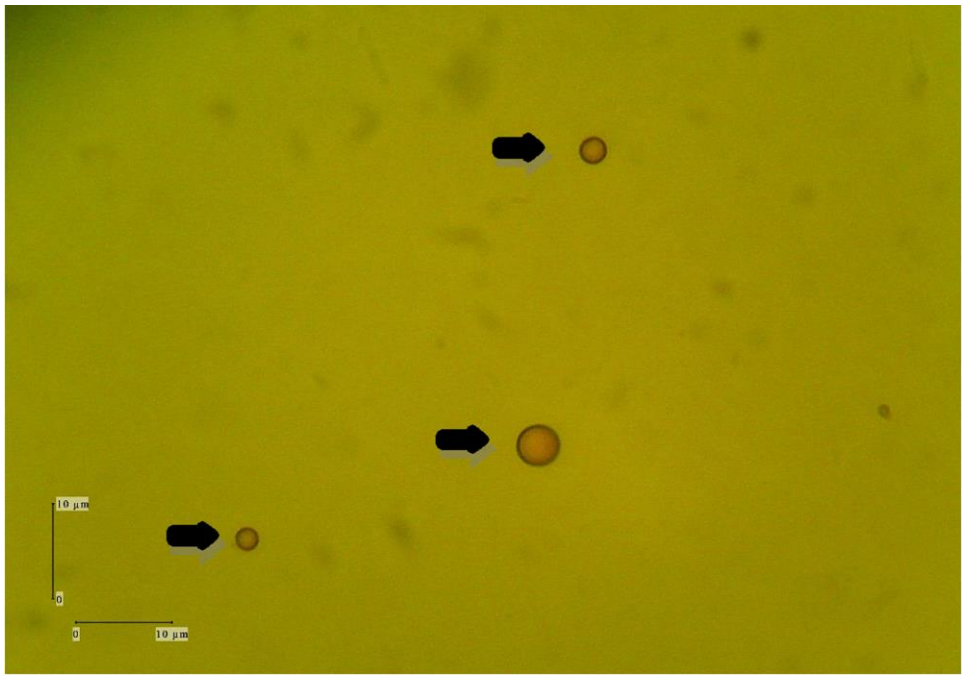


Fig 1. Microscopic images of vacuolar form of *Blastocystis sp.* (black arrows) with iodine staining.

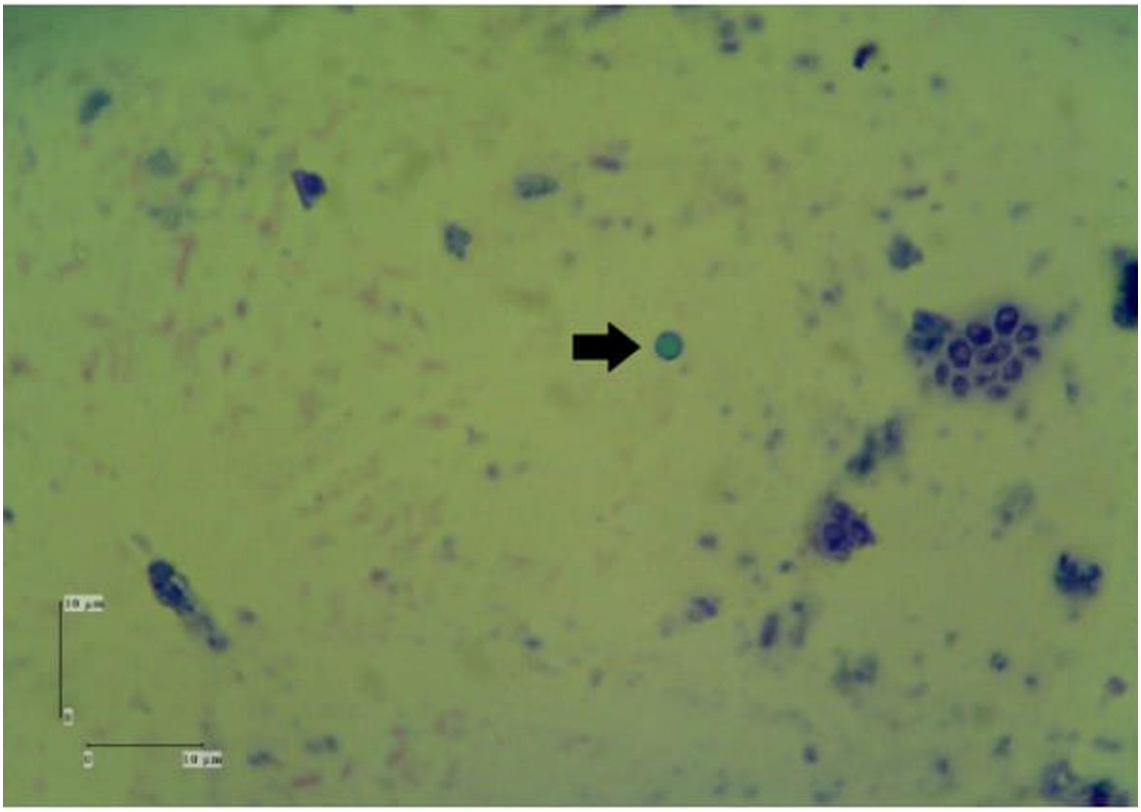


Fig 2. Microscopic images of vacuolar form of *Blastocystis sp.* (black arrows) with giemsa staining

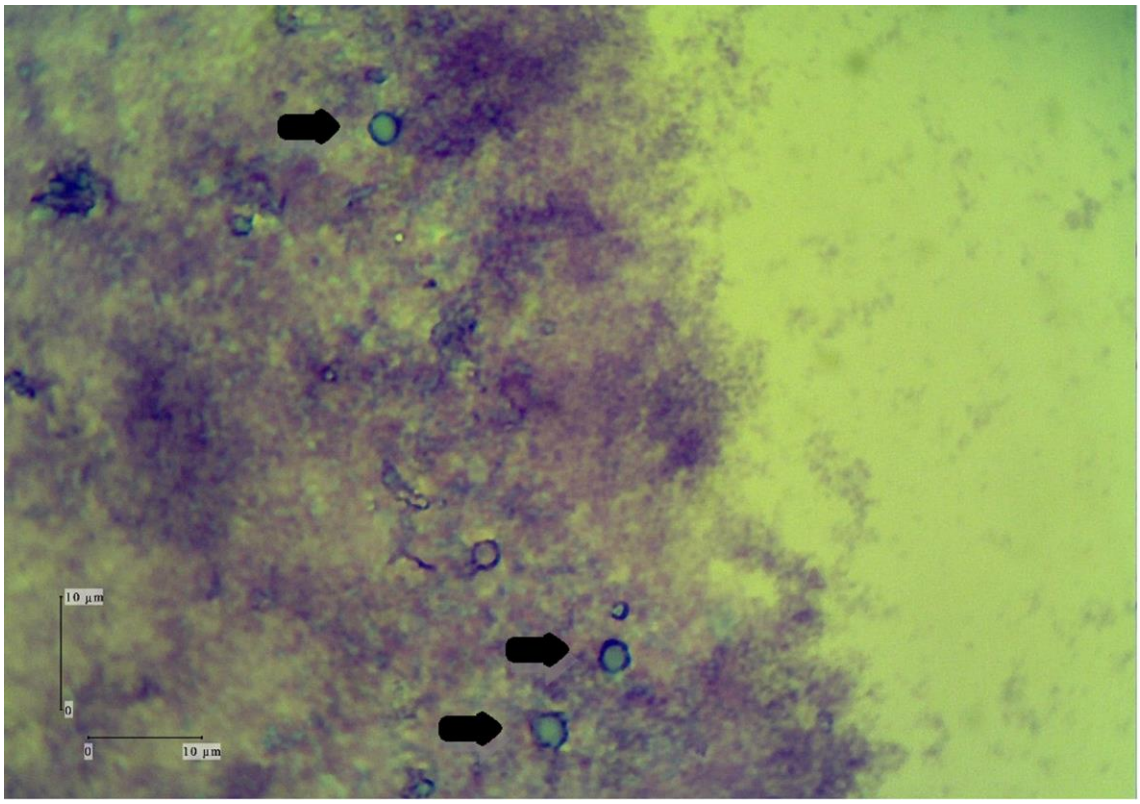
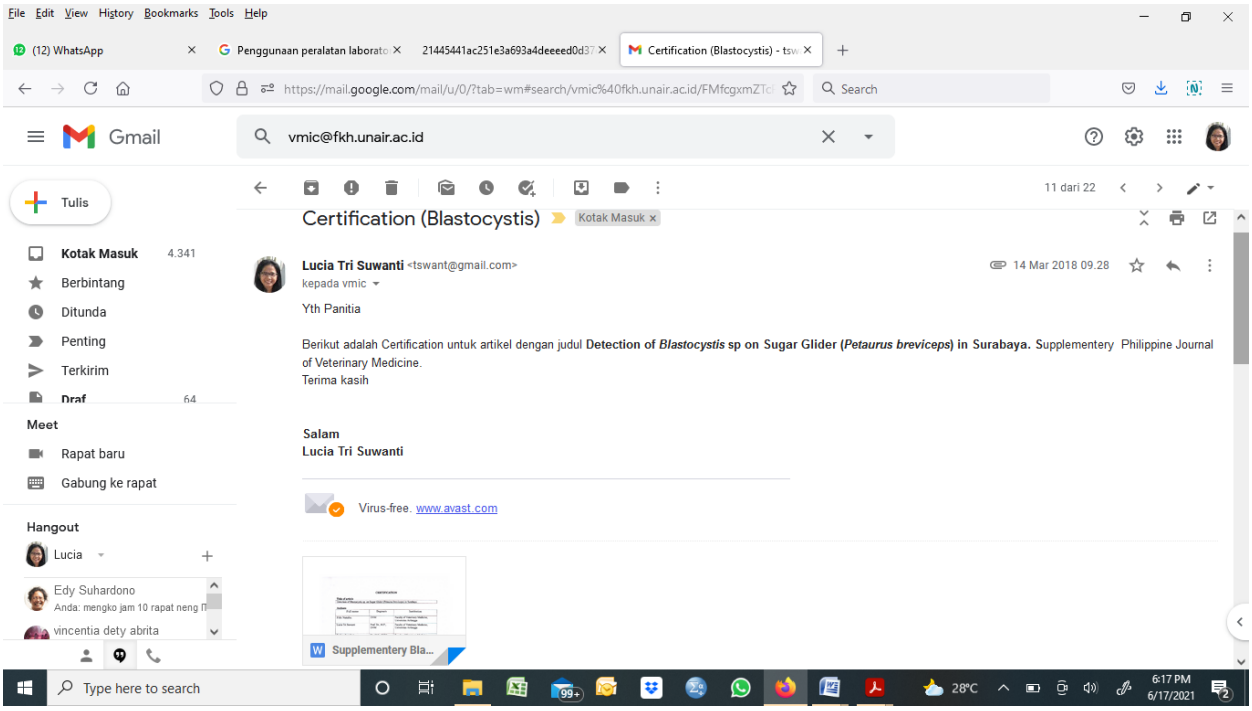


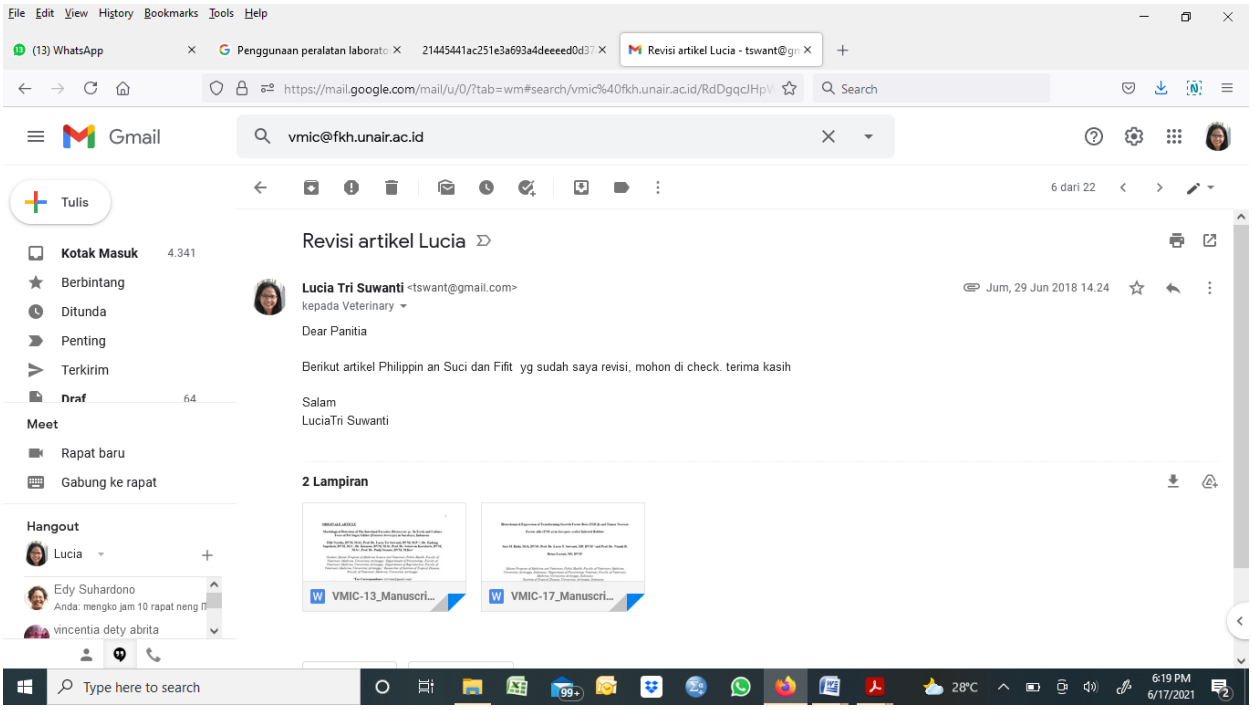
Fig 3. Microscopic images of vacuolar form of *Blastocystis sp*. (black arrows) with methylene blue staining

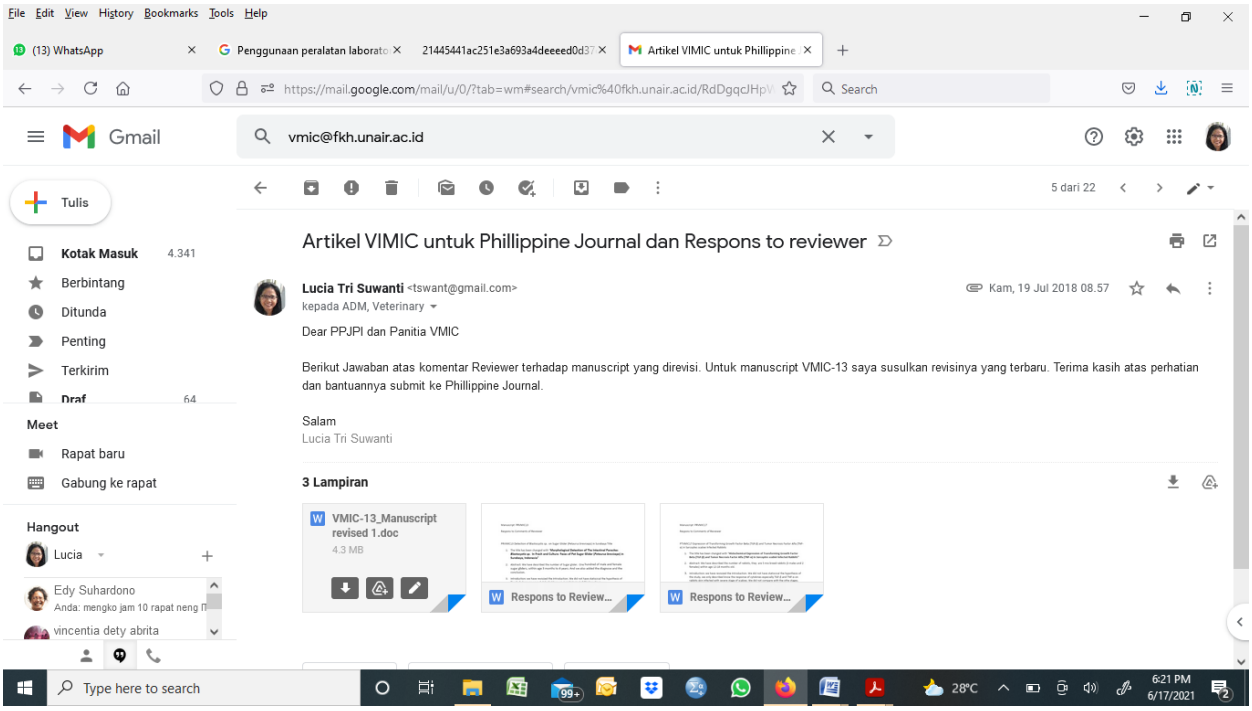
Table 1. Observation of Culture

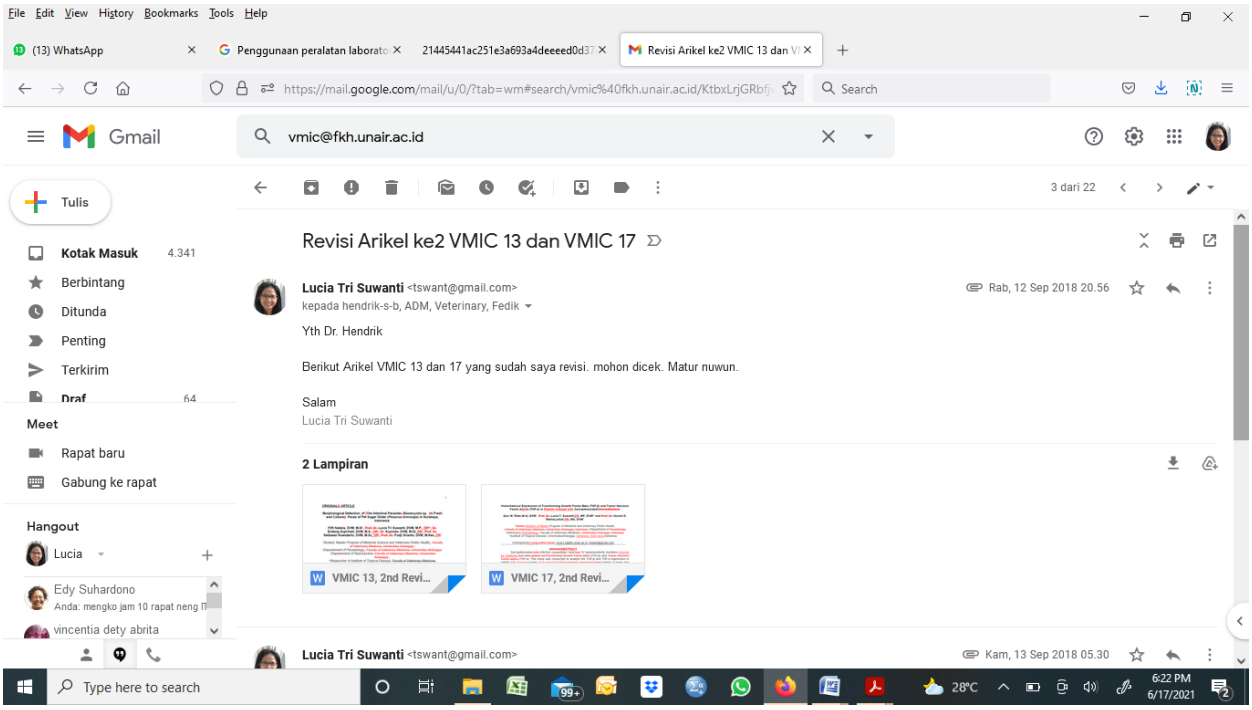
Days	Culture Medium	
	RPMI 1640	Simple Medium
1	Exist, light	Not exist
2	Exist, light	Exist, light
3	Exist, medium	Exist, plentiful
4	Exist, light	Exist, medium
5	Exist, light	Exist, light
6	Dead	Exist, light
7	Dead	Dead

Description: Light: indicated the growth of *Blastocystis sp.* <10 in one field of view; Medium: showed the presence of 10-20 *Blastocystis sp*. in one field of view; Plentiful: showed *Blastocystis sp.* that filled up the one field of view


Certification (Blastocystis)
Lucia Tri Suwanti <tswant@gmail.com>
14 Mar 2018 09.28
Yth Panitia
Berikut adalah Certification untuk artikel dengan judul Detection of Blastocystis sp on Sugar Glider (Petaurus breviceps) in Surabaya. Supplementery Philippine Journal of Veterinary Medicine.
Terima kasih
Salam
Lucia Tri Suwanti
Virus-free. www.avast.com
Supplementery Bla...


Revisi artikel Lucia
Lucia Tri Suwanti <tswant@gmail.com>
Jum, 29 Jun 2018 14.24
Dear Panitia
Berikut artikel Philippin an Suci dan Fifit yg sudah saya revisi, mohon di check. terima kasih
Salam
LuciaTri Suwanti
2 Lampiran
VMIC-13_Manuscri...
VMIC-17_Manuscri...


Artikel VIMIC untuk Phillippine Journal dan Respons to reviewer
Lucia Tri Suwanti <tswant@gmail.com>
kepada ADM, Veterinary
Kam, 19 Jul 2018 08.57
Dear PPJPI dan Panitia VMIC
Berikut Jawaban atas komentar Reviewer terhadap manuscript yang direvisi. Untuk manuscript VMIC-13 saya susulkan revisinya yang terbaru. Terima kasih atas perhatian dan bantuannya submit ke Phillippine Journal.
Salam
Lucia Tri Suwanti
3 Lampiran
VMIC-13_Manuscript revised 1.doc
4.3 MB
Respons to Review...
Respons to Review...


Revisi Arikel ke2 VMIC 13 dan VMIC 17
Lucia Tri Suwanti <tswant@gmail.com>
kepada hendrik-s-b, ADM, Veterinary, Fedik
Rab, 12 Sep 2018 20.56
Yth Dr. Hendrik
Berikut Arikel VMIC 13 dan 17 yang sudah saya revisi. mohon dicek. Matur nuwun.
Salam
Lucia Tri Suwanti
2 Lampiran
VMIC 13, 2nd Revi...
VMIC 17, 2nd Revi...
Lucia Tri Suwanti <tswant@gmail.com>
Kam, 13 Sep 2018 05.30

ORIGINAL ARTICLE

Morphological Detection of the Intestinal Parasite *Blastocystis* sp. in Fresh and Cultured Feces of Pet Sugar Glider (*Petaurus breviceps*) in Surabaya, Indonesia

Fifit Natalia, DVM, MSi[1], Lucia Tri Suwanti, DVM, MP, DR[2,4], Endang Suprihati, DVM, MS, DR[2], Kusnoto, DVM, MSi, DR[2], Setiawan Koesdarto, DVM, MSc, DR[2], Pudji Srianto, DVM, MKes, DR[3]

[1]Student, Master Program of Medicine Science and Veterinary Public Health;
[2]Department of Parasitology;
[3]Department of Reproduction;
[4] Researcher of Institute of Tropical Disease, Universitas Airlangga, Surabaya, East Java, Indonesia

***For correspondence:** tswant@gmail.com

ABSTRACT

Many deadly sugar glider diseases remain underdiagnosed. Thus, this study aimed to detect the presence of *Blastocystis* sp. in sugar gliders (*Petaurus breviceps*). Fresh stools were taken from 100 3-month to 4-year old male and female sugar gliders from enthusiasts and breeders. Samples were directly observed in wet mount, stained with iodine, methylene blue, and giemsa, and cultured on simple and RPMI 1640 media. Results showed high detection of the parasite: 87% on wet mount, 94% on iodine staining, and 100% on methylene blue, giemsa staining and cultured media. *Blastocystis* sp. in sugar glider can be described as vacuolar, granular, cyst, and amoeboid, wherein vacuolar form predominated, with size 0.38–2.95 μm (average of 1.46 μm). The parasite lived for 6 days in simple culture medium and 5 days on RPMI 1640 medium. Growth peak was marked on the third day for both media. This study is the first to report *Blastocystis* sp. in sugar gliders, revealing its presence in both fresh and cultured sugar glider stools.

Key words : *Blastocystis* sp., culture medium, protozoan, staining, sugar glider

INTRODUCTION

Recently, sugar gliders have become in demand exotic pets to animal lovers in the world, including Indonesia (Catro, 2013). However, study on their diseases is still limited, especially on the presence of *Blastocystis* sp. The main problem often

faced by sugar glider lovers and breeders is the case of sudden death of spiders shortly after having shown signs of diarrhea or bloating—a condition that veterinarians to this day are unable to diagnose. In fact, according to Corriveau (2014), enteritis typically pesters sugar gliders.

Blastocystis sp. is an intestinal parasite generally found in both human and animal feces. The parasite lives in the digestive tract of humans, livestock, birds, rodents, reptiles, dogs, pigs, cats, and other animals (Duda *et al.,* 1998; Yoshikawa *et al.,* 2004*;* Yoshikawa *et al.,* 2016). It causes infection with clinical symptoms such as loss of appetite, constipation, diarrhea, urticaria, flatulence, and irritable bowel syndrome (IBS) (Tan, 2008; Casero *et al*. 2015). Moreover, some researchers noted other asymptomatic cases and skin disorders (Ramirez *et al*., 2017; Khademvatan *et al*., 2018).

Parasitological surveys have often detected *Blastocystis* sp. in patient stool samples. Several methods are used for its detection: wet mount, staining, and culture methods. Common stains used are iodine lugol, iodine, giemsa, trichrome, acid-fast and iron haematoxylin, a modification of Ziehl Neelsen? (Leelayoova *et al*., 2002; Stensvold *et al*., 2007; Tan, 2008; Zhang *et al.,* 2012; Prasetyo, 2015). Meanwhile, there is also a number of culture media: Jones', Dulbecco's, RPMI 1640, 199, formol ethyl acetate concentration, and Boeck and Drbohlav (Sakhsirisampant *et al.,* 2003; Tan, 2008; Zhang *et al.,* 2012). Culture method *in vitro* can be used to identify *Blastocystis* sp. for diagnosis in clinical and field studies. By using culture method, identification of diseases becomes more accurate, while also characterizing various forms of *Blastocystis* sp., such as vacuolar, cyst, granular and amoeboid (Dogruman *et al.,* 2010; Zhang *et al.,* 2012).

This study was conducted to detect *Blastocystis* sp. in fresh stool of sugar glider by staining and culture methods. This is the first research to detect *Blastocystis* sp. in sugar gliders, intended to assist veterinary practitioners in diagnosing possible infections.

MATERIALS AND METHODS

The study was conducted from August to October 2017. One hundred fecal samples from 3-month to 4-year old sugar gliders were obtained from enthusiasts and breeders in Surabaya, Indonesia. Age, sex, health status (healthy, presence of diarrhea or bloating), feed type, and feeding frequency were recorded. Fecal samples were fresh stools that were just released during observation of health status. About 1 g of feces was collected per sugar glider and stored in sterile Eppendorf tubes® containing 1 ml of 2.5% potassium dichromate (Merck, Germany).

Experiments were conducted at the Laboratory of the Department of Veterinary Parasitology, Faculty of Veterinary Medicine, Universitas Airlangga. Fecal samples were centrifuged at 1500 rpm for 5 min, and the pellets of each sample were smeared on 4 object glasses, one for wet mount and 3 were stained using iodine (povidone-iodine, Mahakam Beta Farma, Indonesia), methylene blue (Merck, Germany), and 20% giemsa solution (Merck, Germany). The remaining pellets were resuspended with aquadest to a volume of 0.5 ml for culture. The suspension was divided into two, one mixed with simple culture medium and the other with RPMI 1640 medium (Gibco® Life Technologies™, USA) to a volume of 1.5 ml. Cultures were incubated at 37°C. Development of protozoan in the culture was observed daily. According to Mohammed *et al.* (2015), the composition of simple medium are 500 ml ringer solution (Otsu-RL® Otsuka, Indonesia), 0.5 gm yeast extract (Merck, Germany), 5 g peptone (Merck, Germany), 20 ml boiled rice water and 50-100 mg oxytetracyclin (Vet-oxy LA, Sanbe, Indonesia.) RPMI medium contains 10.4 g RPMI 1640 in 1000 ml double-distilled water (Zhang *et al*., 2012). Morphology of *Blastocystis* sp. was observed and measured under a light microscope (Nikon® E100, Japan) connected to a camera (Optilab® MTN001, Indonesia).

RESULTS AND DISCUSSION

Only a few *Blastocystis* sp. can be detected in fresh stools, where vacuolar form dominated (Fig. 1a-d); meanwhile, all forms, (vacuolar, granular, cyst, and amoeboid) were found in cultured samples, and vacuolar form was also the most evident (Fig. 1d). The diameter of *Blastocystis* sp. in sugar glider was 0.38-2.95 µm (average=1.46 µm). This is smaller in terms of size compared to *Blastocystis* sp. found in dogs, cats, and humans (Stenzel *et al.,* 1996; Duda *et al.,* 1998).

Microscopic observations showed high detection of parasite on wet mount (87%), iodine staining (94%), and methylene blue and giemsa staining (100%) (Table 3). This suggests that all samples were positive for *Blastocystis* sp., and methylene blue and giemsa staining had the highest occurrence of *Blastocystis*. These results coincide with the study by Zhang *et al.* (2012), wherein staining with methylene blue and giemsa resulted to a more effective method of detection compared to wet mount smears with iodine.

Moreover, cultured stools also showed 100% detection of *Blastocystis* sp., growing both in simple and RPMI 1640 media. The development of *Blastocystis* sp. in cultured media was observed for 7 days (Table 2). Growth in RPMI 1640 medium was relatively faster but as was the occurrence of death. In simple medium, growth was seen on day 2, alive until day 6; while on RPMI medium, growth was already visible on day 1 , surviving only until day 5. Third day marked the peak of growth of *Blastocystis* for both media. The simple medium can then be considered a new, affordable, fast and easy to produce culture medium, without adding either human or horse serum (Mohammed *et al*., 2015).

Such high occurrence of *Blastocystis* sp. in sugar glider feces raises the question of whether it is actually a commensal protozoan or a pathogen. A review by Parija and Padukone (2016) argues that although *Blatocystis* has been identified a century ago, its taxonomy, biology, and pathogenicity are not yet fully understood. In the recent decades, however, many researchers have focused on the pathogenicity of *Blastocystis* sp. One study, for instance, has shown that stressful conditions can increase the infectivity, pathogenesis and growth of the parasitic *B. hominis* (Chandramathi *et al*., 2014)

As shown in Table 1, *Blastocystis* sp. was found in both sugar gliders with or without clinical symptoms. Twenty-one sugar gliders manifested weakness, diarrhea, and bloating. These observations are similar to human studies. Some researchers have found *Blastocystis* in both symptomatic and asymptomatic patients, and clinical symptoms varied from skin disorders (itching) to intestinal symptoms (nausea, diarrhea, flatulence, and irritable bowel syndrome (Ramirez *et al*., 2017; Khademvatan *et al*., 2018). There are 17 subtypes (ST) of *Blastocystis* in mammals and birds, 9 subtypes (ST1-9) of which can infect humans (Cian *et al*., 2017). In human cases, according to Ramirez *et al*., (2017), clinical outcome of *Blastocystis* sp. infection is not likely associated with a specific subtype of *Blastocystis* sp., but Zulfa *et al*. (2017) argues that ST3 subtype is more likely to be associated with diarrhea in children.

This research also found that the number of *Blastocystis* in sugar gliders with clinical symptoms were higher than those without clinical symptoms (data not shown). Based on the researcher's experience, if sugar gliders show symptoms as

stated, this often leads to sudden death. Sugar gliders with symptoms are assumed to have originated from colonies given raw drinking water and whose cages were only cleaned once a month?. Water quality, contamination of food and drinking water, and sanitation influence the rate at which *Blastocystis* sp. infection can occur (Abdulsalam *et al*., 2012; Canete *et al*., 2012). Further research using molecular markers is needed to understand the dynamics of *Blastocystis* sp. infection and its role in health and disease of sugar gliders.

Several studies have shown that *Blastocystis* infection has the potential to be a zoonotic disease, with the discovery of the same subtype affecting both animals and humans (Osman *et al*., 2015). Transmission of *Blastocystis* infection can be oral or fecal (Yoshikawa *et al*., 2004). Considered as the infective stage, *Blastocystis* cysts in some animals can contaminate water, an easy source of transmission (Lee *et al*., 2012). Thus, *Blastocystis* subtypes in sugar gliders and their zoonotic potential entail further research.

This study illustrates that *Blastocystis* sp. exist in fresh stool of sugar gliders, with the highest occurrence noted for methylene blue, giemsa stained, and cultured media samples. Morphology of *Blastocystis* sp. was vacuolar (dominant form), granular, cyst, and amoeboid. And diameter was 0.38-2.95 μm, with average of 1.46 μm. Day 3 marked growth peak in both media. This is the first report of *Blastocystis* sp. in sugar glider.

ACKNOWLEDGMENT

The researchers would like to thank sugar glider enthusiasts and breeders for their permission to use their pets as experimental models and for their assistance during stool collection. Huge thanks as well to the Department of Veterinary Parasitology, Faculty of Veterinary Medicine, Universitas Airlangga and the acting Dean for providing the facilities which enabled the conduct of the study.

REFERENCES

Abdulsalam A, Ithoi I, Al-Mekhlafi H, Ahmed H, Surin J and Mak J. 2012. Drinking water is a significant predictor of *Blastocystis* infection among rural Malaysian primary school children. *Parasitology* 139: 1014-1020.

Canete R, Diaz M, Garcia RA, Martinez PL and Ponce FM. 2012. Intestinal parasites in children from a day care centre in Matanzas City, Cuba. *PLoS ONE* 7: e51394.

Casero RD, Mongi F, Sánchez A and Ramírez JD. 2015. *Blastocystis* and urticaria: examination of subtypes and morphotypes in an unusual clinical manifestation. *Acta Tropica* 148: 156-161.

Chandramathi S, Suresh K, Sivanandam S and Kuppusamy UR. 2014. Stress exacerbates infectivity and pathogenicity of *Blastocystis hominis*: *in vitro* and *in vivo* evidences. *PLoS ONE* 9(5): e94567.

Cian A, El Safadi D, Osman M, Moriniere R, Gantois N, Benamrouz-Vanneste S, Delgado-Viscogliosi P, Guyot K, Li L-L, Monchy S, Noel C, Poirier P, Nourrisson C, Wawrzyniak I, Delbac F, Bosc S, Chabe M, Petit T, Certad G and Viscoglios E. 2017. Molecular epidemiology of *Blastocystis sp*. in various animal groups from two French zoos and evaluation of potential zoonotic risk. *PLoS ONE* 12 (1): e0169659.

Dogruman AF, Zahide S and Kenneth B. 2010. Comparison of methods of detection of *Blastocystis hominis* in routinely submitted stool samples and also in IBS/IBD patients in Ankara. *PLoS ONE* 5(11): 15484.

Duda A, Stenzel DJ and Boreham PFL. 1998. Detection of *Blastocystis sp.* in domestic dogs and cats. *Veterinary Parasitology* 76: 9-17.

Khademvatan S, Masjedizadeh R, Yousefi-Razin E, Mahbodfar H, Rahim F, Yousefi E and Foroutan M. 2017. PCR-based molecular characterization of *Blastocystis hominis* subtypes in southwest of Iran. *Journal of Infection and Public Health* 11(1): 43-47.

Lee L, Chye TT, Karmacharya BM and Govind SK. 2012. *Blastocystis* sp.: waterborne zoonotic organism, a possibility? *Parasites & Vectors* 5:130-134.

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

Mohammed ST, Sulaiman NM and Kamal SB. 2015. Preparation of simplified culture for culturing *Blastocystis hominis* parasite. *Journal of Biology, Agriculture and Healthcare* 5(20): 112-115.

Osman M, Bories J, El Safadi D, Poirel MT, Gantois N, Vanneste SB, Delhaes L, Hugonnard M, Certad G, Zenner L and 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: 167-170.

Parija SC and Padukone S. 2016. *Blastocystis*: pathogen or passenger? An evaluation of 101 years of research. *Tropical Parasitology* 6(2): 163-164.

Prasetyo RH. 2015. *Opportunistic intestinal parasite infection*. Airlangga University Press, Surabaya, Indonesia 35-38.

Ramírez JD, Flórez C, Olivera M, Bernal MC and Giraldo JC. 2017. *Blastocystis* subtyping and its association with intestinal parasites in children from different geographical regions of Colombia. *PLoS ONE* 12(2): e0172586.

Sakhsirisampant W, Nuchprayoon S, Wiwanitkit V, Yenthakam S and Ampavasiri A. 2003. Intestinal parasitic infestations among children in an orphanage in Pathum Thani province. *Journal of the Medical Association of Thailand* 86(Suppl. 2)**:** S263–S270.

Stensvold CR, Arendrup MC, Jespersgaard C, Mølbak K and Nielsen HV. 2007. Detecting *Blastocystis* using parasitologic and DNA-based methods: a comparative study. *Diagnostic Microbiology and Infectious Disease* 59**:** 303-307.

Stenzel DJ and Boreham PFL. 1996. *Blastocystis hominis* revisited. *Clinical Microbiology Reviews* 9: 563-584.

Tan KS. 2008. New insights on classification, identification, and clinical relevance of *Blastocystis* spp. *Clinical Microbiology Reviews* 21: 639-665.

Yoshikawa H, Yoshida K, Nakajima A, Yamanari K, Iwatani S and Kimata I. 2004. Fecal-oral transmission of the cyst form of *Blastocystis hominis* in rats. *Parasitology Research* 94: 391-396.

Yoshikawa H, Tokoro M, Nagamoto T, Arayama S, Asih PBS, Rozi IE and Syafruddin D. 2016. Molecular survey of *Blastocystis* sp. from humans and associated animals in an Indonesian community with poor hygiene. *Parasitol International* 65(6 Pt B): 780-784.

Zhang X, Qiao J, Wu X, Da R, Zhao L and Wei Z. 2012. *In vitro* culture of *Blastocystis hominis* in three liquid media and its usefulness in the diagnosis of blastocystosis. *International Journal of Infectious Diseases* 16: e23-e28.

Zulfa F, Sari IP and Kurniawan A. 2017. Association of *Blastocystis* subtypes with diarrhea in children. *IOP Conf. Series: Journal of Physics: Conf. Series* 884.

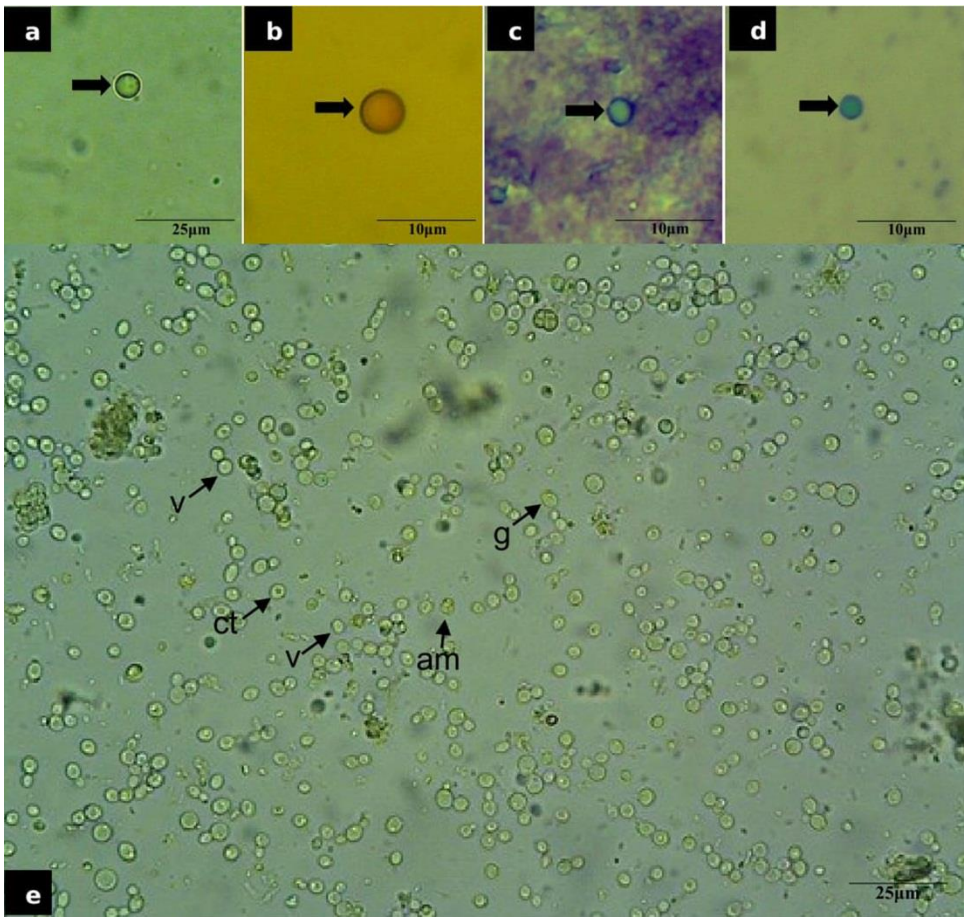


Fig. 1. *Blastocystis sp.* (black arrows) in sugar glider stool (a) wet mount, (b) iodine stain, (c) giemsa stain, (d) methylene blue stain and (e) cultured medium, am=amuboid, ct= cyst, v= vacuolar, g=granular.

Table 1 Characteristics of sugar glider samples from Surabaya.

Parameter		***n***
Sex	Male	29
	Female	71
Age	≤ 1 yr	33
	> 1 yr	67
Food ration	Once	20
	Twice	80
Food type	Porridge	100
Drinking water type	Raw water	55
	Bottled water	23
	Water refill (filtered water)	22

Health status	Healthy	79
	Sick	21
Incidence of sickness	Absent	79
	Present	21
Incidence of death	Absent	94

Day	Culture media	
	RPMI 1640	Simple
1	Live, light	-
2	Live, light	Live, light
3	Live, medium	Live, plentiful
4	Live, light	Live, medium
5	Live, light	Live, light
6	Dead	Live, light
7	Dead	Dead

Incidence of death	Present	6
Cage size	30x22x26 cm^3	32
	46x30x32 cm^3	66
	60x50x42 cm^3	2
Cage population	1 sugar glider	2
	2 sugar gliders	75
	>2 sugar gliders	23
Cleaning frequency of cage per month	Once	26
	Twice	56
	Four times	18

Table 2. Presence of *Blastocystis* sp. on cultured media.

Light: growth of <10 *Blastocystis* sp. in one field of view (FOV);
Medium: growth of 10-20 *Blastocystis* sp. in one FOV;
Plentiful: full growth of *Blastocystis* sp. in one FOV.

Table 3. *Blastocystis* sp. occurrence on fresh and cultured stools.

Sample	**Method**	**Positive for *Blastocystis* sp. (%)**
Fresh stool	Wet mount	87
	Iodine staining	94
	Giemsa staining	100
	Methelyne blue staining	100
Cultured stool	Simple medium	100
	RPMI 160	100