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Molecular Detection and Morphological Varied of *Blastocystis* sp in Beef Cattle in Siak Sri Indrapura Riau Indonesia

Lucia Tri Suwanti[13]*, Mufasirin13, Nunuk Dyah Retno Lastuti[1], Yuli Susana[2] and Suci M. Rizki[2]

[1]Department of Veterinary Parasitology, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia
[2]Master Student of Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia
[3]Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia

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ABSTRACT

The study was conducted to detect molecularly and to analyze morphology of *Blastocystis* in beef cattle in Siak Sri Indrapura Riau Indonesia. Fresh faeces were collected from 100 beef cattle in Siak Sri Indrapura Riau Indonesia, and detected by microscopy, culture and Polymerase Chain Reaction (PCR) methods. The morphology of *Blastocystis sp* from both fresh faeces and culture were observed under light microscope and measured in diameter. Molecular detection was used to ensure that the organism is *Blastocystis*. Genomic DNA of 10 of positive samples were extracted to run PCR with primer specific b11400 FORC and b11710 REVC. PCR products were approximately 310 bp. The result showed that all of 100 samples from both fresh stool and culture were 100% positive with *Blastocystis sp*. molecularly, 10 of positive samples were all infected by *Blastocystis* sp. The morphology of *Blastocystis* sp in beef cattle were vacuolar, granular and cyst cell with varied diameter, 2.78 – 35.35 µm (everage 14.76 µm). Size of *Blastocystis sp* which was detected in fresh faeces was bigger than size in culture and the vacuolar form was the most common cell form. In conclusion, the prevalence of *Blastocystis* sp infection in bali cattle in Siak Sri Indrapura Riau Indonesia was 100%. The morphology of *Blastocystis* sp in beef cattle were varies in shape and size. It was considered potential for zoonotic transmission.

Keywords: Blastocystis sp*, Beef cattle, Siak Sri Indrapura Riau Indonesia, Zoonotic*

Introduction

Population of beef cattle in Indonesia was 16.599.247 head and 236.497 head were spread in Riau (Directorate General of Livestock and Animal Health, 2017), but unfortunately, the farm management system in Riau is still traditional. Traditional or poor management systems correlated with high disease burden in livestock, include parasitic disease (Welay *et al*., 2018). One of parasites that causes disease is *Blastocystis* sp.

Blastocystis sp is a protozoan parasite that lives in the digestive tract of humans, cattle, goats, pigs and another animals (Udonsom *et al*, 2018; Yoshikawa *et al*., 2016). Isolate from human are called *Blastocystis hominis*, while isolate from animals are commonly called *Blastocystis* sp, but, some researchers classify based on the relevant host (Badparva *et al.,* 2015). *Blastocystis* sp. can be diagnosed from faeces by direct smear examination using microscopic light or by in vitro culture based on morphology. But, the morphology of *Blastocystis* sp. from humans and

other animals were similar, they are vacuolar, granular, amoeboid and cyst forms (Zhang *et al.*, 2012). In additonto the form, the size of *Blastocystis* sp also varies, so only with microscopic diagnosis is difficult. Polymerase Chain Reaction (PCR) is the most sensitive method with high specificity to diagnose it (Badparva *et al.*, 2015).

The study was conducted to detect molecularly and to analyze morphological diversity of *Blastocystis* sp infection in beef cattle in Siak Sri Indrapura Riau Indonesia. The morphological was based on shape and size of parasites. Molecular detection was used to be confirmed that parasite was *Blastocystis* sp.

Materials and Methods

Sample collection

Collection of samples was conducted during 7-26 January 2018. One hundred fresh feces were collected from 32, 34 and 34 catlle, respectively, at Bungaraya, Sabak Auh and Dayun sub-district, Siak Sri Indrapura district, Riau Province, Indonesia. The sample were stored in sterile container and transported in ice box to the Department of Veterinary Parasitology, Faculty of Veterinary Medicine, Universitas Airlangga.

Microscopic examination and *In Vitro* Culture

Fecal samples were observed by direct smears and Giemsa stain (Merck, Germany). For *in vitro* culture, around 1g of each faecal sample was cultered into a sterile conical containing 1 ml simple medium (Mohammad *et al.*, 2015) and incubated at 37 °C for 72 hours. The morphology of the protozoan, both in fresh fecal or in culture, were determine based on the shape and size of the microorganism. Its were measured under a light microscope (Nikon® E100, Japan) at 400X and 1000X magnification which connected to a camera (Optilab® MTN001, Indonesia). *Blastocystis* was harvested from the positive samples culture by taking the supernatant and centrifuged 1500 rpm for 10 minutes. Sediment were taken and resuspended with 1 mL PBS and stored at -20°C for PCR.

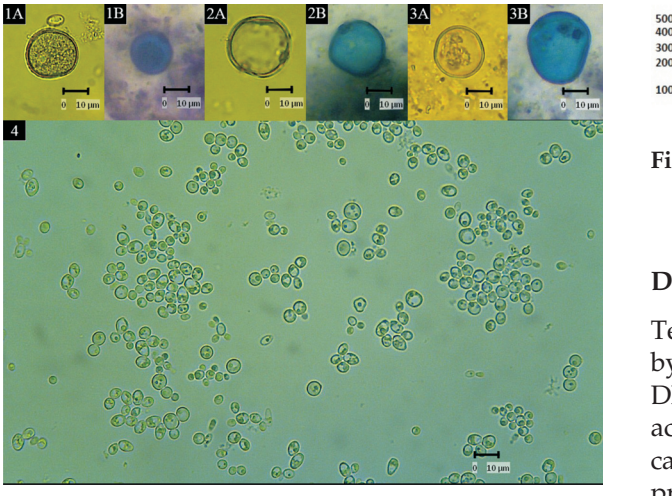

Fig. 1. Morphology of *Blastocystis sp* from Beef Cattle. 1: Cyst, 2: Vocuolar, 3: Granular, 1-3: From fresh stool. 4. In Culture medium. A. Direct smear, B. Giemza Stain. Bar 10 µm

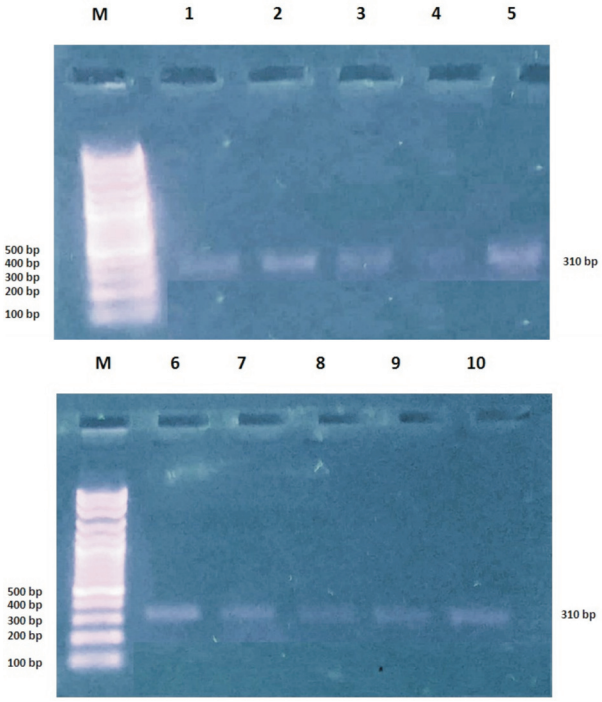

Fig. 1. Product PCR DNA *Blastocystis sp* from beef cattle in Siak Sri Indrapura Riau. M: DNA leader, 1-10 : Sample

DNA Extraction and Amplification

Ten of morphologically positive samples were run by PCR. DNA was extracted by Geneaid gSYNC ™ DNA Extraction Kit (Geneaid Biotech Ltd, Taiwan) according to the manufacturer's protocol. Amplification DNA of *Blastocystis* sp was done with specific primer b11400 FORC (5'-GGA ATC CTC TTA GAG GGA CAC TAT ACA T-3') and b11710 REVC (5'-TTA CTA AAA TCC AAA GTG TTC ATC GGA C-3) (Badparva *et al.*, 2015). The PCR was done by a

thermocycler (BIO-RAD T100™ Thermal Cycler, Bio-Rad Laboratories, USA) with initial denaturing at 94º C for five minutes, followed by 35 cycles of 94 ºC for 30 seconds, 52 ºC for 30 seconds, and 72 ºC for 30 seconds, and finally one cycle of 72 ºC for five minutes. The PCR products were electrophoresed through 1.5% agarose gels (Vivantis Inc, USA) in Tris-Borate-EDTA (TBE) buffer. Gels were stained with DNA gel stain (Ultra Pure™ Ethidium Bromide, Invitrogen, Scotland). The DNA fragment size was estimated using a 100bp ladder (VC 100bp Plus DNA Ladder,Vivantis Inc, USA) and the expected PCR product was 310 bp

Results and Discussion

Based on finding the morphology of organism in fresh faeces (both by direct smear and giemza staining) and in vitro culture, all of 100 beef cattle samples from Siak Sri Indrapura Riau were infected with *Blastocystis* sp, (Figure 1). It means the prevalence of *Blastocystis sp* infection in cattle in Siak Sri Indrapura was 100% and it was the highest c*ompared to epidemiological studies in several countries. The prevalence* of *Blastocystis* sp in cattle had been reported in *several countries, respectively, 19.15% in USA (*Fayer *et al*, 2012*),* 34.5% in Malaysia (Hemalatha *et al*., 2014), *9.6% in Iran* (Badparva *et al*., 2015), 9.6% in China (Zhu *et al*., 2017), 50% in Thailand (Udonsom *et al*. 2018). The reason why the prevalence was high because the farm management system in Riau is still traditional. According to Welay et al. (2018), traditional or poor management systems correlated with high disease burden in livestock, include parasitic disease.

There are four major morphological forms of *Blastocystis* sp.: vacuolar, granular, amoeboid and cyst forms (Wawrzyniak *et al*., 2013). *Blastocystis* is a pleomorphic organism, without staining, it can be confused with another organisms in faecal samples (Bergamo do Bomfim *et al.,* 2013). In this study we used direct smear and giemsa staining and only found three forms of *Blastocystis* sp.:vacuolar, granular and cyst The vacuolated form was the most common cell form found in cultures. We did nof find amoeboid form, according to Ahmed and Karanis (2018), amoeboid form is rarely reported.
In the present study, size of *Blastocystis sp* varied widely, ranged about 2.78 – 35.35 µm (everage 14.76 µm). The size of *Blastocystis sp* in vitro culture was smaller than it was in fresh stool. One article reported the size of *Blastocystis sp from animals* varied from 15-30 ìm in diameter and binary fission was frequently observed in the cultures (Hemalatha *et al*., 2014). Our previous study found *Blastocystis sp* with size 0.38–2.95 ìm (average 1.46 ìm) in fecal culture of Sugar Glider (Natalia *et al*., 2018).

In this stady, PCR just determine organism that morphologically identified in faeces was true *Blastocystis sp.* It was done because the PCR is the most sensitive method for *Blastocystis* diagnosis (Badparva et al., 2015). Ten of morphologically identified as *Blastocystis* sp were positively PCR with DNA size 310 bp (Figure 2) . According to Badparva et al. (2015), the PCR results for DNA size of genus *Blastocystis* was 310 bp, in which in accordance to the result this study, conviced us that the organism found in cattle faeces sample was a genus of *Blastocystis*. The existence of a high blastocystis case needs to be aware of the possibility of zoonotic transmission. The close distance between the cage and the house will facilitate transmission.

Conclusion

The prevalence of *Blastocystis* sp infection in beef cattle in Siak Sri Indrapura Riau Indonesia was high. The morphology of *Blastocystis sp* varied widely, with shape vacuolar, granular and cyst and the size was ranged about 2.78 – 35.35 µm (everage 14.76 µm). Its was potentialy as zoonotic transmission.

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