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The Structure of *Toxoplasma gondii* Cyst by Quick-freeze/Deep-etch Technique

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The cyst forming coccidia are a large group of genera that are heteroxenous – the asexual phase of the life cycle leads to the formation of cysts in different tissues of the intermediate host [1]. Since the morphology of cysts varies greatly from genus to genus, their ultrastructural characteristics are particularly useful for species and genus determination [2]. *Toxoplasma gondii* is one of the most important clinical opportunistic pathogens for immunocompromised patients, such as recipients of organ transplant and those infected with HIV [3]. In these individuals the reactivation of the infection from the breakdown of tissue cysts can lead to lethal encephalitis. Despite the key role played by the cyst in the persistence of the infection, the ultrastructure and biogenesis of this important life stage are poorly understood. In this work, we analyzed the fine structural aspects of cysts isolated from brains of mice chronically infected for 2 months. These cysts were quick-frozen, freeze-fractured, and deep-etched. Afterwards, a metal replica was made over the fractured material. These metal replicas revealed that the cyst wall was formed by a rigid compact layer, localized in the most outer part of the structure. Beneath, there was another layer in contact with the cyst matrix, three times thicker than the former, presenting a spongy-like arrangement, which will be referred as inner layer. The wall also presented numerous vesicles of different sizes and tubules within and in connection with the inner layer. Quick frozen/ deep-etched cysts also showed the presence of large irregular vesicles of approximately 300 nm in the cyst matrix and in the vicinity of cyst wall. In the same replicas we were able to observe a network interconnecting the parasites, formed by small vesicles and tubules of similar dimensions of the intravacuolar network found in tachyzoites' parasitophorous vacuoles. Metal replicas depicted secretory process by the bradyzoites, that will lead to the formation of this network observed. This work was supported by CNPq and Faperj.

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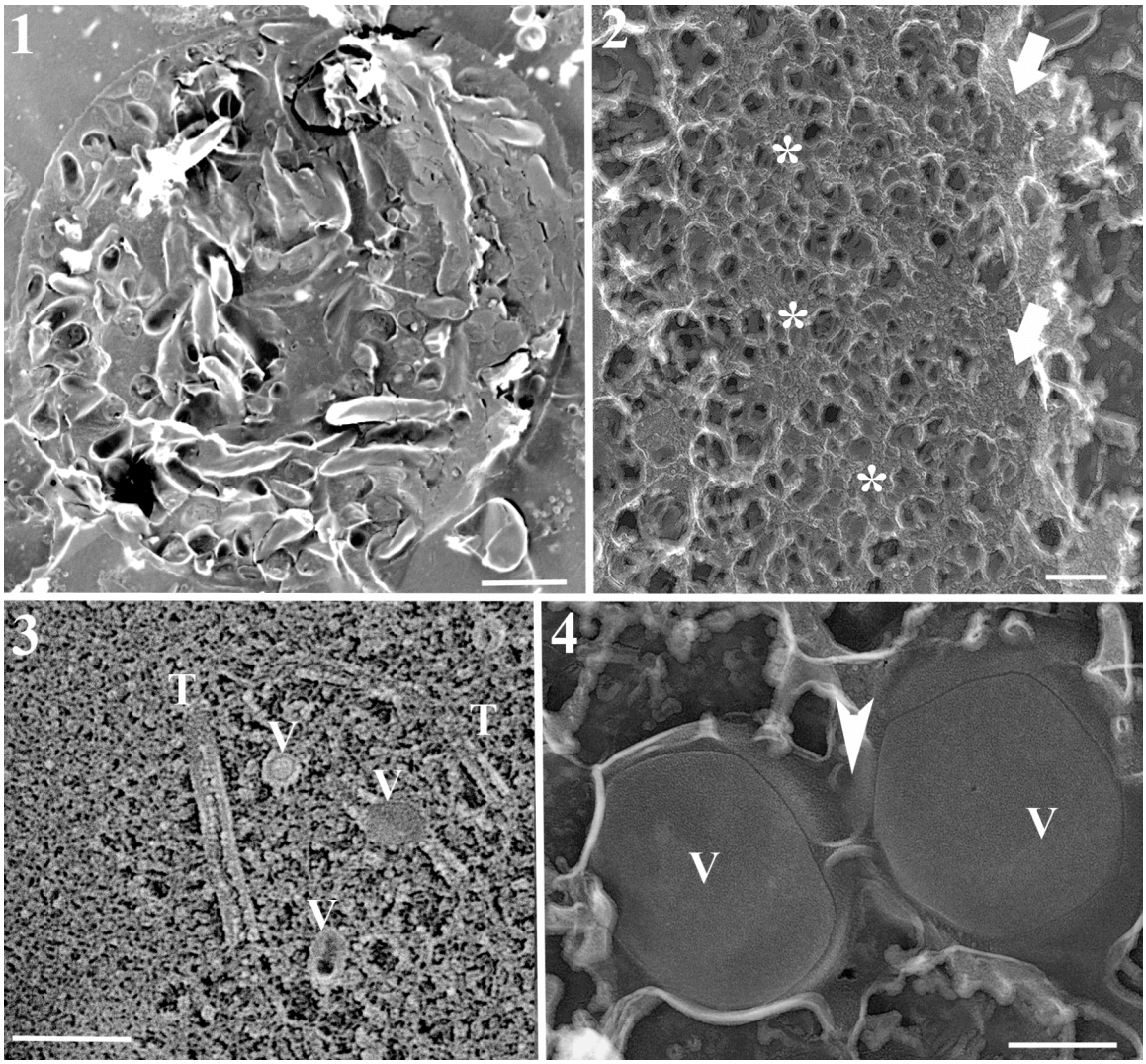

FIG. 1. General view of the isolated tissue cyst of *Toxoplasma gondii* quick-frozen, freeze-fractured, and deep-etched. Bar = 10 µm.

FIG. 2. The structure of the cyst wall. The cyst wall appears to be arranged in two layers: an outermost more compacted (white arrows) and an innermost more loosen (asterisks), presenting a "spongy-like" aspect. Bar = 0,5 µm.

FIG. 3. Vesicles (V) were seen nearby the cyst wall, as well as within the granular material that from structure. Tubules (T) were also present both near the cyst matrix, and within the granular material. Bar = 0,5 µm.

FIG. 4. Large vesicles were also seen within the cyst matrix. Their surfaces were smooth. Bar = 0,5 µm.

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