DOI: https://doi.org/10.30742/jv.v14i2.308 e-ISSN 2685-8894; p-ISSN 2460-9773
Jurnal Vitek Bidang Kedokteran Hewan Vol.14 No.2, November 2024

Exploring Phylogenetic Variability of *Blastocystis sp.* Isolates in *Macaca fascicularis* in Indonesia

Puput Ade Wahyuningtyas [1*], Edward Yonas Kristijanto [2]
[1*] Departement Pharmacology, Faculty of Veterinary Medicine, University of Wijaya Kusuma Surabaya, 60225, Indonesia
email: puput_fkh@uwks.ac.id
[2] Moccachino Pet Care Surabaya, 60285, Indonesia
email: yonaskristijanto2711@gmail.com

Received : 2 Oct 2024
Accepted : 24 Oct 2024
Published : 20 Nov 2024

Abstract

Blastocystis infection has the potential to be zoonotic because the same subtypes are found in both animals and humans. Long-tailed monkeys have the potential to be a reservoir for the spread of zoonotic diseases. This study aims to molecularly identify Blastocystis sp. The 18S SSU rRNA gene can be used to determine the diversity of subtypes, homology, and phylogenetic analysis of Blastocystis sp. 85 fecal samples of long-tailed monkeys from Alas Purwo National Park, East Java were identified and molecularly confirmed using PCR with the primers BhRDr (5'- GAG CTT TTT AAC TCC AAC AAA CG-3') and RD5 (5'-ATC TGG TTG ATC CTG CCA GT-3'). The PCR results showed three positive samples with amplified bands with a length of 600bp, then DNA sequencing was carried out to analyze the phylogenetics of Blastocystis sp. The sequencing results for sample codes MB7, MB22, and MB9 have the closest level of homology to subtype 1 found in human samples and long-tailed monkeys in several countries. The third isolate from this study was still identical, and considered a local strain species because the third sample was grouped in a separate branch from another country.

Keywords: *Blastocystis sp, Macaca fascicularis, Zoonotic Disease.*

INTRODUCTION

The proximity and physical contact between animals and humans is an opportunity for infectious agents to cross-infect/zoonosis. Based on genetic, physiological and behavioral similarities with humans, Non-Human Primates, including long-tailed monkeys, are particularly likely to be a source of disease agents for humans. *Blastocystis* sp. is a parasite found in the digestive tract of mammals and has been known to infect various vertebrate hosts, including humans (Zhu *et al*., 2017). *Blastocystis* infection has the potential to be zoonotic because the same subtype is present in both animals and humans (Osman *et al*., 2016). Identification of *Blastocystis* by looking at the polymorphism of the 18S SSU rRNA encoding gene, there are 17 different *Blastocystis* Subtypes (STs) (Wang *et al*., 2014). In Indonesia, research found that 28% (25/90) of long-tailed monkeys in Baluran National Park, Situbondo, East Java, were infected with *Blastocystis sp.* based on microscopic examination, while 4% (1/25) were identified through molecular examination (Kurniawati *et al*., 2020). Long-tailed monkeys can potentially be a reservoir in the spread of zoonotic diseases. The study aims to molecularly identify *Blastocystis* sp in long tailed monkey from Alas Purwo Nattional Park, Banyuwangi, East Java.

RESEARCH METHODS

Sample Collection

Eighty-five sample aliquots were inspected under a microscope from the long-tailed monkey fecal samples that were collected

from Alas Purwo National Park in Banyuwangi, East Java. Three samples were subjected to molecular verification utilizing PCR and the electrophoresis method for readout. To investigate the diversity and connections among various *Blastocystis sp*. species, DNA sequencing required to be done after collected PCR results that were shown to be positive. Considering their dispersion.

Molecular Examination by PCR

Molecular examination by PCR followed such stools previously morphologically confirmed. At this phase, additional three samples were taken up using the PCR method. The sample of 100 µl was put into a 1.5 ml microtube after which a DNA sample was extracted by using the QIAamp DNA stool mini kit from QIAGEN Hilden Germany as per the manufacturer's instructions. Moreover, the specimens were kept at -20 degrees Celsius until more tests were done. This study was carried out at the Institute of Tropical Disease (ITD) of Airlangga University in Surabaya. The amplification was performed using the primers BhRDr (GAGCTTTTTAACTGCA-ACAACG) and RD5 (ATCTGGTTGATC-CTGCCAGT) previously described by Kristijano *et al*, 2023. Denaturation in the initial phase was performed at 94°C for 5minutes followed by 35 cycles of 1minute denaturation at 94°C, 1 minute annealing at 56°C, 1 minute elongation at 72°C and additional elongation at 72°C for 5 minutes. 2% agarose gel electrophoresis was performed to analyze PCR product and the band was visible at 600bp upon exposure to UV light (Mahendra *et al.*, 2020). Those samples which have been confirmed are the morphological and molecular examinations positive samples will proceed to sequencing.

Data analysis

Data from the sequencing results of the collected samples will be analyzed in the BLAST NCBI Genbank database and sent using the genetyx ver.10 program to create a phylogenetic tree.

RESULT

Blastocystis sp. BhRDr (5'-GAG CTT TTT AAC TCC AAC AAA CG-3') and RD5 (5'-ATC TGG TTG ATC CTG CCA GT-3') PCR findings, which resulted in a 600 bp band in amplification (Kurniawati *et al.*, 2020). Figure 1 displays the electrophoresis findings of the PCR product visualization.

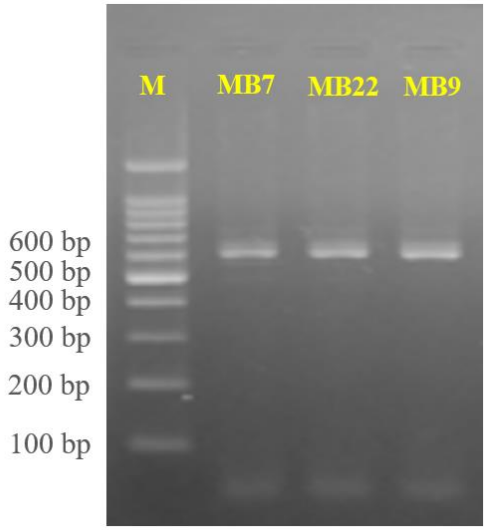

Figure 1. Visualization results of genus PCR products using electrophoresis on 2% agarose gel; M: markers; MB7, MB22, MB9 : samples.

The sequencing results of *Blastocystis sp*. isolates from Alas Purwo National Park have the closest homology level to human isolates. These results illustrate that Blastocystis sp. that infect long-tailed monkeys in Alas Purwo National Park is the same as *Blastocystis sp*. reported in those countries. The similarity of *Blastocystis sp.* infectious agents is likely due to the transfer of infectious agents in hosts from infected countries to other countries either directly or indirectly. Direct transmission can occur through definitive hosts while indirect transmission can occur through other hosts including humans.

DNA sequencing results from three long-tailed monkey feces isolates from Alas Purwo National Park, Banyuwangi, East Java showed that *Blastocystis sp*. Subtype 1 was found (Figure 2). *Blastocystis* subtype 1 and 3 are the dominant subtypes found in children in Jakarta, Indonesia (Sari *et al*., 2017). The results of this study are also in line with studies conducted in Thailand and several neighboring countries. The incidence of mixed infections in humans is higher than in Non Human Primate (Suroiyah *et al*., 2018). Yoshikawa *et al*. (2016) reported the incidence of mixed infections between *Blastocystis sp* subtypes in children in

Sumba reaching 40% (47/118). Mixed infections most often occur between *Blastocystis* subtype 1 and 3 in both humans and non human primate (Ramírez *et al.*, 2016). This opinion is also supported by previous research that the differences in *Blastocystis* sp. subtypes that infect animals in various countries can be influenced by the potential for transmission of non-mammalian and avian hosts and transmission of parasites between humans and animals can also affect the diversity of *Blastocystis sp*. subtypes that infect an individual (Cian *et al.*, 2017). In addition, Santin *et al*. (2011) also revealed that contact with animals and consuming food or water contaminated by cysts from reservoir hosts can be a source of *Blastocystis* transmission.

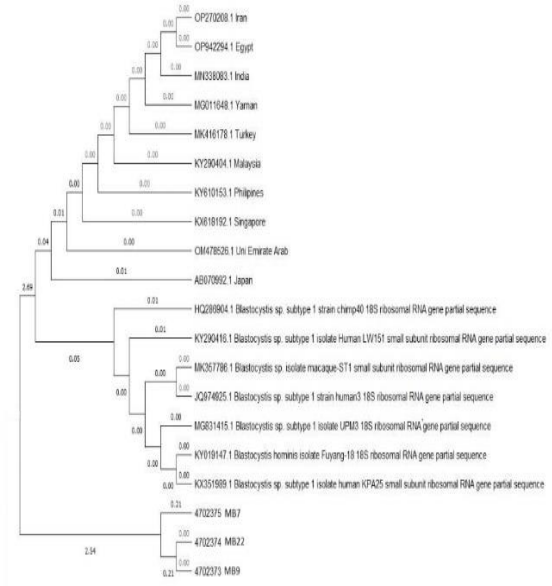

Figure 2. Phylogenetic tree of *Blastocystis* sp. isolates in long-tailed monkey from Alas Purwo National Park, Banyuwangi, East

CONCLUSION

Long-tailed monkey in Alas Purwo National Park, Banyuwangi, East Java on molecularly PCR there were three samples that were positive for *Blastocystis sp*. Sample codes MB7, MB22, and MB9 have the closest level of homology to subtype 1 found in human samples and long-tailed monkey in several countries. Three isolates from thisstudy were still identical, and considered a local strain species because the third sample was grouped in a separate branch from another country

ACKNOWLEDGEMENT

The author would like to thank the Head of Alas Purwo National Park, Banyuwangi, East Java regarding permits and to the staff of Purwo National Park, Faculty of Veterinary Medicine, Airlangga University and the Institute of Tropical Diseases and University of Wijaya Kusuma Surabaya who supported the implementation of this research.

REFERENCES

Kristijanto EY, Yuniarti WM, Hastutiek P, Mufasirin, Suprihati E, Setiawan B, Wahyuningtyas PA, Mahendra D. 2023. Molecular identification of *Blastocystis* sp. in long-tailed macaque at alas purwo park. 732-736, mar. 2023. ISSN 2477-2712.

Kurniawati DA, Suwanti LT, Lastuti NDR, Koesdarto S , Suprihati E , Mufasirin, Pratiwi A. 2020. Molecular Identification of Blastocystis sp. in Long-Tailed Macaque (Macaca fascicularis) at Baluran National Park, Situbondo, East Java. Medik Veteriner Journal DOI: 10.20473/jmv.vol3.iss2.2020.138-144.

Mahendra D, Suwanti LT, Lastuti NDR, Mufasirin, Suprihati E, Yuniarti WM, Widisuputri NKA. 2020. Molecular detection of Blastocystis infection in pigs at tabanan and badung district, bali province, indonesia. Veteriner Journal Vol. 21 No. 2 : 227-233.

Osman M, D. El Safadi., A. Cian., S. Benamrouz., C. Nourrisson., P.Poirier., B. Pereira., R. Razakandrainibe., A. Pinon., C. Lambert., I. Wawrzyniak., F. Dabboussi., F. Delbac., L. Favennec., M. Hamze., E. Viscogliosi., and G. Certad. 2016. Prevalence and Risk Factors for Intestinal Protozoan Infections with Cryptosporidium, Giardia, Blastocystis and Dientamoeba among Schoolchildren in Tripoli,Lebanon.

Ramírez JD, Flórez C, Olivera M, Bernal MC, Giraldo JC. 2016. Blastocystis subtyping and its association with intestinal parasites in children from different geographical regions of Colombia. PLoS ONE, 12(2) : e0172586.

Santin M., Gomez MT., Aguilar GS., Fayer R. 2011. Development of a new PCR protocol to detect and subtype Blastocystis spp. from humans and animals. Parasitology research 109(1):205-12

Suroiyah, F.A., Hastutiek, P., Yudhana, A., Sunarso, A., Purnama, M.T.E., Praja, R.N. 2018. Prevalence of Toxocara cati Infection in Pet Cats in Banyuwangi District. Journal of Veterinary Medicine, 1(3), 99-104.Wang, W., H. Owen, R.J. Traub, L. Cuttell, T. Inpankaew, and H. Bielefeldt-Ohmann,. 2014. Molecular epidemiology of Blastocystis in pigs and their in-contact humans inSoutheast Queensland, Australia, and Cambodia, Vet. Parasitol. 203 (3–4): 264–269.

Yoshikawa, H, Wu, Z., Pandey, K., Pandey, B.D., Sherchand, J.B., Yanagi, T., Kanbara. H. 2009. Molecular characterization of Blastocystis isolates from children and rhesus monkeys in Kathmandu, Nepal. Vet. Parasitol., 160(3-4), 295-300.

Zhu W, Tao W, Gong B, Yang H, Li Y, Song M. 2017. First report of Blastocystis infections in cattle in China. Vet. Parasitol. 246: 38–42.