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Survey of *Brucella* spp. in Marine Mammals from the Brazilian Coast: a histopathological, immunohistochemical, serological and molecular approach.

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Brucellosis is one of the most important and widespread emerging infectious zoonosis affecting marine mammals. However, in South America the information about this disease is very limited. The aim of this study was to investigate the occurrence of brucellosis in marine mammals of Brazil by using serological, molecular, histopathological and immunohistochemical approaches. Samples kept at the Marine Mammal Tissues Bank (LAPCOM/VPT/FMVZ/USP) as well as samples collected from fieldwork at Northeastern Brazil (Ceará and Bahia States) were analyzed. In a previous step of this research, necropsy reports (when available) and histopathology records were analyzed in order to identify suspicious cases and select target tissues for immunohistochemistry (IHC). Selected tissues of those cases as well as frozen samples of additional cases of interest were tested through molecular methods (conventional and real-time polymerase chain reaction [PCR], totalizing 124 autopsied animals [118 cetaceans (65 Delphinidae, 34 Pontoporidae, 8 Balaenopteridae, 7 Kogiidae, 2 Balaenidae, 1 Physeteriidae, 1 Ziphiidae), 4 Otariidae, 1 Mustelidae, 1 Trichechiidae] tested. Additional serological assays (competitive-enzyme-linked immunosorbent assay [C-ELISA] and 2-mercaptoethanol [2-ME], this last in RBT- or C-ELISA-positive) were conducted in individuals tested previously by Rose Bengal test [RBT] and additional cases (some of them were also tested by PCR). A total of 84 individuals were tested by serology (63 cetaceans and 21 manatees). Only cetaceans were positive for at least one of the applied tests: 8% (3/63) to RBT; 15.9% (10/63) to C-ELISA; 10% (1/10) to 2-ME; and 3.4% (4/118) to PCR/qPCR. On cases positive for at least one test, postmortem gross data, histopathology and immunohistochemistry (polyclonal anti-*B. abortus* antibody) were analyzed to characterize associated lesions in exposed or infected cetaceans, revealing a repertoire of *Brucella*-type lesions in part of the specimens. These results provides evidences of exposure and infection by *Brucella* spp. without associated lesions, as well as cases suggestive of acute/chronic brucellosis in cetaceans of Brazil. This study constitutes the first large-scale investigation of *Brucella* spp. Brazil, widening the distribution and host range of this pathogen while raising new concerns about conservation of marine mammals in South America. *Special thanks to the Society for Marine Mammalogy for the support provided through the Small Grant in Aid to Research.*