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TESTUDINES INTRANUCLEAR COCCIDIUM: THE PARASITE THAT BREAKS A BIOLOGICAL PARADIGM. IMMUNOHISTOCHEMICAL CHARACTERIZATION OF LESIONS AND HOST IMMUNE RESPONSE

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Testudines Intra-Nuclear Coccidiosis (TINC) is an infectious emerging disease in chelonians, caused by a coccidium ascribed to the *Eimeriidae* family [1]. Vertebrates coccidia commonly have endozoic cytoplasmic development, although at least 11 species of *Eimeria*, *Isospora* and *Cyclospora* are caryotropic, having intranuclear life stages [2]. This represents a unique behavior where an eukaryotic cell (parasite) enters into the nucleus of another eukaryotic cell (host), breaking a biological paradigm and opening new questions about parasite-host interaction. The aim of the present work was to characterize tissue and cellular lesions, uncovering the host defense and immune response mechanisms. In order to explore these aspects we performed immunohistochemistry (CD3, CD21, F4/80, S100, IL-1 and TUNEL assay using formalin-fixed paraffin-embedded tissues (kidney, intestine, liver, lung, spleen) from five dead patients ascribed to the species *Astrochelys radiata* (n=2), *Cuora aurocapitata* (n=1), *Stigmochelys pardalis* (n=2) with TINC generalized infection positive histology. TINC PCR was also performed in three patients from formalin-fixed paraffin-embedded tissues to verify the presence of the parasite. Anamnesis referred lethargy, anorexia and hematological data showed mild to severe anemia and leukocytosis. Histology revealed intranuclear protozoans (different endozoic forms) mainly localized in liver, kidney and lungs. Parasitism was accompanied by small foci of hepatic and renal tubular epithelial cells death with interstitial lymphomonocytic infiltrate and numerous activated melanomacrophage centers (MMCs). In particular, immunohistochemistry results revealed $CD3^+$ T cells and $F4/80^+$ macrophages. $S100^+$, IL-1 $^+$ intranuclear structures, morphologically compatible with parasite endozoic forms, were highlighted in hepatic, bile ducts and renal tubular epithelial cells. Parasite infected cells generally showed $TUNEL^+$ signal. PCR results confirmed the presence of the parasite in one patient. Lesions immunophenotyping unveils the cell-mediated nature of the host immune response characterized by a strong antigen presenting cells multi-organ surveillance, as suggested by the numerous activated MMCs. The parasite high potential systemic invasion is probably the result of an evolved strategy that takes advantage from the phagocyte cells network as a cellular “Trojan horse”. The $S100^+$, IL-1 $^+$ intranuclear structures reveals from one side the existence of parasite motility adaptation in the eukaryotic nuclear context and from the other the host response, activating the caspase-1 dependent trigger to pyroptosis, with DNA fragmentation as demonstrated by the TUNEL assay.

[1] Hofmannová et al. Intranuclear coccidiosis in tortoises — discovery of its causative agent and transmission, European Journal of Protistology, 67:71-76, 2019. [2] Alvarez et al. Development of a quantitative PCR for rapid and sensitive diagnosis of an intranuclear coccidian parasite in Testudines (TINC), and detection in the critically endangered Arakan forest turtle (*Heosemys depressa*), Veterinary Parasitology, 193:66-70, 2013.