MEDICINE AND PHARMACY

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PARASITIC INVASION OF *BLASTOCYSTIS SPP.*: REGULARITIES OF PRIMARY GROWTH OF BLASTOCYSTIS SP. IN FIVE TYPES OF NUTRIENT MEDIA

Abstract. *The work was to determine growth regularities of Blastocystis sp. primary cultures in five types of nutrient media and to identify the most effective media for amoeboid morphoforms detection in short-term cultures of parasites. Established primary growth regularities of three cultures of Blastocystis sp. and their long-term subcultivation in mJones's, mLE, RPMI, IMDMEM and RPMI / IMDMEM media justify the appropriateness of the optimal medium type selection taking into consideration the goal and objectives of the proposed study.*
Keywords: *Blastocystis sp., primary growth, nutrient media, amoeboid forms.*

Blastocystis sp. is the most prevalent intestinal protozoan parasite in humans and many animals [1-3]. Unfavorable colonization of *Blastocystis* sp. in human intestine is associated with development of various inflammatory diseases and irritable bowel syndrome (IBS), which may be accompanied by allergic reactions [4-7].

Currently, microscopic, cultural (*in vitro* parasite cultivation), immunological and molecular genetic methods are used for *Blastocystis* sp. detection in stool samples [5, 7, 8]. Cultural methods are characterized by a high degree of sensitivity and specificity [5, 6, 8-10].

They are widely used in epidemiological studies to evaluate *Blastocystis* sp. prevalence, to determine their sensitivity to drugs, and to obtain parasite antigens [4, 5, 8, 12, 13-17]. Cultural methods are also used to study blastocystosis pathogenesis and pathogen strains of various origins and their virulent potential, including the intensity of the amoeboid morphoforms formation, which microscopic methods fail to detect directly in faecal samples [11, 12, 16, 18, 19]. A vast majority of previous studies of *Blastocystis* sp. *in vitro* growth in different media were performed using axenic and stabilized xenic cultures of parasites.

The goal of this study was to determine growth regularities of *Blastocystis* sp. primary cultures in five types of nutrient media and to identify the most effective media for amoeboid morphoforms detection in short-term cultures of parasites.

Materials and Methods. Three fresh stool specimens of IBS-D (Rome IV) patients were used as inoculum. The specimens contained ≥ 5 cells of *Blastocystis* sp. observed in the field of view under light microscopy with ×400 magnification.

Blastocystis sp. identification was carried out by means of microscopy of the faecal smears, which were stained by Wheatley's modification trichrome stain and by Heidenhain's iron-hematoxylin stain. The inoculation dose of faecal samples homogenate (1:10 dilution in PBS pH = 7.4) was 200 μl per tube with 5 ml of liquid media Jones's (mJones's), RPMI-1640 (RPMI), Iscove's modified DMEM (IMDMEM), RPMI/IMDMEM (mixture of equal volumes of RPMI and IMDMEM media) and a liquid phase of the modified two-phase LE medium (mLE, modification Boeck and Drbohlav's).

All types of media contained antibiotics (ampicillin 12 mg/ml and streptomycin 4 mg/ml) and 10% inactivated horse serum. *Blastocystis* sp. culture growth was carried out under anaerobic conditions at 37 ºC for 10 days.

The specifics of *Blastocystis* sp. primary growth in five types of nutrient media were characterized by the following indicators: parasite cells generation time, (T_g)

measured in hours (h); maximum concentration of *Blastocystis* sp. viable cells, (MCVC) measured in milliliter (ml); time, required to achieve MCVC in parasite cultures, measured in days (PTD, peaking time in days); preservation of typical phenotypic traits of the *Blastocystis* sp. cell of various forms (CPFC); percentage of amoeboid forms in the parasite cultures (PAF); suitability for long-term subcultivation *Blastocystis* sp. (SLTS).

Determination of T_g, MCVC and PTD was based on the results of counting the number of *Blastocystis* sp. viable cells in microvolumes of their cultures. The number of viable cells of Blastocystis sp. in all tubes was determined immediately after inoculation of fecal homogenate and then daily during the cultivation period.

Quantification of *Blastocystis sp.* cells was done in a hemocytometer using trypan blue exclusion test. CPFC parameter was evaluated by a phase contrast microscopy of *Blastocystis* sp. suspensions with ×600 magnification.

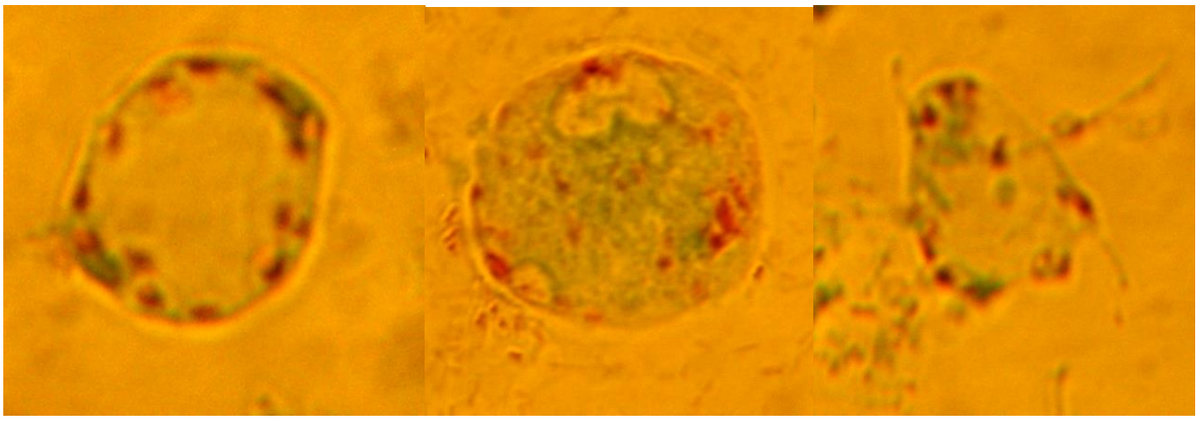
The value of the PAF index for each type of medium was determined by calculating the percentage of amoeboid morphoforms in 300 counted parasite cells in suspensions smears stained by modified Field's stain method. The value of SLTS parameter is determined by sustaining the growth of *Blastocystis* sp. subculture during 10 sequential passages in the same type of media.

Results & Discussion. In all faecal homogenates (FH) prepared for seeding, the dominant cell morphologies of *Blastocystis sp*. were vacuolar (85-95%) and granular (5-15%), and single amoeboid forms (< 1%) could be detected in one of the three (FH) (Fig. 1).

T_g was relatively the longest ($p < 0,05$) during the first day of cultivation (phase of adaptation and beginning of the growth) in all types of media. Its average value was (27,9±5,7) h, (23,5±3,9) h, (20,4±4,6) h, (28,3±6,0) h and (23,2±4,1) h in mJones's, mLE, RPMI, IMDMEM and RPMI/IMDMEM, respectively.

Since the second day of cultivation till the time of the maximum concentration of *Blastocystis* sp. cells in suspensions was achieved (exponential growth phase), T_g was reduced to the minimum values in these media, respectively: (19,5±3,0) h, (18,9±4,6) h, (17,8±2,5) h, (22,3±4,7) h and (18,5±3,8) h. In the subsequent stages

of *Blastocystis* sp. cultivation (phase of stationary growth and the beginning of accelerated cell death) the value of T_g slightly increased again, but by the end of the observation period, on Day 10, it did not reach the value of the phase of adaptation and the beginning of the growth.



A **B** **C**

Fig. 1. **Vacuolar (A), granular (B) and amoeboid (C) forms of *Blastocystis sp*. in GZF smears, persistently stained with Whitley's modified trichrome (light microscopy with total magnification ×1000, reference mark 10 µm)**

It was established that MCVC value can differ significantly ($p<0,05$) during *in vitro* culturing of Blastocystis sp. authentic cultures in different types of media. For the parasite strains used in the experiments, the average MCVC value reached: $(25,5\pm6,7)\times10^5$ cells/ml, $(32,0\pm7,8)\times10^5$ cells/ml, $(56,6\pm9,0)\times10^5$ cells/ml, $(36,6\pm8,4)\times10^5$ cells/ml and $(50,1\pm9,4)\times10^5$ cells/ml in the media mJones's, mLE, RPMI, IMDMEM and RPMI/IMDMEM, respectively. Meanwhile, PTD was 3 days in mLE and RPMI media, 4 days in − mJones's and RPMI/IMDMEM and 5 days in IMDMEM. A wide range of *Blastocystis* sp. morphological forms was revealed in all types of media: vacuolar, granular, amoeboid, in the stage of division, polyvacuolar, avacuolar, precysts, cysts and others. A vast majority of parasites visualized in cell suspensions were characterized by distinct identification features typical for certain morphoforms of these protozoan organisms. Thus, according to CPFC parameter, mJones's, mLE, RPMI, IMDMEM and RPMI / IMDMEM media are quite suitable for cultivation of *Blastocystis* sp. clinical strains and for

establishing specifics in dynamics of parasites morphoforms changes during different stages of culture growth. Amoeboid forms are the stages in the life cycle of *Blastocystis* sp. and play a pathophysiological role in the incidence and course of blastocystosis. It is rather difficult to identify amoeboid cells of *Blastocystis* sp. by microscopic methods directly in stool samples, instead they are easily detected in the cultivation of parasites *in vitro*. The results of our studies showed a significant effect of the type of medium on the intensity of amoeboid cells generation by the same strains of *Blastocystis* sp. ($p<0.05$). Generally, the PAF measurement reached a maximum value in one day or two days after MCVC was achieved in cultures on: (8,5±4,7) %, (14,2±3,8) %, (14,9±4,4) %, (17,8±5,5) % and (19,8±5,4) % in mJones's, mLE, RPMI, IMDMEM and RPMI/IMDMEM, respectively. The former four types of media were the most suitable for long-term subculture of *Blastocystis* sp. strains (SLTS=30).

Conclusion. Established primary growth regularities of three cultures of *Blastocystis* sp. and their long-term subcultivation in mJones's, mLE, RPMI, IMDMEM and RPMI / IMDMEM media justify the appropriateness of the optimal medium type selection taking into consideration the goal and objectives of the proposed study.

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