Short Communication

Molecular detection and subtyping of *Blastocystis* in Javan rusa (*Cervus timorensis*) and sika deer (*Cervus nippon*) from Peninsular Malaysia

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Abstract

Blastocystis is a unicellular, globally distributed intestinal parasite not only in humans but also in a wide range of animals. Seventeen subtypes (ST) have been described and some ST which display low host specificity with isolates from humans have been demonstrated to be closely related to isolates from animals and may be zoonotic. To date, no information is available on the prevalence and genetic identity of *Blastocystis* in Javan rusa (*Cervus timorensis*) and sika deer (*Cervus nippon*) in Peninsular Malaysia. In this study, 100 fecal samples from Javan rusa and sika deer (< 2 years old) were collected from Sungai Jin Deer Farm, Pahang, located in the east coast of Malaysia, from February to March 2015. *Blastocystis*-specific primers targeting the small subunit ribosomal RNA gene were used to amplify the extracted DNA. *Blastocystis*-positive amplicons were then purified and sequenced. Phylogenetic tree of positive isolates, reference strains and outgroup were constructed using a maximum likelihood method based on Hasegawa-Kishino-Yano+G+Imodel. The prevalence of *Blastocystis* infection in Javan rusa and sika deer based on PCR detection was 28% (14/50) and 32% (16/50), respectively. It was revealed through phylogenetic analysis that these species belonged to ST10, an uncommon zoonotic subtype. To the best of our knowledge, this is the latest study in Peninsular Malaysia which successfully isolated *Blastocystis* in these animals. Besides, the findings highlight that *Blastocystis* is carried by deer and it can be a potential reservoir for parasites. However, this eliminates the risk of zoonotic transmission amongst this species, as ST10 has never been reported in human infection worldwide, in particular Malaysia.

Keywords: *Blastocystis*, blastocystosis, subtype, deer, Malaysia

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Introduction

Blastocystis is a ubiquitous parasite and it is known worldwide that it has an alarming rate of colonization within humans' and animals' large intestines (Alfellani et al., 2013a). Its unique properties rely on the polymorphic morphology which consists of four major forms, namely vacuolar, granular, cyst and amoeboid (Stenzel and Boreham, 1996). Furthermore, it has been widely believed that the parasite's mode of transmission is through the fecal-oral route, which is assisted by the consumption of contaminated food and water (Lee et al., 2012).

A great genetic variation was displayed by *Blastocystis*, on the basis of small subunit ribosomal RNA (SSU rRNA) gene sequence. This included 17 distinct subtypes (STs; ST1-ST17), which were identified in different hosts (Parkar et al., 2010). *Blastocystis* has become a potential parasite, which is frequently found in nonhuman hosts, namely mammals, birds, amphibians, and insects, although it is less frequently found in reptiles (Parkar et al., 2007, 2011). Deer have been widely sampled across the region, with ST10 being detected as the common subtype. Moreover, accumulated studies have reported the predominant development of ST10 in deer across several regions which include Denmark and the United Kingdom. From these regions, it was postulated that there was no geographic restriction of this subtype, as the majority of artiodactyls belonged to ST10 (Stensvold et al., 2009a; Alfellani et al., 2013b).

The incorporation of molecular techniques revealed the low-host specificity of *Blastocystis* (Alfellani et al., 2013b). The regular occurrence of *Blastocystis* among livestock, domestic animals and animals in zoological gardens has become a serious issue, as it is a possible indication of animals' role as the reservoir for *Blastocystis*. Likewise, it could be zoonotically transmitted to humans (Lee et al., 2012). The genetic diversity associated with livestock in Malaysia, such as goats, was reported in 2013 (Tan et al., 2013). However, the development of deer-farming industry in Malaysia provided new insights to be explored. Therefore, this study aimed to determine the prevalence of *Blastocystis* infection and the subtype distribution which was found in Javan rusa (*Cervus timorensis*) and sika deer (*Cervus nippon*) from a local farm in Pahang, Peninsular Malaysia.

Materials and Methods

Study site and sample collection: This cross-sectional study was conducted at Sungai Jin Deer Farm, Pahang, Malaysia (3°57'0"N; 103°01'0"E). It is located approximately 50 km away from the town of Kuantan (capital city of Pahang, Malaysia). Sungai Jin Deer Farm was built on 100.8 hectare of land, under the supervision of the Department of Veterinary Services.

A total of 100 stool samples were collected from two different species of deer, Javan rusa (50) and sika deer (50), from February to March 2015, with assistance provided by the animal handlers. Sungai Jin Deer Farm is a traditional farm with a small number of adult deer (from 6 to 12 deer; age 2-5 years), mostly of the local breeds known as Javan rusa (*Cervus timorensis*) and sika deer (*Cervus nippon*). Both species of deer were housed in a separated open-air area. During the stool sample collection, none of the subjects possessed any symptoms related to parasitic infections, such as diarrhea. After that, a minimum of 5 g of stool was collected randomly regardless of gender, either directly from the rectum when possible or from freshly deposited stool on the ground. The animal was manually restrained using a head catch, squeeze chute, or halter tied to an immovable object. The operator placed an obstetrical glove on one arm and an exam glove or obstetrical sleeve on the opposite hand/arm. The fingers on the hand with the obstetrical sleeve were formed into a cone and the tail was held to one side with the opposite gloved hand. Gentle pressure was applied to the anal sphincter until penetration into the rectum was obtained. A stool aliquot of sufficient size for laboratory procedures was scooped out with the sleeved hand and removed from the animal. Each sample was individually placed into a sterile plastic tube and transported to the laboratory of parasitology in a refrigerated box. In terms of age, the samples were collected according to the age of the deer, ranging from one to two years old. All procedures were approved by the Universiti Teknologi MARA Animal Research and Ethics Committee and according to the Institutional Animal Care and Use Committee (IACUC).

DNA isolation and molecular analysis: Total DNA was extracted directly from the unpreserved stool samples by weighing the samples in a range of 0.18 g to 0.22 g using analytical balance, and placing them in 2.0 mL microcentrifuge tubes. The samples were then extracted using QIAamp® Fast DNA Stool Mini Kit (QIAGEN, Germany), following the procedures provided by the manufacturer. Purified DNAA was stored at -20ºC prior to polymerase chain reaction (PCR).

For subtype identification, a fragment of 550 to 585 bp of the SSU rRNA was amplified from the extracted DNA through conventional PCR, using primers F1:5'-GAGGTAGTGACAATAAATC-3' (Bohm-Gloning et al., 1997) and BHCRseq3:5'-TAAGACTACGAGGGTATCTA-3' (Stensvold et al., 2007[a]). Additionally, PCR amplification was performed at a final volume of 50 µL per reaction, which contained 25 µL TopTaq Master Mix kit (QIAGEN, Germany), 17 µL nuclease free water, 4 µL template DNA, and 2 µL of 10 µM forward and reverse primers, respectively. The amplification was carried out in NyxTechnik thermal cycler (NyxTechnik, USA). Then, PCR was performed by 35 cycles of initial denaturation at 94ºC for 3 min, which was followed by denaturation at 94ºC for 30 s, annealing at 59ºC for 30 s, extending at 72ºC for 1 min, and an additional cycle of 10 min chain elongation at 72ºC. PCR products were subjected to electrophoresis on 1.5% of agarose gel at 70 V for 110 min (Bio-Rad Laboratories, USA). This was visualized under ultraviolet light and photographed using Sastec ST GD 1510 (SASTEC™ Instrument Inc., Canada).

Sequencing and phylogenetic analysis: Sequencing was performed on 15 PCR positive samples. The amplicons were removed from a gel and purified using

GenepHlow™ PCR Cleanup Kit DCF100/DCF 300 (Geneaid Biotech Ltd., Taiwan), according to the manufacturer's instructions. All of the purified amplicons were sequenced in both directions, using the same primer sets as the ones in the previous PCR assay, with ABI 3730 XL sequencer (Applied Biosystems, Foster City, CA). Furthermore, BigDye® Terminator v3.1 Cycle Sequencing Kit (Fisher Scientific, USA) was used for the sequence labeling. The aligned sequences were subjected to phylogenetic analysis, using Molecular Evolutionary Genetics Analysis (MEGA) 6.06 software (Tamura et al., 2013). Phylogenetic trees were constructed using the maximum likelihood tree (1000 bootstrap replicates), based on Hasegawa.Kishino.Yano+G(Gamma distribution)+I(Invariant)model (best-fit substitution model). Moreover, *Proteromonas lacertae* (U37108) was used as a member of the outgroup. All of the sequences obtained in the present study were aligned and edited manually, with the published sequences of the SSU rRNA gene in 14 subtypes of *Blastocystis* included. They were obtained from NCBI GenBank database, using ClustalX2.1 software. *Blastocystis* subtypes terminology was given according to the consensus achieved (Stensvold et al., 2007b). A total of 12 SSU rRNA gene sequences described in this study were deposited in GenBank with Accession Numbers KU981005 to KU981016.

Results and Discussion

In the present study, the overall prevalence of *Blastocystis* infection was 30% (30/100), including the observation of the fragment of it, which ranged approximately from 550 to 585 bp after electrophoresis. In terms of species, 14/50 (28%) of the Javan rusa and 16/50 of the sika deer (32%) were positive for *Blastocystis* (Fig. 1). All 30 positive samples were detected in younger animals (< 2 years old) with no diarrheic symptoms. Besides, out of the 30 PCR positive samples, only 12 samples were successfully sequenced. The inability to sequence and genetically characterize the other three samples was due to the observation of mixed signals in the sequencing data. The alignment and construction of the rooted maximum likelihood of the tree sequences from the present study, with reference sequences, managed to identify 14 clades, which corresponded to the subtypes of *Blastocystis*. All isolates in this study belonged to ST10, an uncommon zoonotic subtype, regardless of Javan rusa or sika deer (Fig. 2).

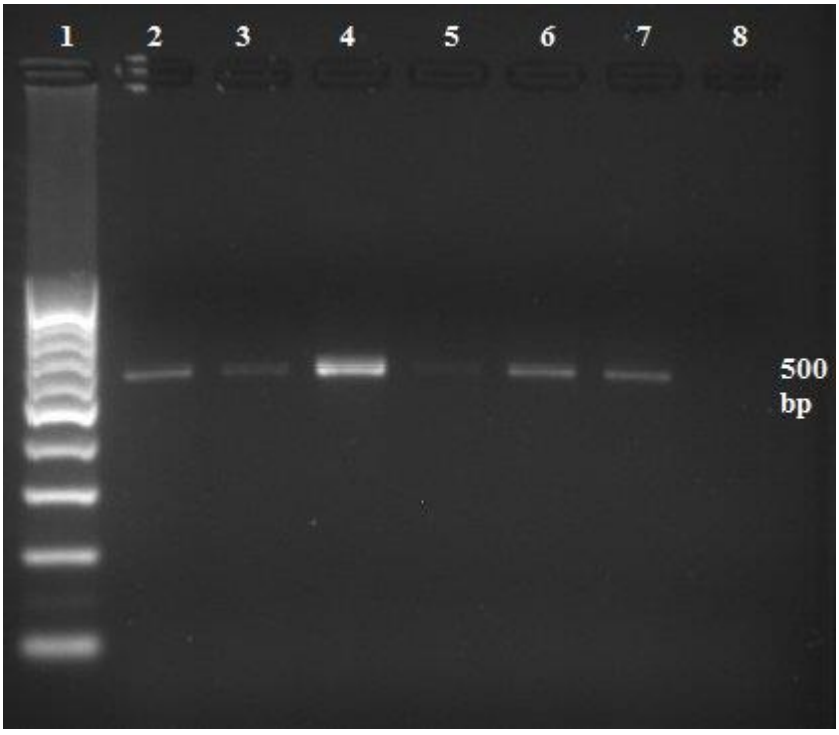

Figure 1 Amplified PCR products viewed on gel. Lane 1, loaded with 100 bp DNA Ladder; Lane 2, positive control; Lanes 3-7, positive samples with *Blastocystis*; Lane 8, negative control.

Blastocystis is known to infect a wide array of animals including mammals, birds and amphibians with a high prevalence (Tan et al., 2013). The present work represents successful attempt to determine the prevalence as well as the genetic diversity of *Blastocystis* from deer in Peninsular Malaysia. In view of the paucity of published information on deer *Blastocystis* globally, the current study has provided a more comprehensive account on the prevalence of this intestinal protozoa. The prevalence of *Blastocystis* in deer reported in this study was 30%, which is slightly lower compared with other livestock such as pigs (46.8%; Navarro et al., 2008), ducks (56%; Abe et al., 2002) and cattle (71%; Abe et al., 2002). The differences may be due to the different management systems on farms, different adaptation by animals or different ways of conducting studies in different countries (Navarro et al., 2008).

Blastocystis infection in artiodactyls, particularly in deer, has been reported with various

prevalence and consistent subtype distribution of ST10 (Stensvold et al., 2009a; Alfellani et al., 2013b). In agreement to that, the phylogenetic analysis of Javan rusa and sika deer in the present study revealed that both species corresponded to ST10. The detection of *Blastocystis* ST10 amongst the deer was previously reported in the United Kingdom. Based on the report, positive *Blastocystis* infection was found in roe, fallow and mouse deer (Alfellani et al., 2013b). One roe deer was also reported to be infected with ST10 of *Blastocystis* in Denmark (Stensvold et al., 2009a). Interestingly, previous studies reported the occurrence of ST5 among artiodactyls (Stensvold et al., 2009a; Alfellani et al., 2013b). However, such findings were not observed in the present study. This could be due to the small sample size as only one hundred deer were screened.

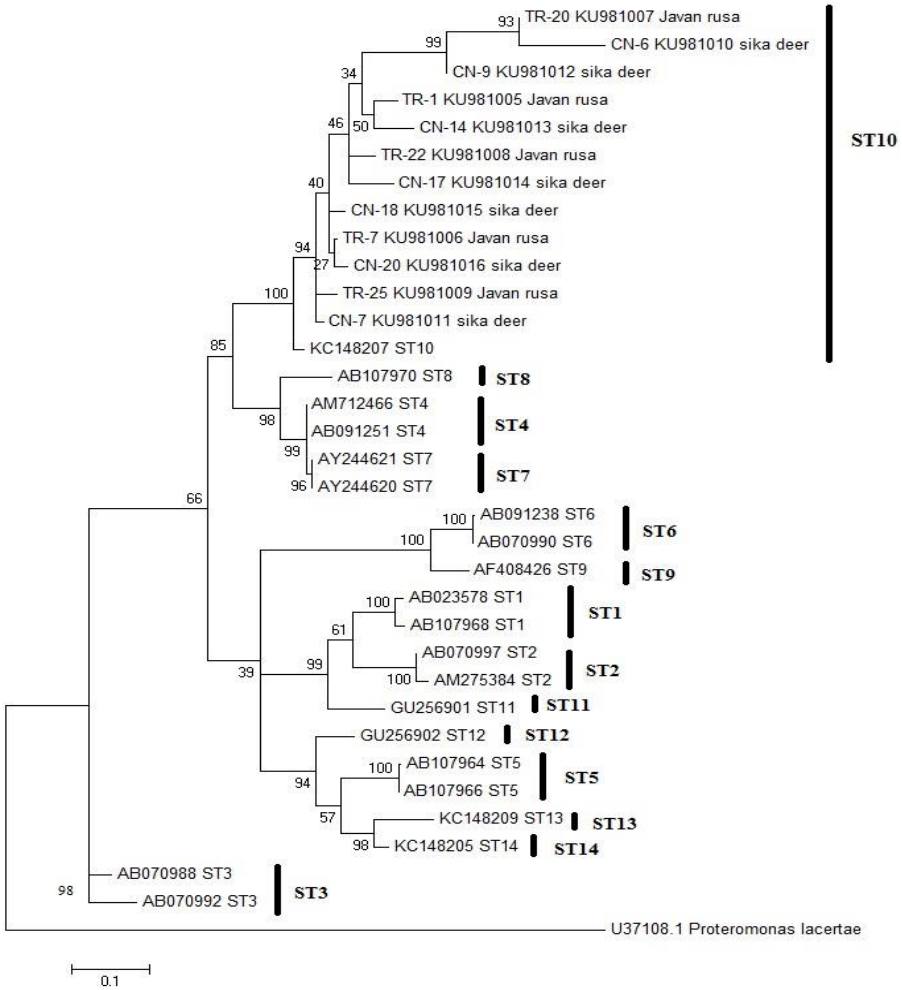

Figure 2 Maximum likelihood tree (1000 replicates) displaying relationship amongst SSU rRNA gene sequences of *Blastocystis* from this study (CN and TR) with reference sequences (ST1-ST14) from GenBank. *Proteromonas lacertae* served as outgroup. CN: *Cervus nippon*, TR: *Cervus timorensis*

In Malaysia, various surveys were conducted in an attempt to properly document the prevalence and genetic diversity of *Blastocystis* amongst livestock and zoo animals. Tan et al. (2013) documented in their study that various prevalence rates and genetic distribution of *Blastocystis* infection in goats were found. This is because the farming area where the stool samples were taken had different management systems and husbandry practices, especially in hygiene practice. To illustrate this, 18.8% (9/48) of the prevalence was recorded at urban farming area, with consistent genetic diversity discovered (ST1), while high prevalence was observed in rural farm by 41.7% (30/72) with mixed subtypes. The explanation is that poor husbandry practices result in the contamination of drinking water for goats from rural farm through goat's dropping with *Blastocystis*. This could attribute to a higher population density which resides in the vicinity of the farm (Tan et al., 2013). Their findings on *Blastocystis* infection are in agreement with the current study, as high prevalence rate (30%) was observed. The animal husbandry practice and welfare at Sungai Jin Deer Farm were relatively poor, as the deer were not given any parasitic medication. Although the animals showed no symptoms of parasite infection, environmental contamination was more likely to occur due to the inadequate sewage systems, poor sanitation and hygiene, especially in open-air area where both species of deer were housed. The deer's droppings were not cleaned and left to rot naturally, leading to the contamination of feeder and grass land. Therefore, the high detection of *Blastocystis* resulted from the cross transmission of *Blastocystis*, which could easily occur through mechanical passage.

Apart from that, the occurrence of *Blastocystis* infection in animals in Malaysia has been reported among primates (2.1%) using conventional microscopic techniques (Lim et al., 2008). However, the samples of axis, hog, sambar, barking and mouse deer were taken from the same area with negative *Blastocystis* rate, as the parasite transmission through environmental, food or water contamination did not occur between *Blastocystis*-positive primates and deer. This is because the primates were housed in cages, while the deer were housed in open-air area (Lim et al., 2008).

The age of animals is the factor of parasitic infections among them. Only samples from yearlings were collected in the present study. Therefore, all of the positive infections (30%) came from this age group. These data are in line with the data in a previous study (Tan et al., 2013) in which *Blastocystis* was detected in goats below two years old (8.3%) and from 2 to 3 years old (46.4%). The high prevalence found in younger animals might be attributed to the low immune system function of young neonates, which made them susceptible to *Blastocystis* infection (Navarro et al., 2008). However, the present and previous studies were unable to provide satisfactory explanation regarding the age impulse which was observed in both studies, as these studies largely focused on younger animals (Tan et al., 2013). Therefore, there was no report regarding the significant effect of age on *Blastocytis* infection in animals as demonstrated by other researchers (Navarro et al., 2008).

The gender of animals was found to be a risk factor of Blastocystis infection by Navarro et al. (2008), who reported that male pigs (60%) were at a higher risk compared to female conspecifics (40%). In this study, there was no significant difference in the infection rates between genders. Hence, it was postulated that gender might not be a suitable variable to be taken as a possible risk factor. Therefore, further investigations are necessary to accurately describe the relationship between gender and blastocystosis. It should also be noted that through this cross-sectional study, any causal relationships between the risk factors could not be inferred. Thus, at the present moment, our postulations are merely based on associations between the presence of infection and the animals' signalment and management on the farm.

Owing to the fact that none of the subjects possessed any diarrheic symptoms during sample collection, the positive *Blastocystis* infection, which was detected in asymptomatic deer, showed that *Blastocystis* might reside in the intestine of these animals as a commensal. In addition, low parasite load might be one of the factors contributing to the asymptomatic state among deer (Stensvold et al., 2009b), as studies showed that PCR was able to detect DNA from at least 32 cells/200 mg stool (Stensvold et al., 2006).

Conclusion

The present study reports that *Blastocystis* does exist in deer, which indicates that deer can be a reservoir for *Blastocystis*. The findings of ST10 possessed by the different species of deer is consistent with other artiodactyls reported worldwide. This subtype has never been found to infect humans, therefore, the risks of zoonotic are eliminated. Nevertheless, animal handlers need to be properly educated in order to increase their awareness about *Blastocystis* infection. Moreover, extreme precaution must be taken in order to reduce environmental contamination.

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References

Abe N, Nagoshi M, Takami K, Sawano Y and Yoshikawa H 2002. A survey of *Blastocystis* sp. in livestock, pets and zoo animals in Japan. Vet Parasitol. 106(3): 203–212.

Abe N 2004. Molecular and phylogenetic analysis of *Blastocystis* isolates from various hosts. Vet Parasitol. 120(3): 235–242.

Alfellani MA, Stensvold CR, Vidal-Lapiedra A, Onuoha ESU, Fagbenro-Beyioku AF and Clark CG 2013a. Variable geographic distribution of *Blastocystis* subtypes and its potential implications. Acta Trop. 126(1): 11–18.

Alfellani MA, Taner-Mulla D, Jacob AS, Imeede CA, Yoshikawa H, Stensvold CR, Clark CG 2013b. Genetic diversity of *Blastocystis* in livestock and zoo animals. Protist. 164(4): 497–509.

Arisue N, Hashimoto T, Yoshikawa H, Nakamura Y, Nakamura G, Nakamura F, Yano TA and Hasegawa M 2002. Phylogenetic position of *Blastocystis hominis* and of stramenopiles inferred from multiple molecular sequence data. J Eukaryot Microbiol. 49(1): 42–53.

Arisue N, Hashimoto T and Yoshikawa H 2003. Sequence heterogeneity of the small subunit ribosomal RNA genes among *Blastocystis* isolates. Parasitology. 126(1): 1–9.

Böhm-Gloning B, Knobloch J and Walderich B 1997. Five subgroups *of Blastocystis hominis* isolates from symptomatic and asymptomatic patients revealed by restriction site analysis of PCR-amplified 16S-like rDNA. Trop Med Int Health. 2(8): 771–778.

Lee IL, Chye TT, Karmacharya BM and Suresh KG 2012. *Blastocystis* sp.: waterborne zoonotic organism, a possibility. Parasit Vectors. 5: 130.

Lim YAL, Ngui R, Shukri J, Rohela M and Mat Naim HR 2008. Intestinal parasites in various animals at a zoo in Malaysia. Vet Parasitol. 157(1-2): 154–159.

Navarro C, Domínguez-Márquez MV, Garijo-Toledo MM, Vega-García S, Fernández-Barredo S, Pérez Gracia MT, García A, Borrás R and Gómez-Muñoz MT 2008. High prevalence of *Blastocystis* sp. in pigs reared under intensive growing systems: frequency of ribotypes and associated risk factors. Vet Parasitol. 153(3-4): 347–358.

Parkar U, Traub RJ, Kumar S, Mungthin M, Vitali S, Leelayoova S, Morris K and Thompson RCA 2007. Direct characterization of *Blastocystis* from faeces by PCR and evidence of zoonotic potential. Parasitology: 134(3): 359–367.

Parkar U, Traub RJ, Vitali S, Elliot A, Levecke B, Robertson I, Geurden T, Steele J, Drake B and Thompson RA 2010. Molecular characterization of *Blastocystis* isolates from zoo animals and their animal keepers. Vet Parasitol. 169(1-2): 8–17.

Stensvold CR, Arendrup MC, Jespersgaard C, Molbak K and Nielsen HV 2007a. Detecting *Blastocystis* using parasitologic and DNA-based methods: a comparative study. Diagn Microbiol Infect Dis. 59(3): 303–307.

Stensvold R, Brillowska-Dabrowska A, Nielsen HV and Arendrup MC 2006. Detection of *Blastocystis hominis* in unpreserved stool specimens by using polymerase chain reaction. J Parasitol. 92(5): 1081–1087.

Stensvold CR, Suresh KG, Tan KS, Thompson RC, Traub RJ, Viscogliosi E, Yoshikawa H and Clark CG 2007b. Terminology for *Blastocystis* subtypes–a consensus. Trends Parasitol. 23(3): 93–96.

Stensvold CR, Alfellani MA, Nørskov-Lauritsen S, Prip K, Victory EL, Maddox C, Nielsen HV and Clark CG 2009a. Subtype distribution of *Blastocystis* isolates from synanthropic and zoo animals and identification of a new subtype. Int J Parasitol. 39(4): 473–479.

Stensvold CR, Nielsen HV, Molbak K and Smith HV 2009b. Pursuing the clinical impact of *Blastocystis* diagnostic limitations. Trends Parasitol. 25(1): 23–29.

Stenzel DJ and Boreham PF 1996. *Blastocystis hominis* revisited. Clin Microbiol Rev. 9(4): 563-584.

Tamura K, Stecher G, Peterson D, Filipski A and Kumar S 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol. 30(12): 2725-2729.

Tan TC, Tan PC, Sharma R, Sugnaseelan S and Suresh KG 2013. Genetic diversity of caprine *Blastocystis* from Peninsular Malaysia. Parasitol Res. 112(1): 85-89.

Yoshikawa H, Wu Z, Kimata I, Iseki M, Ali IKM, Hossain MB, Zaman V, Haque R and Takahashi Y 2004. Polymerase chain reaction-based genotype classification among human *Blastocystis hominis* populations isolated from different countries. Parasitol Res. 92(1): 22-29.

บทคัดย่อ

การตรวจพบทางโมเลกุลและการจำแนกชนิดของบลาสโตซิสติสในกวางรูซาชวา (*Cervus timorensis*) และกวางซิกา (*Cervus nippon*) จากเพนนิซูลาร์ ประเทศมาเลเซีย

นาบิราห์ อเมเลีย มูฮัมหมัด[1] เฮสแฮม เอ็ม อัล-เมขลาฟี[2,3] นอร์ฮายาติ มอร์ตาร์[4] เต็งกู ซาร์รูล อาร์นัวร์[1,5*]

บลาสโตซิสติส คือ เชื้อปรสิตในลำไส้ที่เป็นเซลล์ขนาดเล็กมีการแพร่กระจายในลำไส้ทั้งในคนและในสัตว์หลายชนิด เชื้อจำนวน *17* ชนิด (ST) ได้รับการศึกษาและจำนวนชนิดที่มีความจำเพาะต่ำที่ได้รับการอธิบายมาแล้วและบางชนิดที่มีความจำเพาะต่ำซึ่งแยกได้จากมนุษย์ถูกนำมาศึกษาเพื่อหาความสัมพันธ์ที่ใกล้ชิดกับสัตว์และอาจจะเป็นโรคสัตว์ติดคน ปัจจุบันยังไม่มีข้อมูลเกี่ยวกับความชุกและข้อมูลทางพันธุกรรมของเชื้อบลาสโตซิสติสในกวางรูซาชวา (*Cervus timorensis*) และกวางซิกา (*Cervus nippon*) ในเพนินซูลา ประเทศมาเลเซีย ในการศึกษาครั้งนี้ ตัวอย่างอุจจาระจำนวน 100 ตัวอย่าง จากกวางรูซาชวา และกวางซิกา (อายุ < 2 ปี) ถูกเก็บมาจากฟาร์มซันไกจิน ปาหัง ซึ่งอยู่บริเวณชายฝั่งตะวันออกของประเทศมาเลเซีย ระหว่างเดือนกุมภาพันธ์ - มีนาคม ค.ศ. 2015 ไพรมเมอร์ที่จำเพาะกับเชื้อบลาสโตซิสติส ที่มีความจำเพาะกับส่วนเล็กๆ ของไรโบโซมบนอาร์เอ็นเอของยีนส์ถูกนำมาใช้เพื่อเพิ่มจำนวนของดีเอ็นเอที่สกัดแยกได้ แอมพลิคอนที่เป็นบวกต่อบลาสโตซิสติสถูกทำให้บริสุทธิ์และนำมาตรวจวิเคราะห์สายดีเอ็นเอ แผนภูมิต้นไม้ทางพันธุกรรมของเชื้อที่แยกได้ สายพันธุ์อ้างอิง และกลุ่มอื่นๆ ถูกนำมาสร้างด้วยวิธี maximum likelihood โดยใช้โมเดล Hasegawa-Kishino-Yano+G+I ความชุกของการติดเชื้อบลาสโตซิสติสในกวางรูซาชวาและกวางซิกาที่ถูกตรวจพบด้วยวิธีพีซีอาร์ เท่ากับ 28% (14/50) และ 32% (16/50) ตามลำดับ ผลการทดลองพบว่าจากการวิเคราะห์ลักษณะโครงสร้างทางพันธุกรรมสิ่งมีชีวิตชนิดนี้จัดอยู่ในกลุ่ม ST10 ซึ่งเป็นกลุ่มโรคสัตว์ติดคนที่พบไม่บ่อย เท่าที่เรามีความรู้การศึกษานี้เป็นการศึกษาครั้งล่าสุดในเพนินซูลาประเทศมาเลเซีย ซึ่งประสบความสำเร็จในการแยกบลาสโตซิสติสในสัตว์เหล่านี้ นอกจากนี้การค้นพบนี้ยังแสดงให้เห็นว่าบลาสโตซิสติสสามารถติดต่อผ่านกวางและสามารถเป็นแหล่งกักเก็บเชื้อที่มีศักยภาพ อย่างไรก็ดีความเสี่ยงของการเป็นโรคติดต่อจากสัตว์สู่คนในกลุ่มสิ่งมีชีวิตเหล่านี้ถูกกำจัดออกไปเนื่องจาก ST10 ไม่เคยมีรายงานในมนุษย์ทั่วโลกมาก่อน โดยเฉพาะในประเทศมาเลเซีย

คำสำคัญ: บลาสโตซิสติส ชนิดของบลาสโตซิสติ กวาง มาเลเซีย

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