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Short communication

Suitability of selected culture media for *Blastocystis* spp.

A. Piekara-Stępińska, M. Gorczykowski, J. Piekarska

Department of Internal Medicine and Clinic of Diseases of Horses, Dogs and Cats. Division of Parasitology, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, Norwida 31, 50-375 Wroclaw, Poland

Abstract

Blastocystis is a common enteric protozoan of humans and various species of animals. Culture and microscopic examination of fecal samples is the conventional method for identifying four major forms of Blastocystis (vacuolar, granular, non-vacuolar or cystic). In this article, we compared eight liquid media for cultivation of *Blastocystis spp.* Study material included fecal samples from clinically healthy pigs. Significant differences in the growth of *Blastocystis* on individual media were observed.

Keywords: *Blastocystis* spp., in vitro culture, zoonosis, Jone's medium

Introduction

Blastocystis is a common gastrointestinal protozoa belonging to the Phylum *Stramenopiles* (Tan 2008). It was first detected in a sample of human feces in 1912 (Yoshikawa et al. 1998) and is now found in mammals, birds, amphibians, reptiles and even arthropods (Stensvold et al. 2009). Low host species specificity of *Blastocystis* suggested a possibility of parasite transmission between different species (Rivera et al. 2008). Microscopic diagnostics of fecal smears, stained with trichrome, Giemsa, Gram or Wright stains (Stenzel et al. 1996) allows for observation of its various forms, such as vacuolar, granular, non-vacuolar or cystic (size varies from 2 to 200 μm). The culture methods are highly effective due to the rapid growth of the protozoa and are useful for obtaining samples with a higher concentration of the requested genetic material for molecular testing. The aim of the study was to verify the usefulness of selected media in the diagnostics of *Blastocystis.*

Materials and Methods

Study material included two collective fecal samples from six clinically healthy pigs grown at two different breeding stations. Microscopic examination of both collective fecal samples confirmed the presence of single cells of *Blastocystis. Blastocystis spp.* were multiplied in a liquid medium of a composition based on Jones' medium (Jones 1946), buffered with 1 x concentrated Dulbecco's Phosphate-Buffered Saline (DPBS), pH = 7.2, without Ca^{2+} or Mg^{2+} ions (IIiTD PAN Wrocław). Newborn Calf Serum heat inactivated (Gibco) or heat inactivated (30 min, 56°C) collective

Correspondence to: A. Piekara-Stępińska, e-mail: agnieszka.piekara@upwr.edu.pl, tel./fax: +48 71 32 05 245

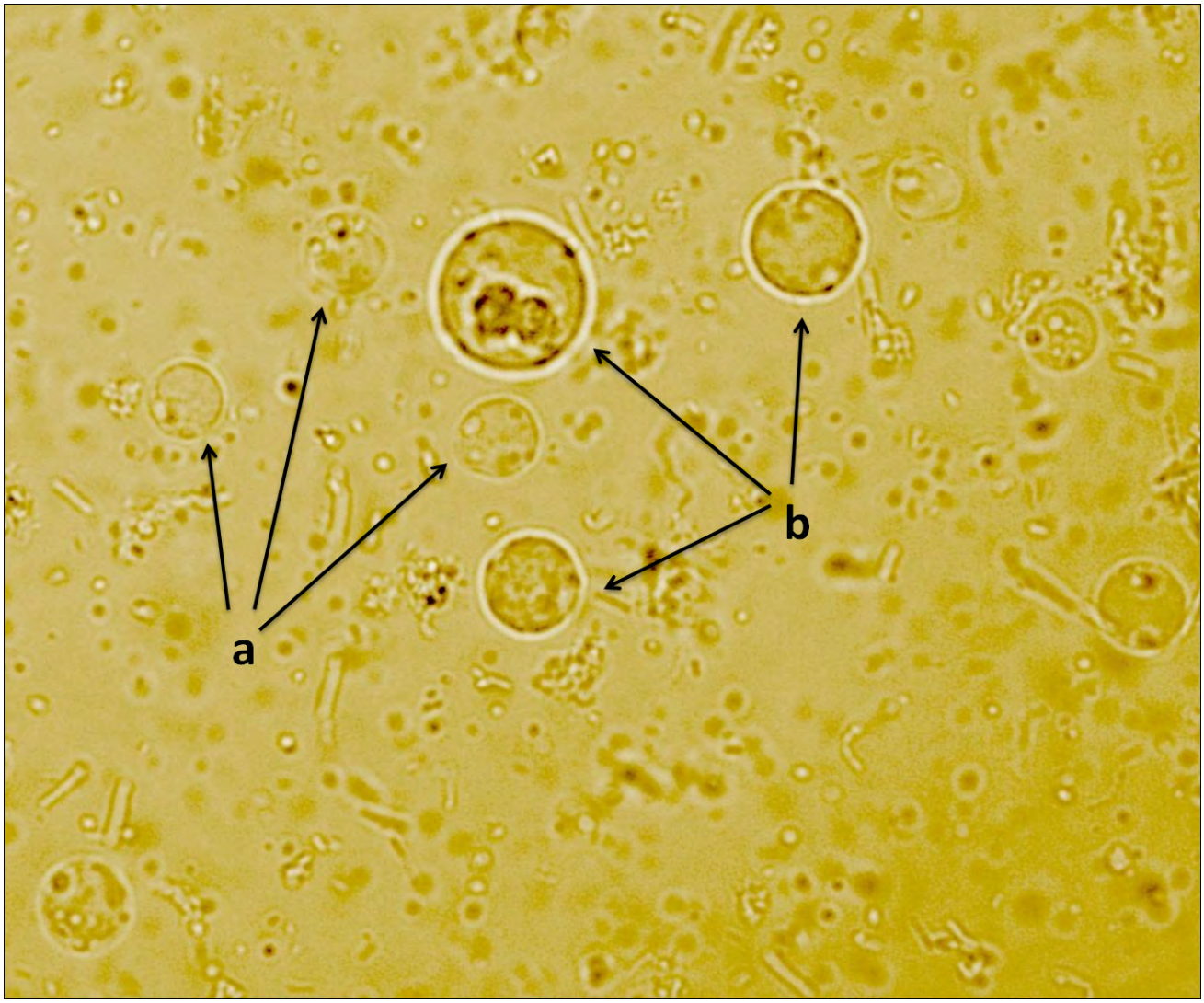

Fig. 1. Vacuolar (a) and granulat (b) cells of Blastocystis showing extensive variation in size; × 400

rabbit serum obtained from our own adult animals were used for serum supplementation. The media were also supplemented with Bacto-Peptone (Difco), yeast extract (Biocorp), and rice starch made from ground rice and heated for two hours at 160°C prior to supplementation. The experiment involved eight liquid media (Table 1). All media were autoclaved at 121°C for 20 min before adding the serum or rice starch. No additives inhibiting the growth of bacteria or fungi were used. Six milliliters of each medium were poured into sterile, screw-on Falcon tubes. A lump of feces of the size of a pin head was added to each tube and incubated for 48 h at 37°C. Survival and/or proliferation of the protozoa was evaluated daily based on a microscopic observation of a drop of the sediment in fresh preparations at 200-400x magnification.

Results and Discussion

Significant differences in the growth of *Blastocystis* on individual media were observed after 24 hours and both vacuolar and granular forms were detected (Table 1, Fig 1). The most intense growth was observed in the media supplemented with yeast extract and Bacto-Peptone (No. 4), where the vacuolar form prevailed (20-39 μm). Slightly less intense growth was detected in the original Jones' medium (No. 1), where the most common forms were vacuolar cells of the size of 20-25μm. In media No. 2, 3, 6 and 7 only single *Blastocystis* cells, both vacuolar (20-40 μm) and granular (50- 62um) were detected or no growth (No. 8) was observed at all. Our studies with modified Jones' media identified medium No. 4 (supplemented with 0.1% (w/v) yeast extract, 0.1% (w/v) Bacto-Peptone and 10% calf serum) and No. 5 (supplemented with 0.25% (w/v) yeast extract and 10% calf serum) as recommended for

Table 1. Growth of *Blastocystis* on individual media observed after 24 hours

No	Medium	Growth (after 24 h)	
		Sample 1	Sample 2
1	DPBS + 0.1% yeast extract + 10% calf serum	+++	+
2	DPBS + 0.1% yeast extract + 10% calf serum + rice starch	+	+
3	DPBS + 0.1% yeast extract + 10% rabbit serum	+	+
4	DPBS + 0.1% yeast extract and Bacto-Peptone + 10% calf serum	++++	+++
5	DPBS + 0.25% yeast extract + 10% calf serum	++	-
6	DPBS + 10% calf serum + rice starch	+	+
7	DPBS + 10% rabbit serum + rice starch	+	+
8	DPBS + 20% calf serum + rice starch	-	-

- no growth
+ (0-1 *Blastocystis* cells in the field of view)
++ (2-3 *Blastocystis* cells in the field of view)
+++ (4-5 *Blastocystis* cells in the field of view)
++++ (>5 *Blastocystis* cells in the field of view)

Blastocystis culture. Medium No. 2 (0.1% (w/v) yeast extract, calf serum and 20 mg of rice starch per tube) provided less abundant growth of the protozoa. An unexpected result was a lack of *Blastocystis* cells in the medium containing a higher amount of calf serum (substrate No. 8) than the other media. *Blastocystis* growth was detected in a medium of identical composition (No. 6) but containing 10% less calf serum. Lack of growth on medium No. 8 might be due to overgrowth of bacterial flora. There are numerous commercial media available for maintaining xenic and axenic cultures. *Blastocystis* may be grown on LE medium (containing eggs), Robinson's medium (containing Bacto-Peptone that, as demonstrated in this study, may positively affect the growth abundance of *Blastocystis*) or TYSGM-9 medium (supplemented with yeast extract and porcine gastric mucin) (Clark 2002). Routine diagnostics is mainly based on xenic media and the axenic ones are usually used for research purposes (Clark and Stensvold 2016).

Current studies suggest a key role for selecting a proper medium in the diagnostics of *Blastocystis* spp. In our study, its growth was particularly intense in the medium enriched with Bacto-Peptone. Medium composition determined not only growth intensity but also frequency of specific morphological forms.

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