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Balantioides coli: morphological and ultrastructural characteristics of pig and non-human primate isolates

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Abstract

Balantioides coli is a ciliated protozoon that inhabits the intestine of pigs, non-human primates and humans. Light microscopy studies have described over 50 species of the genus *Balantioides* but their validity is in doubt. Due to the limited information about this genus, this study is aimed to identify morphological characteristics of *Balantioides coli* isolated using fluorescence microscopy and both scanning (SEM) and transmission electron microscopy (TEM). Trophozoites isolated from the feces of pig and macaque were washed and subjected to centrifugation. These cells were fixed with paraformaldehyde for immunofluorescence. Other aliquots of these trophozoites were fixed with glutaraldehyde, post fixed with osmium tetroxide and processed for SEM and TEM. Immunofluorescence studies revealed microtubules with a longitudinal distribution to the main axis of the parasite and in the constitution of cilia. SEM demonstrated a high concentration of cilia covering the oral apparatus and a poor presence of such structures in cytopyge. TEM revealed in the plasma membrane, several associated structures were observed to delineate the cellular cortex and mucocysts. The cytoskeleton of the oral region was observed in detail and had an organization pattern consisting of microtubules, which formed files and nematodesmal networks. Organelles such as hydrogenosomes like and peroxisomes were observed close to the cortex. Macronuclei were observed, but structures that were consistent with micronuclei were not identified. Ultrastructural morphological analysis of isolates confirms its similarity to *Balantioides coli*. In this study were identified structures that had not yet been described, such as hydrogenosomes like and cytoskeletal structures.

Keywords

Balantioides coli, ciliates, SEM, TEM, hydrogenosomes like, peroxisomes

Introduction

*Balantioides s*p., which was formerly known as *Balantidium*, is a protozoon that belongs to the Subphylum Ciliophora, Class Ciliata, Subclass Holotrichia, and Order Trichostomatida (Zaman 1978). In this genus, more than 50 species have been described, but its taxonomic validity remains in doubt (Schuster and Ramirez Ávila 2008). Some species were identified based only on the trophozoites´ morphology and the host species in which they occur, such as the *Balantidium suis* reported by McDonald in 1922 (Levine 1940). *Balantioides coli* have been the best-studied species because it can parasitize a wide variety of animals and can determine a zoonotic transmission cycle. Domestic pig and non-human primates, particularly those of the Old World, are considered the most important reservoirs for human infection (Schuster and Ramirez Ávila 2008).

Although the parasite is still very well known in the scientific academy as *Balantidium coli*, currently, with advances in laboratory techniques of molecular biology, it has been proposed to change the name of the species *Balantidium coli* to *Balantioides coli*. Through molecular analyses, it was observed that the species *Balantidium coli* was grouped in a position that was phylogenetically distinct from *Balantidium entozoon*, the species that was the first to be described taxonomically. This demonstrated that *Balantidium coli* should therefore belong to another genus, and for this reason it was

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suggested that its name should be changed to *Neobalantidium coli* (Pomajbiková *et al*., 2013). In 2014, Chistyakova *et al*. then proposed that the species *Balantidium coli* should be reintegrated into the genus *Balantioides*, nomenclature that had already been attributed by Alexeieff in 1931.

The transmission of *B. coli* occurs primarily by the ingestion of cysts via direct contact between hosts or indirect transmission or the ingestion of contaminated food or water (Thompson 2011). *B. coli* are primarily a parasite of the large intestine, which mainly inhabits the cecum and colon. These clinical manifestations can progress quickly to death, and most patients have reported symptoms of dysentery (Zaman 1978; Solaymani – Mohammadi and Petri Jr 2006). The highest prevalence of balantidiosis has been found in tropical developing countries and is considered a neglected parasitic disease (Schuster and Ramirez Ávila 2008).

Morphological descriptions of the evaluative forms of the parasite, mainly trophozoites of *B. coli*, have been reported by many studies that used light microscopy of biological samples without chemical conservation or associated with permanent staining techniques (Levine 1940; Auerbach 1953; Krascheninnikov and Wenrich 1958). It is known that trophozoites are pleomorphic (Levine 1940; Auerbach 1953). This characteristic makes their taxonomic identification difficult based only on morphological analysis. Moreover, with this tool, it is difficult to demonstrate cortical structures and organelles of the parasite in most cases, and thus, it is necessary to use more advanced methods such as fluorescence and electron microscopy.

Ultrastructural data of evaluative forms of *B. coli* are still very scarce, particularly for those forms isolated from pigs. The most-cited information from an ultrastructural analysis for this parasite is obtained from a study by Zaman (1978). In addition to that study, descriptions of *B. coli* isolated from pig feces were done in Poland (Skotarczak 1997; Skotarczak 1999) and the Philippines (Nilles-Bije and Rivera 2010). Thus, to obtain more information about this genus, the present study aimed to identify morphological characteristics of two strains of *B. coli*, one isolated from the feces of pig and other from non-human primate, by fluorescence, scanning and transmission electron microscopy.

Materials and Methods

All procedures were performed according to the guidelines established by the Colégio Brasileiro de Experimentação Animal (COBEA), by Fundação Oswaldo Cruz – Fiocruz Committee of Ethics for the Use of Animals (license CEUA LW57/12) and by also with the assent of Sistema de Autorização e Informação em Biodiversidade, SISBIO – IBAMA, license 31900-2.

Parasites

From January 2013 to August 2015, two strains of *Balantioides* sp. were isolated and maintained in culture medium xenic Pavlova (1938) modified by Jones (1946) and supplemented with inactivated bovine fetal serum and a sterile rice starch suspension. These strains were morphologically analyzed using fluorescence microscopy and both scanning and transmission electron microscopy. One strain was isolated from a sample of pig (*Sus scrofa*) feces raised in a family-type production system located in Rio Bonito, Rio de Janeiro. The other strain was isolated from fecal material of Cynomolgus macaque (*Macaca fascicularis*), an animal that belongs to a non-human primate colony maintained in specific enclosures within Fundação Oswaldo Cruz/RJ. Isolation and maintenance of the parasite were performed according to the protocol of Barbosa *et al.* (2015). Trophozoites maintained in Pavlova modified medium were incubated at 36°C, and the strains were replaced from new and fresh media every 48 hour.

One aliquot of 1 mL culture was transferred to a watch glass with 3 mL of phosphate buffered saline, pH 7.2 (PBS). Using a Pasteur pipette glass and a stereoscopic microscope Diag Tech XTL 6445® at a magnification of 100 to 200 X, the trophozoites were collected individually and placed in 15-mL centrifuge tubes. Finally, four 15-mL centrifuge tubes were obtained from each protozoan strain, with each tube containing 500 trophozoites. These tubes were centrifuged at 814 g for 10 minutes, and the supernatant was discarded. Immediately after, the pellet was suspended in 3-mL PBS and centrifuged. This washing procedure was performed three consecutive times.

Fluorescence Microscopy

The isolates were fixed in 4% formaldehyde (PFA) for 5 minutes at room temperature and the pellet was then washed by centrifugation with PBS. Next, the membrane of the trophozoites was permeabilized at 30 minutes with PBS solution containing 0.5% Triton X-100 and 4% BSA. Next, the pellet were washed by centrifugation in PBS + 4% BSA solution.

To identify the microtubule, parasites were incubated with an anti-β-tubulin primary antibody at a concentration of 1:200 in PBS + 4% BSA solution for one hour at 37°C. Then, three washes were performed by centrifugation in PBS + 4% BSA followed by incubation with Alexa Fluor 488 and secondary antibody diluted 1:1000 in PBS + 4% BSA for 1 hour at 37°C. Next, the samples were washed in the same saline solution. Microscope slides were mounted with Prolong Gold containing DAPI, a nuclear marker (4', 6-diamidino-2-phenylindole, dihydrochloride), sealed with nail polish and stored at 4°C. The samples were then analyzed with a fluorescence microscope AXIO Image A.2 Zeiss®, and photomicrographs were obtained with a digital camera Axio Cam MRC.

Electron microscopy

The material of each strain culture was fixed for one hour with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer containing 3.5% sucrose and 2.5 mM $CaCl_2$ (pH 7.2) and washed in the same buffer. The specimens were then centrifuged and postfixed for 2 hours at 4°C with a solution of 1% O_SO_4 in 0.1