

**MORPHOLOGICAL AND ULTRASTRUCTURAL OBSERVATION
OF LYMPHOCYSTIS DISEASE (LCD) AND LYMPHOCYSTIS
DISEASE VIRUS (LCDV) DETECTION IN FISH TELEOST
(*Aequidens plaggiozonatus*) FROM AMAZON, BRAZIL***

*OBSERVAÇÃO MORFOLÓGICA E ULTRAESTRUTURAL DA DOENÇA
LINFOCÍSTICA E DO VÍRUS DA DOENÇA LINFOCÍSTICA (LCDV) EM
PEIXE TELEÓSTEO (*Aequidens plaggiozonatus*) DA AMAZÔNIA, BRASIL*

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ABSTRACT. Videira M.N., Velasco M, Matos P.S., Tortelly R., De São Clemente S.C. & Matos E. **Morphological and ultrastructural observation of lymphocystis disease (LCD) and lymphocystis disease virus (LCDV) detection in fish teleost (*Aequidens plaggiozonatus*) from Amazon, Brazil** [Observação morfológica e ultraestrutural da doença linfocística e do vírus da doença linfocística (LCDV) em peixe teleosteo (*Aequidens plaggiozonatus*) da Amazônia, Brasil]. *Revista Brasileira de Medicina Veterinária*, 33(4):215-219, 2011. Laboratório de Pesquisa Carlos Azevedo, Universidade Federal Rural da Amazônia. Av. Presidente Tancredo Neves, Nº 2501 Bairro Montese Belém, 66.077-530, PA, Brasil. Email: edilson.matos@ufra.edu.br

Lymphocystosis or lymphocystis disease (LCD) is a disease produced by a virus of Iridoviridae family. The contamination occurs by virus penetration through lesions of the skin, leading to the formation of tumors. 50 samples of *Aequidens plaggiozonatus* were analyzed, coming from Peixe-Boi River, Pará, Brasil. Fragments were removed from the regions that showed clusters of cells present in the lymphocystic disease and then processed by light microscopy and by transmission and scanning electron microscopy. The prevalence of 36% for LCD was noticed. The lesions were diffusely distributed, spotted on the fins, opercular region and oral cavity, showing growth in the shape of cluster cells, with secular membrane. Several inclusion bodies were observed, highly basophils in the cytoplasm, corresponding to hexagonal icosahedral virus particles. This study highlights the first description of the Lymphocystic disease in The Amazon region, found in *Aequidens plaggiozonatus*.

KEY WORDS. Lymphocystosis, Ultra-structure, *Aequidens plaggiozonatus*, Amazon.

RESUMO. A Linfocistose ou doença linfocística (LCD) é uma doença produzida por um vírus da família Iridoviridae. A contaminação ocorre pela penetração do vírus através de lesões da pele, levando à formação de tumores. Foram analisados 50 exemplares de *Aequidens plaggiozonatus*, pro-

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venientes do Rio Peixe-Boi, Pará, Brasil. Fragmentos de tecidos das regiões que apresentaram aglomerados de células presentes na doença linfocística foram retirados e processados por microscopia de luz e microscopia eletrônica de varredura e de transmissão. Foi observada prevalência de 36% para a LCD. As lesões estavam distribuídas difusamente nas nadadeiras, região opercular e cavidade oral, apresentando um crescimento em forma de aglomerados de células, com membrana sacular. Vários corpos de inclusão foram observados, altamente basófilos no citoplasma, correspondente a conglomerados de partículas do vírus icosaédricas hexagonais. Este estudo destaca a primeira descrição da LCD na região amazônica, encontrados em *A. Plagiozonatus*.

PALAVRAS-CHAVE. Linfocitose, Ultra-estrutura, *Aequidens plagiozonatus*, Amazônia.

INTRODUCTION

Lymphocystosis or lymphocystic disease (LCD) is a chronic and self-limiting disease, which affects many teleost fish species in the most diverse aquatic habitats all over the world (Hossain et al. 2007, Wolf 1962).

LCD is caused by a virus of Iridoviridae family. The contamination occurs by virus penetration through skin lesions in addition Zhan et al. (2010) postulate that the transmission can also occur horizontally by predation. The virus invades the connective tissue leading to the formation of tumors, with hyperplasia of the fibroblasts, mainly located in the fins, being able to affect other organs such as skin, eyes, gills and internal organs (Roberts 1981, Peters & Schmidt 1995).

According to Grinwis et al. (1995), the infection rate is increased depending on populational strain, malnutrition, in host spawning period or pollution of aquatic ecosystems.

Tidona & Darai (1999) pointed out that this disease is described in over 100 fish species of different aquatic habitats. It has caused severe problems in the growth of soila (*Paralichthys olivaceus*) and has been strongly spreading itself in Japan and China (Sun et al. 2000, Hossain et al. 2007).

In Brazil, lymphocystosis has been reported in the South, infecting *Paralichthys orbignyanus* with lesions on the skin, ocular and gular region in specimens from cropping system (Gusmão et al. 2006).

The final diagnosis of the infection by LCDV has been done by transmission electron microscopy, together with other macro and micro features of lymphocystosis.

MATERIAL AND METHODS

Fish and infection spotting

Several whitish irregular aggregations, spotted on the fins – dorsal, caudal and pectoral (Figure 1) and oral cavity (Figure 2) were removed from the freshwater fish *Aequidens plagiozonatus* (Cichlidae) (common name: cará pixuna). These fish were collected in the Amazon region (01° 07' 17, 65" S 47°18" 48, 35" W), near the city of Peixe Boi, Pará State, Brazil. They measured between 15-29cm length, were taken alive to the laboratory, where they were anesthetized with MS 222 (Sandoz AG, Basel), and subsequently necropsied. The prevalence of the infection was of 36% (18 out of 50 examined fishes).

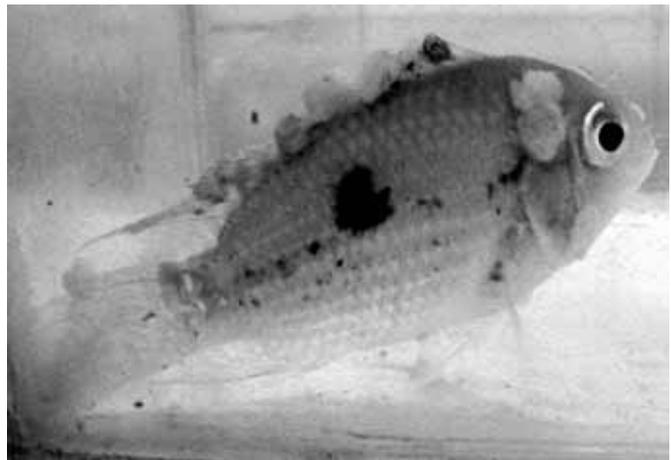


Figure 1. Sample of *Aequidens plagiozonatus* presenting several nodules typical of LCD, diffusely dispersed all over its tegumental surface, especially in the fins.

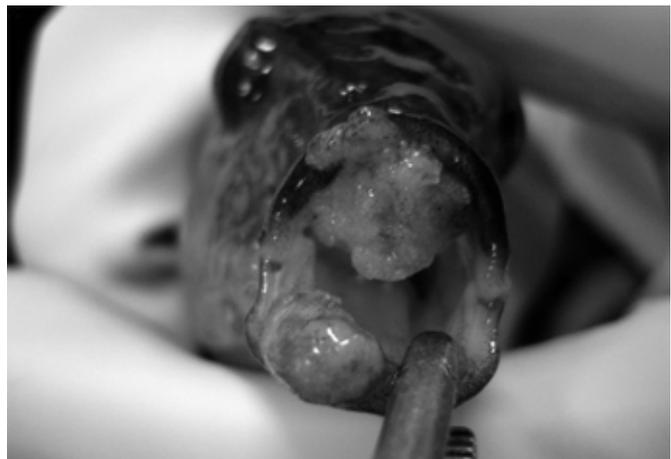


Figure 2. Sample of *Aequidens plagiozonatus*, presenting nodular mass in the oral cavity, formed by many lymphocystic cells.

Light Microscopy

Small fragments were observed fresh and removed from the regions that presented cell clusters typical of the lymphocystic disease and fixed in Davidson (formaldehyde, 95% alcohol, acetic acid and water) for analyses in LM. These fragments were processed and stained by Hematoxylin-Eosin (HE) and Trichomic of Gomori, and soon after, photographed in light microscope Zeiss Primo Star with a Canon adapter A610/A620 52 mm (Germany). With other fragments were carried out imprint and observed between slide and coverslip in LM.

Electron Microscopy

In order to be used by transmission electron microscopy (TEM), small fragments of infected tissues were fixed in glutaraldehyde 3%, buffered with sodium cacodylate (pH 7,2) for 12h at 4°C, washed overnight in the same buffer and postfixed in osmium tetroxide buffered at 2% in the same solution and temperature for 3h. After being dehydrated in a growing series of ethanol and propylene oxide, the fragments were immersed in EPON™ resin. The ultrathin cuts were contrasted with an aqueous solution

of uranyl acetate and lead citrate and examined in electron microscope LEO 906E, working at 80 Kv.

Some fragments were fixed the way previously described, dehydrated in the growing ethanol series, dried to the critical point, covered with a thin film of gold and photographed at the scanning electron microscope (SEM) LEO 1459 VP.

RESULTS

Of the total analysed specimens (n=50) and kept in aquariums, 36% (18 samples) showed soft tissue lesions as commonly found in the lymphocystic disease. These were revealed approximately 42 days after being seized, altering the nodules extension among the animals.

These lesions were diffusely distributed but predominantly found at the fin filaments: pectoral and caudal, in the opercular region and the oral cavity. It was also observed that the stress due to the infection originated behavioral changes, alterations in the feeding and color standard in the fishes.

Macroscopically, the lesions presented palpable gelatinous protuberances with irregular surface, some of them lightly rose-colored indicating abundant vascularization. The gross lesions or nodules

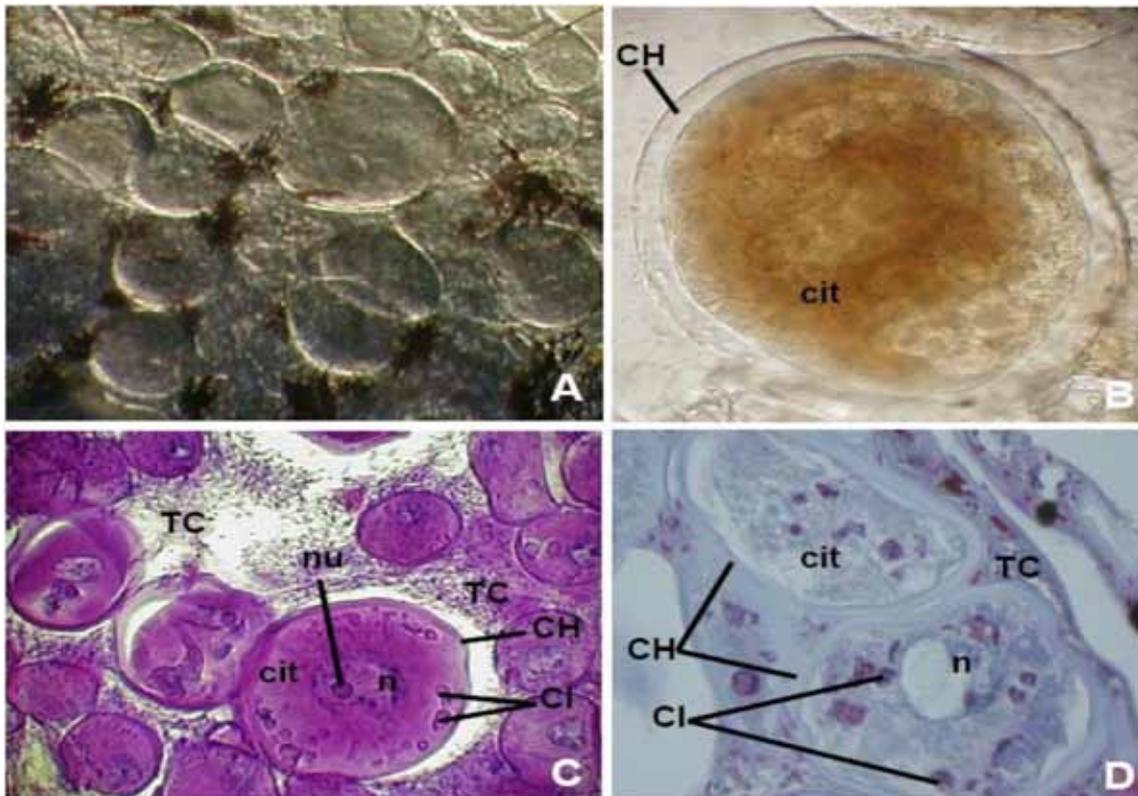


Figure 3. A. Imprint of lymphocystic cells (400X). B. Photomicrograph of a lymphocystic fibroblast observed fresh between slides and coverslips, noticing a big membrane thickness, forming a hyaline capsule (CH) (1000X). C-D. Micrography of the stained lymphocystic cells, respectively, by Hematoxylin-Eosin and trichomic of Gomori; TC – connective tissue; n - nucleus; nu - nucleolus; cit - cytoplasm; CI – viral inclusion corpuscles; CH – hyaline capsule (400X).

presented growing clusters of cells, kept by a thin sacular membrane.

In the *imprint*, it was observed lymphocystic cells and a thin membrane coating and delimiting these cells, grouped in clusters (Figure 3A). Through the light microscopy, (Figure 3B), it was verified that the virus of the LCDV reaches the dermal connective tissue. The fibroblasts presented cytomegalia, with spheroidal or ovoid shapes, with an exacerbated thickness in the outer membrane forming a retractile hyaline capsule.

In histological sections colored by HE and trichomic of Gomori, many inclusion bodies were observed, substantially basophil in the cytoplasm, strongly stained by hematoxylin, found peripherally, near the membrane (Figure.3C, 3D).

Many icosahedral hexagonal viral particles were observed by TEM in the cytoplasm of the lymphocystic cells. It was possible to distinguish viral particles of several bodies and materials highly electron-dense. Many viral particles were seen in different phases of development (Figure 4). Viral particles were found near to the rough endoplasmatic reticulum, a protein synthesizer, probably used in the production of viral capsule.

Through SEM, it was observed more thoroughly a hyaline capsule, as well as a spherical morphology of the fibroblasts and the contiguous fibers (Figure 5).

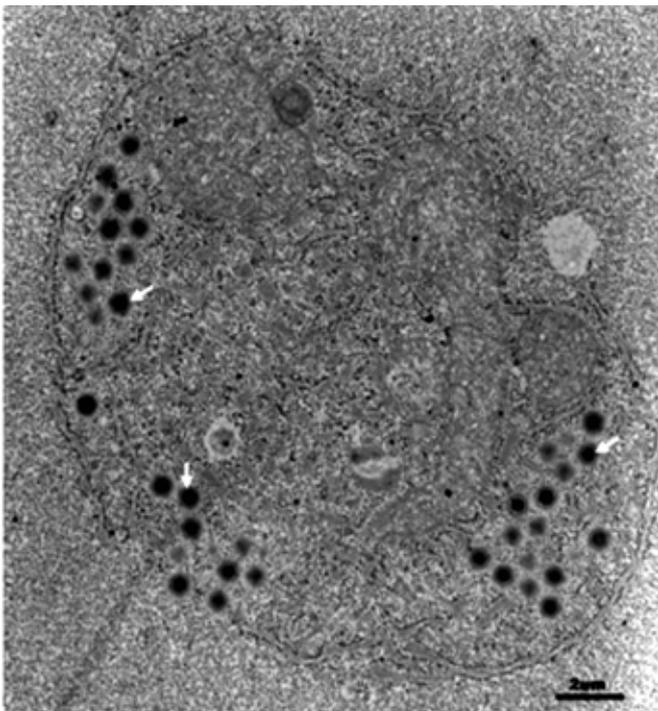


Figure 4. Photomicrograph showing the viral particles in the cytoplasm of the cell (arrow). TEM.

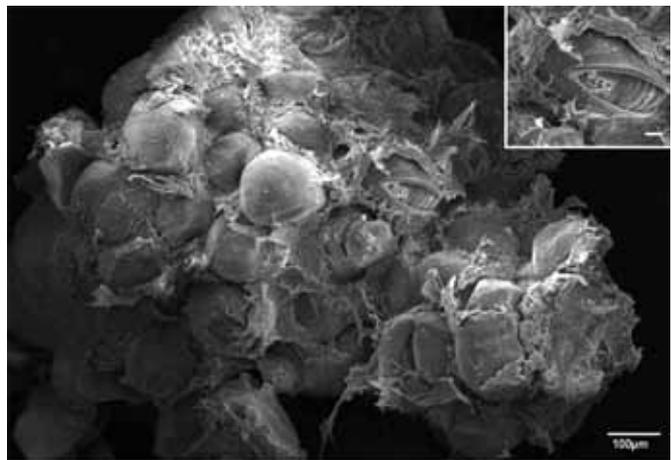


Figure 5. Grouping of cells lymphocystis. (100µm). In detail (20µm) is observed showing disrupted cell thickening the hyaline capsule. SEM.

DISCUSSION

The virus hatching period reported by Anderson et al. (1988), of 17 days after the first seizure, was different of that observed in the present study where these changes were only noticed after 42 days.

The macro and microscopic features produced by LCDV confirm the observations made by McAllister (1988), Ferguson (1989) and Sindermann (1990), such as the morphology of the nodules (macroscopically) and the presence of the hyaline capsule and viral inclusion corpuscles, and observations of Cheng et al. (2007), include cellular hypertrophy, cell enclosure by a distinctive hyaline capsule, enlarged nucleus and granular-appearing cytoplasm with prominent inclusions. Differently, Rahmati-Holasoo et al. (2010) did not been observed suppurative dermatitis.

The disease rarely conducts to death, but promotes changes in the morphological standard, which disfavors business, mainly when the goal is adornment species, lowering the added value in aquaculture systems (Roberts 1981). When located on the gills, LCDV compromises gas exchanges, and when it occurs on the fins, hinders the mobility of the animal, making it more susceptible to predators in natural habitats (Hossain et al. 2007). When the infection is spotted in the oral region it produces a feeding difficulty leading to malnutrition and weight loss, exposing the animal to death by starvation, as observed in the present study.

The highest viral rate, observed in the region of the fins, is related to the low concentration of mucus, since according to Kubitza & Kubitza (2004), mucus is one of the most important barriers to pathogenic organisms, for containing substances of

neutralizing action, as well as immunoglobulins and enzymes.

We have observed hypertrophied fibroblasts, called lymphocystic cells or lymphocysts presenting a thick hyaline capsule, increased nucleus, and bodies of basophil viral inclusion as previously described by Anderson et al. (1988).

Host cells infected with LCDV use the apoptosis as a defense mechanism against viral infections, leading to the destruction of the cells containing viral genetic material, reducing or eliminating the spread of the virus and its propagation in the host (Everett & McFadden 1999). Nonetheless, many virus during its evolutionary process created mechanisms to prevent or at least to slow it down, which would increase the viral replication. This mechanism was shown by Lin et al. (2008) for Iridovirus, that have a gene that encodes an homologous protein of Bcl-2 cellular, which is an inhibitor of apoptosis, conducting to an uncontrolled cellular proliferation of the fibroblasts and generating the observed tumors.

Curiously, lymphocystic nodules have not been noticed in animals of other species, even those captured in the same environment and under the same conditions. Thus, LCDV could be considered as a host-specific virus ratifying previous observations of Anderson et al. (1988).

CONCLUSION

The final diagnosis of the infection by LCDV was confirmed by transmission electron microscopy, together with macro and micro features of lymphocystis. To conclude, we show here, for the first time in the Amazon region, a morphological and ultrastructural description of lymphocystic disease in *Aequidens plagiazonatus*.

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