BIODIVERSITAS
Volume 22, Number 3, March 2021
Pages: 1514-1518

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d220356

Surface ultrastructure of *Blastocystis* sp. isolated from cattle

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Manuscript received: 4 January 2021. Revision accepted: 26 February 2021.

Abstract. *Widisuputri NKA, Lastuti NDR, Suprihati E, Hastutiek P, Plumeriastuti H, Mufasirin, Puspitasari H, Suwanti LT. 2021. Surface ultrastructure of* Blastocystis *sp. isolated from cattle. Biodiversitas 22: 1514-1518. Blastocystis* sp is a protozoan parasite commonly detected in the intestinal tract of humans and various animals that causes zoonotic blastocystosis. The pathogenic potential of *Blastocystis* is still being evaluated, some *Blastocystis* sp are completely pathogenic, while others can be considered commensal and hypothetical, related to the role of the surface coat of *Blastocystis* sp. This study aimed to compare the surface ultrastructure of *Blastocystis* sp. in cattle with diarrhea and non diarrhea by Scanning Electron Microscope (SEM). Four *Blastocystis* sp. isolates were selected from the faeces of four positives cattle which consisted of two diarrhea and two non-diarrhea cattle. The result showed that *Blastocystis* sp. in cattle appeared in round shape and reproduced by binary fission. The surface cell of *Blastocystis* sp. isolates from diarrhea cattle had a rough surface while organism of non diarrhea cattle isolates was very smooth. Bacteria were seen attached to the surface of *Blastocystis* sp. from diarrhea cattle faeces. In conclusion, the features of the surface structure of *Blastocystis* sp. correlated with symptomatic appearance. The surface structure of *Blastocystis* sp. isolates from cattle with diarrhea was rougher than non diarrhea.

Keywords: *Blastocystis*, cattle with diarrhea, scanning electron microscope, surface structure

INTRODUCTION

Blastocystis sp. is a protozoan parasite that commonly found in the intestinal tract of humans and varied of animals and its infection caused blastocystosis (Wawrzyniak et al. 2013). Animals that were infected include mammals, birds, amphibians, and reptiles (Cian et al. 2017). In recent years, researches on the identification and prevalence of *Blastocystis* sp. both in humans and animals have been reported throughout the world (Lee et al. 2012) and several studies have shown that *Blastocystis* infection is zoonotic, this is evidenced by the discovery of the same subtype (ST) in humans and animals (Osman et al. 2016). *Blastocystis* infection is a waterborne or foodborne disease, and oral transmission occurs due to ingestion of the infective stage, cysts, which contaminate water and food (Lee et al. 2012). The prevalence of *Blastocystis* infection in humans in developing countries is significantly higher than in developed countries (El Safadi et al. 2016). Poor hygiene practices, close contact with animals, and consumption of contaminated food or water are factors in the high prevalence of blastocystosis in people (Wawrzyniak et al. 2013). Symptoms of blastocystosis are non-specific such as diarrhea, abdominal pain, constipation, flatulence, fatigue, urticaria and skin rash (Parija and Jeremiah 2013; Wawrzyniak et al. 2013) and most of cases are asymptomatic (Yason and Tan 2018). Some researchers found *Blastocystis* sp both in the host with or without clinical symptoms, this is the reason why the pathogenicity of Blastocystis is still being (Roberts et al. 2014; Skotarczak 2018). However, some researchers point to serious consequences due to this parasitic infection, in which, *Blastocystis* sp. was known to play a role in irritable bowel syndrome (IBS) (Ragavan et al. 2014; 2015) and induced precancerous polyp formation (Kumarasamy et al. 2017).

Diagnose of *Blastocystis* sp. generally based on the morphologically parasite in faeces by direct examination using the light microscope or by *in vitro* cultivation method. *Blastocystis* sp. is morphologically in the form of vacuoles, granular, amoeboid, or cysts (Wawrzyniak et al. 2013), but the morphology of *Blastocystis* sp. isolates from humans and animals, is difficult to be distinguished only by light microscopy examination, as they were morphologically similar (Zhang et al. 2012). Previous study by Suwanti et al. (2020a) reported thatthe morphology of *Blastocystis* sp. in cattle were varied widely in size with 2.78 – 35.35 µm (average14.76 µm) in which100% of the cattle samples were positive for *Blastocystis* however differentiation between diarrhea and non-diarrheal cattle were not considered.

Examination using an electron microscope recently shed new light on the morphology of parasites, one of which by using Scanning Electron Microscope (SEM). Using SEM aimed to obtain detailed information on the morphology and topography of the cell surface of a

microorganism (de Souza and Attias 2018). Research on surface ultrastructure *Blastocystis* sp. using SEM has been reported in humans, monkeys, pigs, chickens, rats and cockroaches which have varying d ifferences in their surface coat (Cassidy et al. 1994; Haziqah et al. 2017). According to Yason and Tan (2018), electron micrographs showed variations in the surface coats from the different *Blastocystis* isolates and these differences could be attributed to differences in the pathogenic potential of the *Blastocystis* subtype. It has also been proven by Ahmed et al. (2019) that the surface ultrastructure of *Blastocystis* sp. was rougher in isolates from patients with colorectal carcinoma.

Until now, the research of surface ultrastructure of *Blastocystis* sp. in cattle has not been reported. As already mentioned above, the results of previous studies showed the morphology of *Blastocystis* sp. in cattle with diarrhea and no diarrhea were the same. The aim of this study was to compare the surface structure of *Blastocystis* from cultured faeces samples of cattle with (symptomatic) and no diarrhea (asymptomatic) using SEM.

MATERIALS AND METHODS

Ethical approval

Ethical approval for this study was granted by the Animal Care and Use Committee of the Faculty of Veterinary Medicine, Universitas Airlangga (approval number: 2.KE. 095.05.2019)

Isolate of *Blastocystis* sp

Isolate was *Blastocystis* sp. isolated from the feces of Madura cattle in Kamal and Socah, Bangkalan, Madura, Indonesia. Isolation was carried out in 2018, by taking samples of fresh feces that had just fallen to the ground. A total of 108 samples were examined. The presence of *Blastocystis* sp. in feces was detected by both morphologically and genetically based on the 18S rRNA gene (Suwanti et al. 2020b). The Isolate were stored in the Department of Veterinary Parasitology, Faculty of Veterinary Medicine, Universitas Airlangga.

In Vitro Cultivation

Four *Blastocystis* sp. isolates were subcultured from four positive cattle samples consisting of two diarrhea and two non-diarrhea cattle. Each sample was cultured in RPMI 1640 medium (CP19-2763, Capricorn Saintific, Germany) and incubated at 37ºC for 3 days. The growth of *Blastocystis* sp. in culture was observed morphologically under a light microscope (Nikon® E100, Japan) at 400X magnification. Microscope was connected to a camera (Optilab® MTN001, Indonesia) to capture the image of the parasites. The supernatant of the culture medium was inserted into the tube and centrifuged at 1500 rpm for 10 minutes. Supernatant was removed and pellets was resuspended with phosphate buffered saline (PBS) and stored for SEM observation.

Scanning Electron Microscope (SEM)

Cell of *Blastocystis* sp. were washed with PBS pH 7 forthree times by centrifugation. It was centrifugated with speed 3000 rpm for 5 minutes. Then each sample was fixed by adding 2.5% glutaraldehyde and post-fixation with 1% osmium tetroxide. The isolate was mounted on a polycarbonate membrane and dehydrated using ethanol in sequence 30%, 50%, 70%, 80%, 90%, and 100%. Each ethanol series was done for 15 minutes and the last step was added with amyl acetate. Critical Point Drying (CPD) was performed using carbon dioxide, then the specimen was coated using a gold coating, and observed using SEM (Ragavan et al. 2014). SEM images were captured at the Faculty of Mechanical Engineering, Institut Teknologi Sepuluh November (ITS) Surabaya.

RESULTS AND DISCUSSION

Morphology of *Blastocystis* in medium culture

On culture media *Blastocystis* sp. most of the vacuolar forms (Figure 1). In previous study, it was found that the diameter of *Blastocystis* sp. in cattles was varied widely with ranged about 2.78 to 35.35 µm (average 14.76µm) and the size of *Blastocystis* sp. in culture was smaller than in fresh stool (Suwanti et al. 2020a). The main morphology of *Blastocystis* sp. has four forms: vacuole, granular, amoeboid and cyst form (Wawrzyniak et al. 2013) and the vacuolar form was the most common cell form found in cultures (Natalia et al. 2018). Under a light microscope, the morphology of *Blastocystis* sp. from healthy cattle (without diarrhea) and diarrhea cattle could not be distinguished. According to Tan et al. (2008), *Blastocystis* poses considerable challenges for diagnostic laboratories. The morphology of *Blastocystis* sp. is difficult to distinguish by using light microscopy only (Zhang et al. 2012). Yanson and Tan (2018) using an electron microscope show variations in the membrane surface from three *Blastocystis* isolates and these differences could be associated with the differences in the pathogenic potential of *Blastocystis* subtypes.

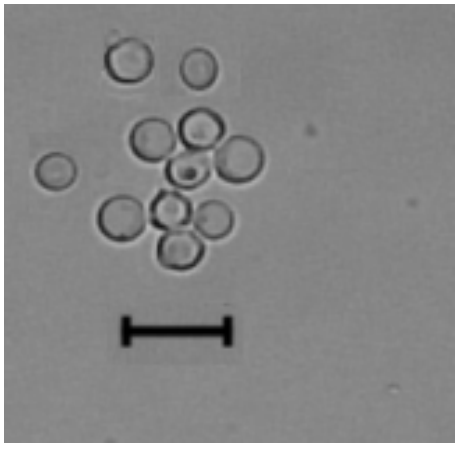

Figure 1. *Blastocystis* sp. in cattle in medium culture. Bar 10 µm

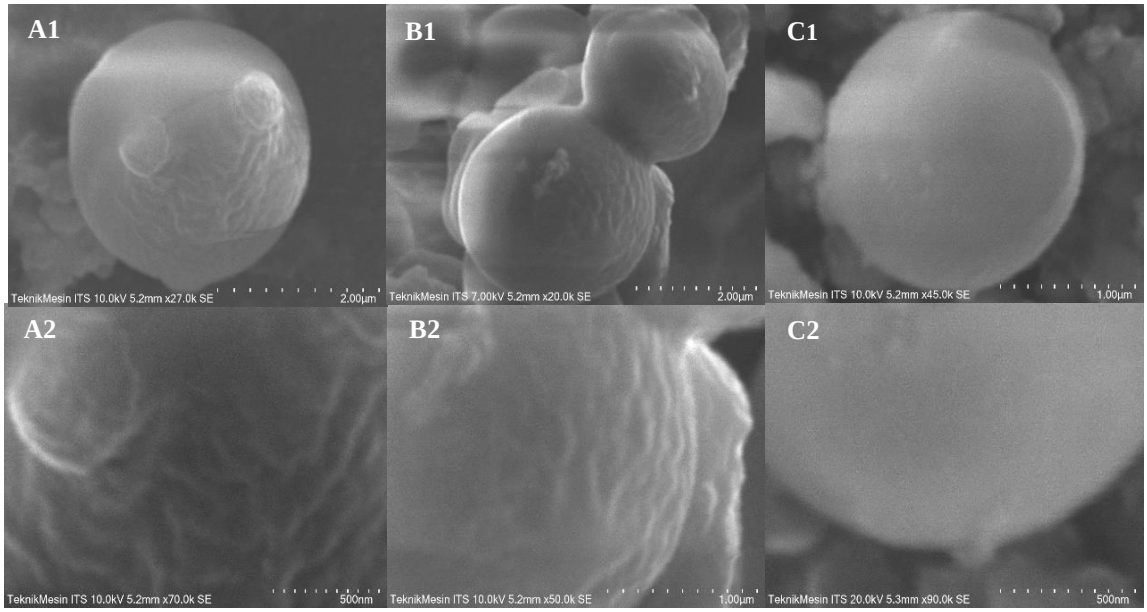

Figure 2. The scanning electron micrograph showing the cell surface of *Blastocystis* sp.isolated from the *in vitro* culture. A and B *Blastocystis* sp. from feces of cattle with diarrhea. C. *Blastocystis* sp.from faeces of asymptomatic cattle. Red arrows are daughter cell. Black arrows are bacteria.

Appearance of the surface structure of *Blastocystis* sp. Using SEM

In this decade, SEM was used widely in the morphological studies of a cell based on the structural surface of the cell, including *Blastocystis* sp. (Boreham and Stenzel 1993; Zaman et al. 1999; Tan et al. 2008; Zhang et al. 2012; Ragavan et al. 2014; Ahmed et al. 2019). SEM revealed the external morphological shape of the cyst and various surface coat structures of the parasite (Zhang et al. 2012). The surface coat was a kind of fibrous substance in varying appearance, rough or fine, which adhere to the surface of the *Blastocystis* cells (Elsayad et al. 2019). This study is the first report on the surface structure of *Blastocystis* sp. in cattle, both in symptomatic (diarrhea) and asymptomatic cattle.

In this study, it was found that *Blastocystis* sp. in cattle appeared in round shape. This result was similar with the morphology of *Blastocystis* in human which presented by Boreham and Stenzel (1993), generally the shape of *Blastocystis*from *in vitro* culture were spherical in shape. Round shape was also found by Ahmed et al. (2019) in addition to other forms, oval and irregular. *Blastocystis* cells often possess one or two nuclei, and occasionally, quadrinucleate cells and cells possessing numerous nuclei have been reported (Tan 2008). According to Zhang et al. (2007), there were five modes of reproduction, namely, binary fission, endodyogeny, plasmotomy, budding and schizogeny. In this study, the reproduction of blastocystis sp is shown in Figures 2.A1 and 2.B1. Figure 2.A1 was probably the initial stage of plasmotomy reproduction. Plasmotomy reproduction occurred during the stage of vacuolar form, which a daughter cell came into being by forming a finger-like cytoplasmic extension from the cellular surface of the mother vacuolar cell (Zhang et al. 2007). Figure 2.B1, *Blastocystis* sp. was seen undergo binary fission similarly reported by Elsayad et al. (2019) and Mehlhorn et al. (2012).

The surface cell of *Blastocystis* sp. isolates from diarrheal cattle had a rough surface as shown in Figure 2.A and 2.B. Whereas organism of non diarrhea cattle isolates was very smooth (Figure 2.C). Elsayad et al. (2019), using SEM micrographs shown different outer morphology of *Blastocystis* in human covered by rough or smooth surface coat, but there was no further explanation regarding the differences. The smooth surface structure of *Blastocystis* sp. isolates from non diarrheal cattle is similar to *Blastocystis* in asymptomatic human isolates as described previously by Suresh et al. (1994). The rough surface cell illustration in diarrheal cattle in this study supported the results of a study previously reported by Boreham and Stenzel (1993) that rough morphology of cells was found on isolates of*Blastocystis* from a diarrhea patient. This cell surface structure may be related to the pathogenicity of organisms related to the symptoms shown. It is suggested that a rough surface *Blastocystis* is more pathogenic than a smooth surface and the features of the surface structure of *Blastocystis* sp. correlated with symptomatic appearance. Tan et al. (2008), Ragavan et al. (2014) and Ahmed et al. (2019) stated that there were differences in the surface ultrastructure of *Blastocystis* isolated from human derivated from symptomatic and asymptomatic persons; *Blastocystis* from symptomatic isolates have coarser surface structure while asymptomatic isolates have a smooth surface structure. Rougher with excessive indentation surface was drawn from isolates derivated from suffering patients IBS (Ragavan et al. 2014) and colorectal carcinoma (Ahmed et al. 2019).

Several studies using SEM to observed *Blastocystis* have indicated that the surface coat is associated with bacteria. The surface coat contains a variety of carbohydrates and has been postulated to play a role in trapping and degrading bacteria for nutrition (Tan 2008). In this study, bacteria were seen attached to the surface of *Blastocystis* sp. from cattle with diarrhea (Figure 2.B_1). The attachment of bacteria to the surface of *Blastocystis* isolated from human samples had been confirmed by SEM studies. Attached bacteria on the surface of *Blastocystis* have been seen in close association with the surface coat and often causing an indentation (Boreham and Stenzel 1993). Although some surface coat functions are not yet known exactly, it is thought to be a mechanism for trapping bacteria for nutritional purposes and attachment to the intestinal epithelial lining (Zaman et al. 1999) and according to Yason and Tan (2018), the surface coat of *Blastocystis* sp. associates with potentially pathogenic of *Blastocystis* subtype. There was a hypothesis that surface coat protects the organism from innate host immune response as well as contribute to greater *adhesion* during colonization (Yason and Tan 2018).

In conclusion, by SEM, most of *Blastocystis* sp. isolated from the *in vitro* cultivation methodof cattle faeces appeared in round shape with and reproduced by binary fission. Meanwhile, the surfaces cell of *Blastocystis* sp. isolates from cattle with diarrhea had a rough surface while *Blastocystis* isolated from asymptomatic cattle isolates were having very smooth surface. Bacteria were seen attached to the surface of *Blastocystis* sp. from cattle with diarrhea. The features of the surface structure of *Blastocystis* sp. correlated with symptomatic appearance. The surface structure of *Blastocystis* sp. isolates from cattle with diarrhea was rougher than non diarrhea. Research on the determinants of the pathogenicity of *Blastocystis* is still needed.

ACKNOWLEDGEMENTS

The authors wish to express gratitude to Directorate General Research and Development Strengthening of Ministry of Research, Technology and Higher Education, for financial support with research grant through the Budget Execution Checklist 2019-2020.

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