***Blastocystis* sp. from food animals in India**

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Abstract *Blastocystis*, a zoonotic protozoan found in the intestinal tracts of a wide range of animals, has not been reported from non-human hosts from India so far. Organisms indistinguishable from *Blastocystis* sp. were identified in the Giemsa stained intestinal scrapings collected from carcasses of piglet and poultry that were brought for necropsy to the Central University Laboratory, Chennai.

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The ‘central vacuole forms’ of the parasite, with number of nuclei ranging from 1 to 12 were identified. The intensity of infection was low, with less than one organism per oil immersion field, indicating that their presence was unconnected to the cause of death. Caecal scraping was found to be more ideal than duodenal scraping for the diagnosis of *Blastocystis*, and can be a potential specimen for definitive diagnosis. Identical organisms were also detected in the dung samples of a buffalo calf which showed clinical signs of diarrhoea. The presence of *Blastocystis* in food animals acquires public health significance, as many subtypes of the parasite from poultry and pigs are transmissible to humans.

Keywords *Blastocystis* sp. · Intestinal scrapings ·
Food animals · Poultry · Pig · Buffalo calf

Introduction

Blastocystis spp. are stramenopile protozoa found in the intestinal tracts of a wide range of animals. The recognized human species, *B. hominis*, is considered an opportunistic pathogen in patients suffering from acquired immuno deficiency disease (Alemu et al. 2011). In recent times, the organism has been shown to be associated with human irritable bowel syndrome (Poirier et al. 2012). The parasite has been isolated from various domestic livestock and birds worldwide. Genetic studies of these isolates have classified them into 12 species comprising of seven distinct clades, with some having a propensity to infect and cause disease in humans (Noel et al. 2005). Human cases of *B. hominis* have been reported from India (Pandey et al. 2012). Though the organism is the most commonly isolated parasite in routine coprological surveys (Tan 2008), it has

not been recorded from non-human hosts in India so far. This paper describes the first, fortuitous finding of *Blastocystis* sp. in the dung samples of a live buffalo calf as well as broiler chicken and pigs during routine necropsy and discusses the public health importance of the finding.

Materials and methods

Direct wet smears were prepared from the dung of a buffalo calf, brought to the large animal outpatient unit of Madras Veterinary College with clinical signs of diarrhoea, and examined under microscope. Dried dung smears were also prepared and stained with Giemsa. Poultry, including chicken and turkey that died at the Poultry Research Station, Nandanam, Chennai and poultry farms from Vellore, and piglet and chicken from the University Research Farm, Chennai, brought to the Central University Laboratory for necropsy, formed the material for this study. A thorough necropsy was conducted on each carcass. Samples were collected to rule out the presence of common pathogens. Smears of the duodenal and caecal mucosal scrapings were prepared on glass slides and stained with Giemsa for routine microscopical examination. Stained smears were examined using oil immersion microscopy for the presence of any parasite stages. A total of 24 broilers, 4 turkeys and 1 piglet were examined.

Results and discussion

Dung from the buffalo calf revealed the presence of numerous spherical, vacuolar organisms of different sizes on bright field microscopy (Fig. 1). The outer thin rim of cytoplasm contained varying numbers of dark nuclei. Giemsa stained smears confirmed the morphology of the organisms as *Blastocystis* sp. Direct fecal smears have been found to be the most insensitive for the detection of *Blastocystis* sp. It was reported that 76 % of positive infections were missed by direct microscopy (Windsor et al 2002). The low rate of infection, wide variation in size and shape and lack of distinct diagnostic features of the organism could be the reason for the above. However, in the present case, the intensity of infection was very high, making diagnosis easy.

Necropsy lesions of the carcasses and subsequent microbiological analyses revealed that most of the birds had died due to outbreaks of Newcastle disease.

Stained smears of the duodenal and caecal mucosa from the 24 chicken revealed the presence of *Blastocystis*-like organisms in 20 (Fig. 2). Three out of the four turkeys, as well as the lone piglet revealed the presence of *Blastocystis* in the caecal smears.

Fig. 1 *Blastocystis* sp. (arrow) in direct wet dung smear from a buffalo calf. The outer, thin rim of cytoplasm contains numerous dark nuclei. The central vacuole contains granular material ($\times 400$)

The sizes of the organisms ranged from 8 to 25 μm . The forms were roughly spherical with an outer rim of cytoplasm encompassing a clear central vacuole. An amorphous, granular material was noticeable in the central vacuole in some forms (Fig. 2A, D). The outer rim of cytoplasm contained nuclei, varying in number from 1 to 12. In larger forms, small, clear vacuoles were noticed in the cytoplasm (Fig. 2D). Apart from the nuclei, numerous purple staining structures were visible in the cytoplasm of some forms (Fig. 2B, C). In some of the smears from broiler chicken, schizont stages of *Eimeria* could be detected (Fig. 2C). Bacteria were associated with some of the *Blastocystis* stages (Fig. 2D).

The morphology of the stages observed in the present study suggest that they are the ‘central vacuole’ or ‘central body’ forms, which is the most commonly encountered form of *Blastocystis* (Tan 2008). The other morphological stages like granular or amoeboid forms of the parasite were not detected. While a wide range of size (2–200 μm) has been reported, most of the parasites encountered herein measured around 8 μm , large forms ($\sim 25 \mu\text{m}$) with numerous nuclei being noticed occasionally. Parasites with clear vacuoles and those with a flocculent material in the vacuole have also been documented earlier. The purple staining structures found in the cytoplasm were presumed to be mitochondria by Stenzel and Boreham (1996), who also documented the presence of bacteria associated with, and engulfed by *Blastocystis* using ultramicroscopy.

The intensity of infection in the positive necropsy samples was low, with less than one parasite appearing per oil immersion field. For causation of disease, presence of five or more parasites per oil immersion field in permanent

Fig. 2 Central vacuolar forms of *Blastocystis* sp. in Giemsa stained intestinal scrapings of broiler chicken. **A** Stage with a single nucleus in a thin rim of cytoplasm around the central vacuole, whose contents appear granular (g) **B** Binucleated stage with clear cyst vacuole. Dark staining structures (arrowheads) are discernible in the cytoplasm. **C** Tetranucleated stage with structures (arrowheads) in the

cytoplasm. An 'extracellular' schizont of *Eimeria* (arrow) can be seen adjacent to the parasite. **D** A large, 12 nucleated vacuolar form with slightly granular (g) vacuole contents. The cytoplasm shows the presence of numerous smaller vacuoles. Bacteria (arrowhead) can be seen associated with the parasite. All bars measure 10 μ m

faecal smears is considered as significant (Tan 2008). *Blastocystis* has been more frequently reported from immunosuppressed humans (Prasad et al. 2000). The etiological diagnosis in most cases of deaths in poultry in the present study was Newcastle disease. The role of Newcastle disease in inducing pronounced immunosuppression is well established. Thus, it is probable that the organisms encountered here were just 'casual bystanders' in a disease episode caused by other pathogens like Newcastle disease virus.

Among the 24 carcasses positive for *Blastocystis*, caecal scrapings were positive in all, while only six duodenal

scrapings revealed the organism. Faecal smears, stained using trichrome, have been considered ideal clinical specimen for the diagnosis of *Blastocystis* (Tan 2008). The present study suggests that, in morbid specimens, in addition to faecal smears, caecal scrapings can be included as potential specimen for the diagnosis of the parasite.

The field of genotype analyses and phylogeny of *Blastocystis* is fluid and fast evolving, with data and information being frequently updated. It is believed that there are many genotypes (subtypes) that can be shared between humans and other animals. Noel et al. (2005) grouped the organism into 7 clades comprising of 12 sub-

types. According to this classification, the poultry isolates belonged to subtypes 1, 6 or 7. Organisms of the subtype 1, 2 or 5 were isolated from pigs. Of these, the subtype 1, which is cross-infective between animals and birds, has been frequently isolated from patients exhibiting symptoms of irritable bowel syndrome (Fouad et al. 2011). Subtype 2, which is also the most commonly isolated subtype from monkeys (Yoshikawa et al. 2009), has also been associated with human infection. The presence of *Blastocystis* in birds and piglets, which are basically meant for human consumption, indicates the possibility of feco-oral transmission to humans associated with the husbandry and/or consumption of these animals and birds. With 75% of the emerging human infections believed to have animal origins, further studies need to be undertaken to ascertain the prevalence of *Blastocystis* in domestic livestock and elucidate the molecular epidemiology of the organism in food animals in India.

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