Surface ultrastructure of Blastocystis sp. isolated from cattle

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Abstract. Widisuputri NKA, Lastuti NDR, Suprihati E, Hastutiek P, Plumeriastuti H, Mufasirin, Pusitasari 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 varoid 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, which, Blastocystis sp. was known to play a role in irritable bowel syndrome (IBS) (Ragavan et al. 2014; 2015) and induced precancerous polyyp 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, 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 that the morphology of Blastocystis sp. in cattle were varied widely in size with 2.78 – 35.35 μm (average 14.76 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 of Blastocystis sp. using SEM has been reported in humans, monkeys, pigs, chickens, rats and cockroaches which have varying differences 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 Isolates 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 37°C 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 for three 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 cattle was varied widely with ranged about 2.78 to 35.35 μm (average 14.76μm) and the size of Blastocystis sp. in culture was culture 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
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 (Elsayed 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, plasmodotomy, budding and schizogony. 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 plasmodotomy reproduction. Plasmodotomy 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 diarrheal 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).

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 feces of asymptomatic cattle. Red arrows are daughter cell. Black arrows are bacteria.
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 *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 method of 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 symmetric 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.

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REFERENCES


