

Brucella ceti infection in a harbor porpoise (*Phocoena phocoena*)

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Running head: *Brucella ceti* in a porpoise

One line summary : Evidence of *Brucella ceti* and associated lesions are described on a harbor porpoise stranded alive on the Belgian coastline.

Keywords: *Brucella ceti*, harbor porpoise, brucellosis

Abstract:

The first case of *Brucella sp.* infection and associated lesions in a harbor porpoise (*Phocoena phocoena*) of the Belgian coast is described. The infection was diagnosed by immunohistochemistry, transmission electron microscopy and bacteriology, and was identified as *B. ceti*. The bacteria location raises the question of abortion and zoonosis risks.

Article:

In cetaceans, *Brucella* infection and related lesions have been described in bottlenose dolphins (*Tursiops truncatus*) (1), in striped dolphins (*Stenella coeruleoalba*) (2-5), in Atlantic white-sided dolphins (*Lagenorhynchus acutus*) (6, 7), in common dolphins (*Delphinus delphi*) (6, 8), in harbor porpoises (*Phocoena phocoena*) (6, 9) and in a minke whale (*Balaenoptera acutorostrata*) (6). Recently, *B. ceti* has been described as being the cetacean *Brucella* sp. strain (10). The present communication describes the first confirmed case of *B. ceti* infection and associated lesions in a harbor porpoise from the Belgian coast.

An adult female porpoise died on the Belgian coast in 2008 and was immediately necropsied (Marine Animals Research and Intervention Network MARIN). The most relevant observations (Table) were emaciation, multiple large acute to chronic skin ulcers around the genital split and between flippers (Figure 1). Internally, mild to severe nematode infestation (right ventricle, pulmonary blood vessels, airways) was associated with acute pulmonary thrombi and severe acute necrotizing pneumonia. The liver was enlarged yellowish with multiple 1-2mm red to dark red spots. The uterus was dilated with a larger left uterine horn and prominent congested blood vessels and a *corpus luteum* was present in the left ovary. On microscopic examination, severe acute necrotizing pneumonia and interstitial subacute to chronic pneumonitis with arteritis mostly associated with lungworms were observed, multiple foci of acute coagulative necrosis were present in the liver and there was a mild multifocal non-suppurative meningitis. In the mammary gland, numerous small acini were present with small amounts of milk in the acini and in ducts. Infiltrate of mononuclear cells under the endometrium suggested an endometritis. Immunohistochemistry (IHC) investigation using a polyclonal antiserum obtained from a rabbit experimentally infected with *B. melitensis* revealed diffuse intracytoplasmic positive staining for *Brucella* spp. mostly primarily in mononuclear and inflammatory cells on various tissues (spleen –Figure 2, lymph nodes, lung,

uterus, liver, pancreas and brain), in lesions, in lungworms and in mammary gland acini and milk. By transmission electron microscopy, very large numbers of relatively small (diameter 380 to 450 nm), intracellular coccoid bacteria suggesting *Brucella* sp. were observed in the genital ulcer. A *Brucella* isolate was obtained from brain and lung. The strain grew on *Brucella* agar supplemented with 5% horse serum in the presence of basic fushin, thionin and growth on safranin O. CO₂ was not required for growth and H₂S was not produced. The isolates showed catalase, oxidase and urease activity. This biotype profile is in agreement with the strain type profile of *B. ceti* (10). MLVA typing showed that the strains belong to genotype 23 when using MLVA panel 1 (8 minisatellite loci: bruce06, bruce08, bruce11, bruce12, bruce42, bruce43, bruce45, bruce55 which are useful for species identification.) (11). Panel 2, was split into two groups, panels 2A and 2B, composed of three (bruce18, bruce19, bruce21) and five (bruce04, bruce07, bruce09, bruce16, bruce30) markers, respectively (12). Using panel 2A, the same profile was obtained as the one described for all *B. ceti* strains isolated from porpoises (11) whereas panel 2B revealed a new genotype (bruce04: 6 repeats, bruce07: 6 repeats, bruce09: 3 repeats, bruce16: 7 repeats, bruce30: 6 repeats), closely related to genotypes ascribed to *B. ceti* strains isolated from porpoises mainly stranded in Scotland (11). The new genotype identified by panel 2B, is possibly associated with southern North Sea porpoises. However, panel 2B contains the more variable loci, and this panel has been given a lower weight in clustering analysis (12).

The results suggest a bacteraemia associated with *B. ceti* and to the authors' knowledge, this is the first description of a generalized *B. ceti* infection in a porpoise. The infection was suspected by electron microscopy, confirmed by bacteriological investigations and immunohistochemistry and finally, the bacteria was identified as *B. ceti*. In Europe, most reported cases of cetacean brucellosis have been reported for the coasts of Scotland and England and were described in striped dolphins, in Atlantic white-sided dolphins, in common

dolphins, in harbor porpoises and in a minke whale (2, 3, 6, 7, 9). Cases of meningoencephalitis associated with *Brucella* sp. infection has been reported for striped dolphins (2, 3) and in one Atlantic white-sided dolphin (7). Necrosis in spleen, liver, lymph nodes associated with *Brucella* sp. infection has also been reported in Atlantic white-sided dolphins (6). In porpoises, *Brucella* sp. has been isolated from different organs without associated pathology with the exception of a coagulative necrosis of the spleen (6) and a testicular abscess (9). Finally, in the minke whale, foci of liver necrosis and inflammation was consistent with *Brucella* sp. (6). In the present study, the enlarged uterine horn, the *corpus luteum* and the presence of milk in mammary acini suggest recent pregnancy and the positive immunolabeling of the endometrium raises the question of a possible abortion. Indeed, *Brucella* sp. is known to be responsible for abortions in terrestrial mammals and, *Brucella*-induced abortions have been described in two bottlenose dolphins with associated placentitis (1) while *Brucella* sp. has been isolated from an aborted bottlenose dolphin fetus (13). *Brucella* antigens were detected in the placenta of a stranded striped dolphin with a seven month dead fetus (5). In addition, a vaginal lithiasis suspected to be the result of ossification of an aborted fetus, in two common dolphins positive for *Brucella* sp. in the uterus has been reported (8). In the present case, a final conclusion cannot be drawn with respect to a possible abortion. Identification of *B. ceti* from milk (as in the present study), as well as in fetal tissues and in secretions of a pregnant dolphin suggest that *B. ceti* has tropism for placental and fetal tissues and that it can be shed externally (4). This finding suggests potential vertical and horizontal transmission to newborns (4). Nevertheless, indirect transmission through parasites should not be excluded, as *Brucella* sp. have been identified from lungworms (14). In addition, the observation of *Brucella* sp. antigens in the milk and in skin ulcers may represent routes of bacterial transmission between individuals and raises the question of the risk of zoonotic transmission when a cetacean is handled on the beach or in rehabilitation centers. All

people handling wild or captive marine mammals, alive or dead or samples collected from the animal should be aware of such risk and take the necessary precaution. To date, four cases of human infection with *Brucella* sp. from marine mammals are known. One was mild and uncomplicated in a laboratory worker while the three others were severe and naturally-acquired infection reported in people without direct contact with marine mammals but with a history of eating raw marine fish or shell-fish (15).

We can conclude that further investigations are needed to improve our knowledge on the prevalence, the impact on individuals and populations, and the zoonotic potential of marine mammal brucellosis. The zoonotic risk should be taken into account by all people in contact (direct or indirect) with marine mammals. Finally, the present case confirms the need of careful monitoring of strandings and of complete post-mortem examinations of stranded marine mammals.

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Figure legends

Figure 1: Longitudinal acute to chronic skin ulcer between flippers of the harbor porpoise

Figure 2: Positive IHC staining in mononuclear cells below the splenic capsule