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Original article

Prevalence and staining characteristics of *Blastocystis* isolates from food animals in Tamil Nadu☆

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ABSTRACT

The present study was undertaken to investigate the prevalence and staining characteristics of *Blastocystis* isolated from food animals. Smears of the duodenal and caecal mucosal scrapings, collected from food animals, were stained with Giemsa, Gram's, modified acid-fast and acridine orange. *Blastocystis* was identified in 295 samples, including faeces and intestinal contents of animals like small ruminants (95), poultry (170) and pigs (30). The prevalence in pigs was found to be high (94.4%) followed by poultry (29.4%) and small ruminants (14%). Various forms of *Blastocystis* such as vacuolar, granular and amoeboid forms were identified by using different stains. The parasites stained with Giemsa were identified by the presence of eosinophilic nucleus and basophilic cytoplasm. In organisms stained with Gram's stain, the cytoplasm of the vacuolar forms took up the counter stain safranine. *Blastocystis* appeared as a pink colored cyst against bluish green background with modified acid-fast staining. The study shows that there is a very high prevalence of *Blastocystis* among the food animals investigated. Simple parasitological procedures, including direct microscopical examination and staining with agents like Giemsa, Gram's and acridine orange can assist identification of the parasites from intestinal contents and faecal material.

1. Introduction

Blastocystis, first described in the early 1900s (Alexeieff, 1911; Brumpt, 1912), is a unicellular, anaerobic, eukaryotic, polymorphic protist, which lives in the intestinal tract of various hosts. The recognized forms of *Blastocystis* are the vacuolar, granular, amoeboid and cyst forms. Avacuolar and multivacuolar stages are less frequently seen (Stenzel and Boreham, 1996). The parasite has been isolated from intestinal tracts of various hosts like insects, reptiles, and mammals. *Blastocystis* spp. is probably the most common human gut protozoan in the world, with more than 50% prevalence in developing countries (Stenzel and Boreham, 1996; Tan, 2008). Various detection methods, including microscopy of saline suspensions of faeces, formal-ethyl acetate concentration technique (FECT), Romanowsky-stained dried smears, permanent staining of sodium acetate acid-formalin (SAF) fixed smear, and *in vitro* culture using Jones' medium have been used to identify *Blastocystis* spp. (Suresh and Smith, 2004).

The pathogenic potential of *Blastocystis* is controversial, with numerous conflicting reports regarding its ability to cause disease; *Blastocystis* is a cause of intestinal disorders due to its significant association with individuals suffering from irritable bowel syndrome or diarrhoea (IBD) (Wawrzyniak et al., 2013). There is only a single report on the presence of *Blastocystis* spp. among animals in India (Sreekumar et al., 2014). There is lacuna in our understanding about the presence of this potential zoonotic organism, especially among the food animals in India. This study aims to fill that void, by conducting parasitological investigations on *Blastocystis* spp. from food animals with the objective to detect *Blastocystis* sp. using parasitological tools in the faeces and intestinal smears of animals and birds slaughtered for food.

2. Materials and methods

2.1. Hosts

Intestinal contents from duodenum and caecum, and faecal material were collected from small ruminants (sheep and goats), poultry and pigs slaughtered at the Perambur slaughter house, local chicken shops, and Postgraduate Research Institute in Animal Sciences (PGRIAS),

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Kattupakkam, respectively. Faecal/dung samples were also collected from live pigs and goats from PGRIAS. In the slaughter houses, the goats were mostly adult, non-descript animals, while sheep belonged to various breeds, including Madras Red, Salem Black and Mecheri. The animals slaughtered at PGRIAS included adult, male Boer-graded goats and Large White Yorkshire cross pigs of both sexes. Various commercial broiler chicken breeds and some native chicken were investigated.

The intestinal contents and faecal material collected from various animals were processed on the same day of collection.

2.2. Direct examination

Direct examination was carried out by placing a match stick-head size of the intestinal content/faecal material in a drop of normal saline on a microscopic slide. A thin smear was prepared and observed under low (10 ×) and high powers (40 ×) of a microscope for the presence of *Blastocystis* spp.

2.3. Staining

Thin smears were prepared by placing a match stick-head size of the intestinal content on one end of the microscopic slide. Using another slide, the content was gently dragged in a zigzag manner, air dried and fixed in methanol. These smears, along with the smears of the duodenal and caecal mucosal scrapings were stained with Giemsa, Gram's and modified acid-fast using standard procedures. Stained smears were examined using 10 ×, 40 × and oil immersion (100 ×) microscopy for the presence of any parasite stages. Acridine orange staining was also carried out according to the procedures of Lauer et al. (1981) and examined using a fluorescent microscope.

3. Results

A total of 107 samples from small ruminants, among which 95 were from slaughtered and 12 were from live animals, 170 chicken (including both free-range and broilers) and 90 pigs, 60 from slaughtered and 30 from live animals, were examined.

Light microscopical examination of faecal materials revealed *Blastocystis* sp. in 50 (29.4%) of the 170 poultry samples. Among the 107 samples from small ruminants, 15 (14%) were positive, including three from slaughtered animals and 12 from live animals. A total of 85 (94.4%) of the 90 pig samples were positive for *Blastocystis* sp., including 27 (90%) from slaughtered animals and 58 (96.6%) from live animals. The prevalence of *Blastocystis* from all three groups of food animals was found to be high, reaching up to 94.4%.

3.1. Direct examination of intestinal contents/faecal material:

On light microscopic examination of unstained wet mounts of intestinal content/faecal material, vacuolar and granular forms of *Blastocystis* cyst were commonly found. Vacuolar forms were recognized by their large size and characteristic appearance of large central vacuole which occupied 70–90% of the cell. The thin cytoplasmic rim contained varying number of dark nuclei (Plate 1A). Amoeboid forms were very rarely found in direct microscopic examination and contained one or more pseudopod like cytoplasmic projections (Plate 1B). Granular forms of *Blastocystis* were identified by the presence of a central mass of granules (Plate 1C). In general, a wide variability in the size and shape of the organism was noted.

3.2. Staining

3.2.1. Giemsa staining

Vacuolar/cyst and granular forms were found in Giemsa stained intestinal/faecal smears. The parasites stained with Giemsa were identified with presence of the eosinophilic nucleus and basophilic cytoplasm. In vacuolar forms with two nuclei, the nuclei were found located at the opposite poles of the cell (Plate 2A). The large central body was found to be devoid of stain and was clear and transparent or sometimes slightly eosinophilic (Plate 2B). Central vacuole occupied more than 80% of the cell volume. The thin cytoplasm was found pushed to the periphery, just under the cell membrane. Granular forms showed the presence of a mass of small sized granules in the central vacuole (Plate 2B). The size of the *Blastocystis* forms encountered varied from 5 to 111 µm.

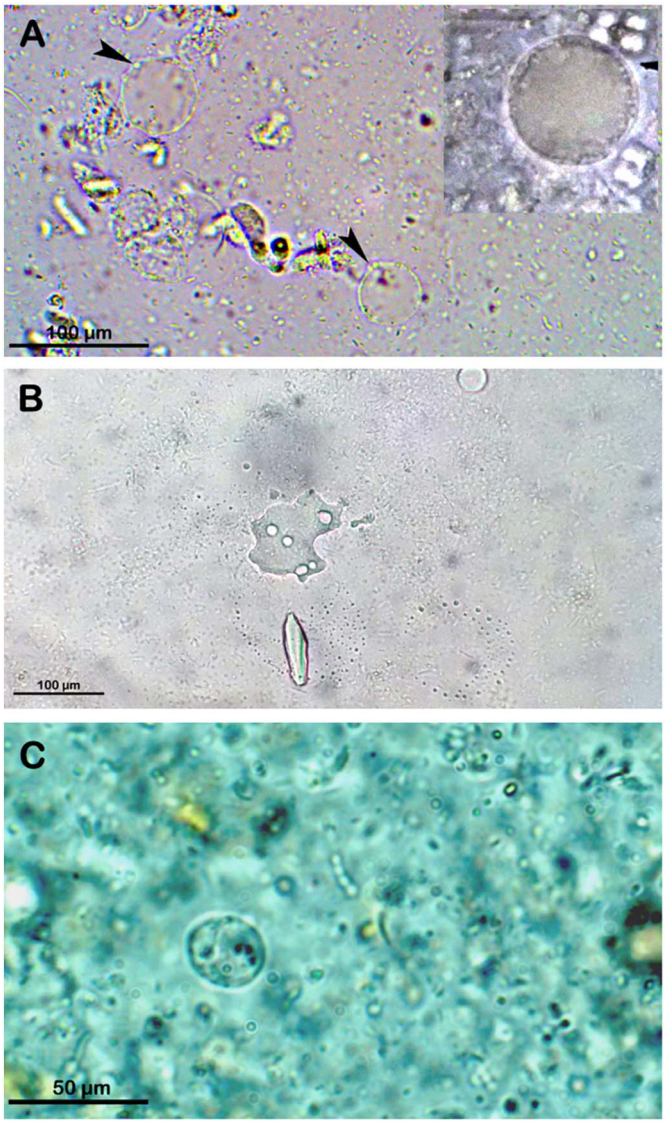

Plate 1. Direct microscopical appearance of *Blastocystis* sp.

A. Vacuolar forms of *Blastocystis* in unstained wet mount from poultry. The nuclei (arrowheads) are located at the peripheral rim of the organism. Note the presence of a large central vacuole. Inset shows vacuolar form of *Blastocystis* with peripheral thin rim of cytoplasm and large central vacuole.
B. Amoeboid form of *Blastocystis*. Note the presence of numerous nuclei.
C. Granular form of *Blastocystis* with numerous granules in the cytoplasm.

3.2.2. Gram's staining

With Gram's stain, the cytoplasm took up the counter stain safranin and was seen as reddish colored peripheral rim around the central vacuole (Plate 3A), which occupied more than 80% of the cell. Some forms showed the presence of non-staining 'halo' areas surrounding the organisms (Plate 3B). In most vacuolar forms, no difference in colour of central vacuole and cytoplasm were noticeable and the parasite was