Marine mammal brucellosis

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1. Introduction

Marine mammals consist of a diverse group of roughly 120 species which live in or depend on the ocean and the marine food chain. They include cetaceans (which contains two suborders: *Mysticeti* (baleen whales) and *Odontoceti* (toothed whales, which includes dolphins and porpoises), pinnipeds (true seals, eared seals and walrus), sirenians (manatees and dugong), polar bear (*Ursus maritimus*) and several species of otters. The polar bear is included because this species spend large parts of the year on the ice around the coastline of the Arctic Ocean, in close association with the marine environment, feeding of its major prey, the ringed seal (*Phoca hispida*) (Born et al., 1997;Stirling, 2009). The sea otter (*Enhydra lutris*), native to the coasts of the northern and eastern North Pacific Ocean, is fully aquatic with no association to the terrestrial environment whereas the marine otter (*Lontra felina*) found in littoral areas of southwestern South America, goes to shore to eat, rest, give birth and rear pups, also feeds exclusively from the sea, and these species are thus considered to be marine mammals (Miller et al., 2001). In addition, some populations of freshwater otters are almost exclusively marine living, and should also be considered as marine mammals, such as the southern river otter (*Lontra provocax*), the North American river otter (*Lontra canadensis*), the European otter and the African Clawless Otter (*Aonyx capensis*) (Estes et al., 2009). Cetaceans have great ecological and commercial value, since they are a fundamental part of the food chain and a source for protein and fat for many people around the world (Endo et al., 2005). The presence of marine mammals in the seas and littorals is a significant indicator for ocean health and gauge the magnitude at which the marine resources are protected. These mammals are also an important tourist attraction in aquariums and littorals (Lloret and Riera, 2008). In addition, dolphins are used in therapies (Antonioli and Reveley, 2005). One frequent phenomenon that brings people in close contact with these attractive animals is the arrival to the shorelines of disoriented dolphins and whales displaying swimming problems. During the last years, these actions and contacts between marine mammals and humans have increased worldwide (Hernandez-Mora et al., 2008;Lloret and Riera, 2008) augmenting the risk of transmission of pathogens from these marine animals to people and possibly terrestrial animals. Within this context, infectious diseases, such as brucellosis, should be taken into consideration in conservation programs.

Based upon what is known about *Brucella* spp. infection of reproductive organs of some cetaceans (e.g. *Phocoena* spp, *Turciops* spp. and *Stenella* spp.), it is likely that brucellosis can negatively impact efforts at protecting and increasing the genetic diversity in sparsely populations, captive collections or endangered species. For instance, it is worth

mentioning that the endangered species *Vaquita* (*Phocoena sinus*), living in the upper Gulf of Baja California, Mexico, inhabits the same area visited by striped (*Stenella coeruleoalba*) and bottlenose (*Tursiops truncatus*) dolphins (http://www.iobis.org), species that have been demonstrated to exhibit severe clinical brucellosis (Hernandez-Mora et al., 2008;Gonzalez-Barrientos et al., 2010).

The Saimaa ringed seal (*Pusa hispida saimensis*), a subspecies of ringed seal (*Pusa hispida*) is the most endangered seal species in the world, having a total population of only about 260 individuals. The only existing population of these seals is found in Lake Saimaa, Finland.   There are three documented species of monk seals. The Caribbean monk seal (*Monachus tropicalis*), last sighted in the 1950s and officially declared extinct in June 2008. The Mediterranean Monk seal is believed to be the world's second rarest pinniped and one of the most endangered mammals in the world with only 350-450 individuals. In 2010, it was estimated that only 1100 Hawaiian monk seals (*Monachus schauinslandi*) remain and is listed as critically endangered. *Brucella* antibodies have been found in the Hawaiian monk seal (Nielsen et al., 2005;Aguirre et al., 2007). However, as for other seal species, no evidence of gross pathology consistent with clinical brucellosis was noted in any of the seropositive animals tested (Nielsen et al., 2005).

Whaling and Sealing

The primary species hunted during modern commercial whaling are the common minke whale (*Balaneoptera acurostrata*) and Antarctic minke whale (*Balaenoptera bonaerensis*), two of the smallest species of baleen whales. The International Whaling Commission (IWC) was set up under the International Convention for the Regulation of Whaling (ICRW) to decide hunting quotas and other relevant matters based on the findings of its Scientific Committee. The IWC voted on 23 July 1982 to establish a moratorium on commercial whaling beginning in the 1985-86 season. Since 1992, the IWC's Scientific Committee has requested that it be allowed to give quota for some whale stocks, but this has so far been refused by the Plenary Committee (http://iwcoffice.org/). Faroese whaling of long-finned pilot whales (*Globicephala melaena*, actually a species of dolphin) is regulated by Faroese authorities but not by the IWC, which does not regulate the catching of small cetaceans. Modern commercial whaling is done for human food consumption. It is worth to note that *Brucella* spp. has been isolated from minke whales in the Atlantic (Clavareau et al.,

1998;Foster et al., 2002) and that *Brucella* DNA has been amplified by PCR from minke whale in the Northern Pacific (Ohishi et al., 2003).

Seal hunting, or sealing, is the personal or commercial hunting of seals. The hunt is currently practiced in five countries: Canada, where most of the world's seal hunting takes place, Namibia, Greenland (Denmark), Norway and Russia. Seal skins have been used by aboriginal people for millennia to make waterproof jackets and boots, and seal fur to make fur coats. Pelts account for over half the processed value of a seal. The European Union banned the importation of any seal product in May 2009, with the exception of seal products resulting from hunts traditionally conducted by Inuit and other indigenous communities and which contribute to their subsistence. The main commercial seal species in the Northern hemisphere are the harp seal (*Phoca groenlandica*) and the hooded seal (*Cystophora cristata*). A high prevalence of antibodies to *Brucella* spp. has been found in hooded seal, which is traditionally consumed by people in Northern Norway (Tryland et al., 2005).

Brucella ceti and *Brucella pinnipedialis* infections in Marine Mammals

Brucella spp. are Gram-negative, facultative intracellular bacteria that can infect many mammalian species including humans. Ten species are recognized within the genus *Brucella*: the six "classical" *Brucella* species, some of which include different biovars: *Brucella abortus* (biovars 1, 2, 3, 4, 5, 6, 7, 9), *Brucella melitensis* (biovars 1, 2, 3), *Brucella suis* (biovars 1, 2, 3, 4, 5), *Brucella ovis*, *Brucella canis*, and *Brucella neotomae* (Corbel and Brinley-Morgan, 1984;Alton et al., 1988) and the recently described *Brucella ceti* and *Brucella pinnipedialis* (Foster et al., 2007), *Brucella microti* (Scholz et al., 2008) and *Brucella inopinata* (Scholz et al., 2010).

The classification for the classical species was mainly based on differences in phenotypic characteristics, host preference(s) and in pathogenicity. Distinction between species and biovars is currently performed by differential laboratory tests (Corbel and Brinley-Morgan, 1984;Alton et al., 1988). The overall characteristics of the marine mammal strains are different to those of any of the six "classical" *Brucella* species (Jahans et al., 1997;Clavareau et al., 1998;Bricker et al., 2000;Cloeckaert et al., 2001) and since 2007, *B. ceti* and *B. pinnipedialis* (infecting preferentially cetaceans and pinnipeds, respectively) are recognized as new *Brucella* species (Foster et al., 2007).

Since the first description of an abortion due to *Brucella* spp. in a captive dolphin in California in 1994 (Ewalt et al., 1994) and the first isolation of *Brucella* spp. in marine

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mammals in their natural habitat, reported in 1994 from stranded harbour seals (*Phoca vitulina*), harbour porpoises (*Phocoena phocoena*) and common dolphins (*Delphinus delphis*) on the Scottish coast (Ross et al., 1994) several studies have described the isolation and characterisation of *Brucella* spp. from a wide variety of marine mammals which rose both conservation and zoonotic concerns.

Brucella ceti and *B. pinnipedialis* have been isolated from cetaceans (*Mysticeti* and *Odontoceti*), true seals inhabiting seas and oceans of Europe, North and Central America and from an European otter (*Lutra lutra*), thus in animals inhabiting almost all the seas covering the globe, but Antarctic waters (Ross et al., 1994;Foster et al., 1996;Ross et al., 1996;Jahans et al., 1997;Garner et al., 1997;Clavareau et al., 1998;Miller et al., 1999;Forbes et al., 2000;Maratea et al., 2003;Watson et al., 2003;Tryland et al., 2005;Dawson et al., 2006;Munoz et al., 2006;Dagleish et al., 2007;Hernandez-Mora et al., 2008;Prenger-Berninghoff et al., 2008;Dagleish et al., 2008;Davison et al., 2009;Jauniaux et al., 2010;Gonzalez-Barrientos et al., 2010). *Brucella* spp. DNA has also been isolated from common minke whale in the western North Pacific (Ohishi et al., 2003).

Anti-*Brucella* antibodies have since then been detected in serum samples from several species of marine mammals from the Northern and Southern Hemispheres (Nielsen et al., 1996;Jepson et al., 1997;Tryland et al., 1999;Nielsen et al., 2001;Van Bressem et al., 2001;Ohishi et al., 2003;Hanni et al., 2003;Nielsen et al., 2005;Dawson, 2005;Rah et al., 2005;Burek et al., 2005;Munoz et al., 2006;Zarnke et al., 2006;Tachibana et al., 2006;Aguirre et al., 2007;Hernandez-Mora et al., 2009;Gonzalez-Barrientos et al., 2010). Although no *Brucella* spp. strain has been isolated from marine mammals in Antarctic waters, anti-*Brucella* antibodies have been identified (Retamal et al., 2000;Blank et al., 2002). No antibodies were detected in marine mammals in New Zealand (Mackereth et al., 2005).

The polar bear is the apex predator in the Arctic marine foodweb, and in the Svalbard area ringed seals, bearded seals (*Erignathus barbatus*) and harp seals are the main preys. Anti-*Brucella* antibodies were found in ringed seals and harp seals in the Svalbard (Tryland et al., 1999). A seroprevalence of 5.4% of anti-*Brucella* antibodies was found in serum samples from 297 polar bears from Svalbard and the Barents Sea (Tryland et al., 2001). Antibodies have also been found in polar bears from Alaska (Rah et al., 2005). To date, there is no indication of disease caused by *Brucella* spp. in polar bear populations.

In terrestrial mammals, horizontal transmission usually takes place through direct or indirect contact with aborted material, most often through ingestion but also through respiratory exposure (aerosols), conjunctival inoculation, udder inoculation during milking

and contamination of damaged skin or mucosal membranes. Mating and lactation pose also a transmission risk (Corbel, 2006). *Brucella* spp. generally does not multiply outside the host (apart from *B. microti*), but can persist in the environment for long periods of time depending on the conditions.

It is not known to which extend these characteristics are also valid for marine mammal *Brucella* infections. Some species of sea mammals are social animals often found in large groups where there is ample opportunity for transmission, e.g. on seal haul-out sites. Some other species are largely solitary animals, only coming together infrequently primarily for mating (venereal transmission) and giving birth thereby creating fewer opportunities for transmission.

Ewalt et al. (1994) documented that *Brucella* spp. isolated from an aborted bottlenose dolphin foetus, may indicate cause of abortion (Ewalt et al., 1994). In 1999, it was reported that two bottlenose dolphins aborted foetuses died as a result of *Brucella* spp. infection at the Space and Naval Warfare Systems Center, San Diego, USA. Placentitis occurred in both cases (Miller et al., 1999). The authors suggested that dolphin brucellosis is a naturally occurring disease that can adversely impact reproduction in cetaceans and may thus play an important role in the population dynamics of these species (Miller et al., 1999). However, to date, abortion has not been reported in cetaceans in their natural habitat, although the isolation of *Brucella* spp. from milk, foetal tissues and secretions, in a stranded striped dolphin (*Stenella coeruleoalba*) has been described in Costa Rica (Hernandez-Mora et al., 2008).

Garner et al. (1997) demonstrated *Brucella* spp. in *Parafilaroides* spp. in the lung of a pacific harbour seal (*Phoca vitulina*) and suggested that transmission in pinnipeds may occur by infected lungworms (Garner et al., 1997). This hypothesis was also suggested following the description of *Brucella* spp. infection within the uterine tissue of lung nematodes *Pseudalius inflexus* collected from the lungs of a stranded juvenile male harbour porpoise in Cornwall, UK (Dawson et al., 2008). Lastly, the presence of *Brucella* spp. was demonstrated by electron microscopy in tattoo like lesions in a stranded porpoise in Belgium. *Brucella* spp. was cultured and identified as *B. ceti* (Jauniaux et al., 2010).

Brucella ceti and *Brucella pinnipedialis* induced pathology

Brucellosis in terrestrial animals is clinically characterised by one or more of the following clinical signs: abortion, retained placenta, orchitis, epididymitis, with excretion of the organisms in uterine discharges and in milk (Godfroid et al., 2005).

It is important to note that pathology induced by *Brucella* spp. is different in cetaceans as to compare to seals. As a general rule, no gross pathology has been associated to *B. pinnipedialis* infections in seals, whereas different acute and chronic pathological changes have been associated with *B. ceti* infection in whales both in *Odontoceti* and *Mysticeti*.

No gross pathology was documented in stranded or by-caught seals in Scotland (Foster et al., 2002), although *Brucella* spp. has been isolated from the testes of a grey seal without any associated pathology (Foster et al., 1996). *Brucella* spp. was isolated from the spleen, gastric lymph node and colorectal lymph node of one stranded, dead, adult hooded seal from the coast of Scotland without any signs of pathology (Foster et al., 1996). In Norway, during scientific sealing operations, hooded seals were sampled and investigated for brucellosis. Despite the high seroprevalence rates, i.e. 35 %, n = 48/137 (Tryland et al., 1999) and 31 %, n = 9/29 (Tryland et al., 2005) and the high number of bacteriological positive animals, i.e. 38 %, n = 11/29 (Tryland et al., 2005) recorded for the hooded seals in the Greenland Sea population, no gross pathological changes have been seen in association with the organism. These results suggest that there is a persistent *B. pinnipedialis* bacteraemia and that limited pathology and immune responses are induced in hooded seals. Sampling occurred in May-June, after the pupping season so that the potential abortificient effect of *B. pinnipedialis* could not be assessed. Moreover, since embryonic diapause (i.e. the blastocyst does not immediately implant in the uterus, but is maintained in a state of dormancy and no development takes place as long as the embryo remains unattached to the uterine lining) occurs in seals, no foetus could be sampled in order to measure early *B. pinnipedialis* infection of the pregnant uterus. The prevalence of seropositive hooded seals in the Northwest Atlantic population is much lower (4,9%) (Nielsen et al., 2001) and no decline in this hooded seal population was observed.

The gross pathology in cetaceans is associated with skin lesions, sub-blubber abscessation, hepatic and splenic necrosis, macrophage infiltration in liver and spleen, epididymitis, spinal discospondylitis, meningitis, lymphadenitis and mastitis. Neurological signs linked to *Brucella* infections have been associated with primary standings of cetaceans. Indeed, *B. ceti* has been isolated from the brain and cerebrospinal fluids of harbour porpoises, a white-beaked dolphin, a white-sided dolphin and, for the most part, stranded striped dolphins. A chronic, non-suppurative meningoencephalitis was found in three young striped dolphins (Gonzalez et al., 2002). *Brucella ceti* was isolated from the mammary gland of sperm whales (*Physeter macrocephalus*) and dolphins, suggesting parasitism of resident macrophages in these glands (Foster et al., 2002), as in the case of terrestrial mammals. In

another report, a minke whale from the western North Pacific, displaying *Brucella* positive serology, showed several nodular granulomatous lesions in the uterine endometrium (Ohishi et al., 2003). These lesions presented significant mononuclear infiltration and had epitheloid and giant cells, suggesting *Brucella* associated pathology. *Brucella ceti* was also isolated from a diseased atlanto-occipital joint of an Atlantic white-sided dolphin (*Lagenorhynchus acutus*) (Dagleish et al., 2007) and in the testes of a harbour porpoise (Dagleish et al., 2008). In one conspicuous case of brucellosis in a pregnant striped dolphin (Hernandez-Mora et al., 2008;Gonzalez-Barrientos et al., 2010) the bacteria was isolated and directly observed by immunofluorescence in placenta, umbilical cord, milk, allantoic and amniotic fluids as well as in multiple foetal organs. In this case, a necrotizing severe placentitis with multiple necrotic foci and a dead fetus close to seven-month gestation was found. Suppurative granulomatous lesions in both female and male reproductive organs have been observed in minke whales and Bryde's whales (*Balaenoptera brydei*) that had anti-*Brucella* spp. antibodies (Ohishi et al., 2003). *Brucella ceti* has also been isolated from the uterus of a striped dolphin, without any associated pathology (Munoz et al., 2006). Notwhistanding these reports, there is currently no information on the occurrence of *B. ceti* abortion in cetaceans in their natural habitat.

Laboratory Diagnostics

Brucellosis does not present pathognomonic lesions. Diagnosis depends partly on clinical investigations but mainly on laboratory testing. Laboratory diagnosis includes indirect tests that can be applied to serum as well as direct tests (classical bacteriology, PCR based methods). Only the isolation of *Brucella* spp. (or *Brucella* spp. DNA detection) allows definite confirmation. Several techniques are available to identify *Brucella* spp. The Stamp staining is still often used and even if this technique is not specific (other abortive agents such as *Chlamydophila abortus*, formerly *Chlamydia psittaci*, or *Coxiella burnetii* are also stained), it provides valuable information for the analysis of abortive material (Alton et al., 1988).

Bacterial isolation is nevertheless always preferable and even required for the typing of the strain. For the definitive diagnosis of brucellosis, the choice of samples depends on the observed clinical signs. For the isolation of *Brucella* spp., the most commonly used medium is the Farrell medium (FM), which contains antibiotics able to inhibit the growth of other bacteria present in clinical samples. While the majority of cetacean isolates will appear on FM after 4 days of incubation, seal isolates will often only be recovered on FM at about 10 days. It is therefore recommended that the incubation period is extended to 14 days before cultures are discarded as negative. Most cetacean isolates will grow in the absence of an increased

CO2 concentration whereas most seal isolates require CO2 for growth. It is therefore, recommended that all primary cultures be incubated in 10% carbon dioxide at 37°C (Foster et al., 2002). The identification and typing of *Brucella* spp. is done by analysis of morphology, staining, control of the biochemical profile (catalase, oxidase and urease), anti-polysaccharide 'O' chain (O-LPS) specific for the A or M epitopes, the lysis by phages, the dependence on CO2 for growth, production of H_2S, growth in the presence of basal fuchsine or thionin, the crystal violet or acryflavin tests (Corbel and Brinley-Morgan, 1984;Alton et al., 1988).

Several PCR based methods have been developed. The best-validated methods are based on the detection of specific sequences of *Brucella* spp. such as the 16S-23S genes, the *IS*711 insertion sequence (which has so far only been detected in *Brucella* spp.) or the bcsp31 gene encoding for a 31Kda protein (Ouahrani-Bettache et al. 1996; Baddour et al. 2008). New PCR techniques allowing the identification and sometimes a quick typing of *Brucella* spp. have been developed and are currently implemented in certain diagnostic laboratories (Bricker and Ewalt, 2005;Le Fleche et al., 2006;Lopez-Goni et al., 2008;Whatmore, 2009;Maquart et al., 2009a)

New techniques such as Single Nucleotide Polymorphism signatures (SNPs, aiming at detecting DNA sequence variation occurring when a single nucleotide in the genome differs between members of a species), MLSA (Multi Locus Sequence Analysis, aiming at directly measuring the DNA sequence variations in a set of housekeeping genes and characterizing strains by their unique allelic profiles) and MLVA (Multi Locus Variability Analysis, aiming at analysing the variability of loci presenting repeated sequences) are currently used for the typing of marine mammal *Brucella* spp. (Le Fleche et al., 2006;Whatmore, 2009;Maquart et al., 2009a).

The earliest molecular studies related to marine mammal *Brucella* strains in the late 1990s confirmed their distinction from classical species associated with terrestrial mammals (Clavareau et al., 1998). A marker specific for the marine mammal strains was identified when amplification of the gene encoding the immunodominant bp26 protein revealed a larger than expected PCR product reflecting the insertion of an *IS*711 element downstream of the gene (Cloeckaert et al., 2000). A PCR based around bp26 has become a well-used test for differentiation of *Brucella* spp. associated with marine mammals from classical species associated with terrestrial mammals. Following molecular characterisation of the omp2 locus a division into two species (labelled *Brucella pinnipediae* and *Brucella cetaceae*), compatible with the classical criteria of host preference and DNA polymorphism at the omp2 locus, was suggested (Cloeckaert et al., 2001). Eventually, two new *Brucella* species labelled (with

corrected etymology) as *B. ceti* for isolates from cetaceans and *B. pinnipedialis* for isolates from pinnipeds were validly published (Foster et al., 2007). This was in line with the decision of the *Brucella* Taxonomic Subcommittee (Osterman and Moriyon, 2006) and would cater for the prospective inclusion of biovars within these two species. Further, MLSA studies suggested that *Brucella* strains from marine mammals corresponded to a cluster of five sequence types (STs) distinct from all previously described *Brucella* species from terrestrial mammals (Whatmore et al., 2007). The first large scale application of both MLVA and MLSA techniques specifically to the marine mammal *Brucella* group was published in 2007 and, examining over 70 isolates, described the clear existence of three groups with distinct host preferences (Groussaud et al., 2007).Recently the largest study to date examined 294 isolates from 173 marine mammals by MLVA (Maquart et al., 2009a).More than hundred genotypes were identified and divided into five clusters that related to previous MLSA findings. On the basis of emerging data, the taxonomic descriptions of marine mammal *Brucella* may need to be reconsidered in the future (Whatmore, 2009).

Taxonomic classification of *Brucella* spp. is very often made difficult by the lack of, or high degree of similarity in, the marker genes traditionally used for this (Foster et al., 2009). Recently such methods were used to compare 32 sequenced genomes from the *Brucella* genus, representing the six classical species, *B. ceti* and *B. pinnipedialis* (Bohlin et al., 2010). The findings were in remarkable consistency to the current taxonomy, indicating that phylogenetic classification of *Brucella* spp. based on MLSA and marker genes (Whatmore et al., 2007) shows a surprising similarity with the actual whole gene content of the *Brucella* organism.

Brucellosis serology in marine mammals is usually performed using the same antigens as in domestic ruminant serology because the *Brucella* immunodominant antigens are associated to the surface "smooth" lipopolysaccharide (LPS) and are to a large extent shared by all the naturally occurring strains of *B. abortus*, *B. melitensis*, *B. suis*, *B. microti*, *B. ceti*, *B. pinnipedialis* and *B. inopinata*. According to their reactivity, three different type of immunochemical techniques have been used: i) direct serological assays, such as agglutination tests (Rose Bengal, RB test) and fluorescence polarization method (FPA), in which the antibodies modified the physical properties of the antigen, a phenomenon that is visually or photometrical recorded in a short period of time; ii) displacing methods, such as competitive ELISA (cELISA) in which the antibodies have to compete with monoclonal antibodies directed against the main epitope associated to the O-chain , and finally; iii) indirect serological assays, mostly designed to detect anti-LPS antibodies, such as protein G-

ELISA (gELISA), protein A-ELISA (aELISA), recombinant protein G/A-ELISA (g/aELISA), antibody-ELISA, using species specific anti-IgG conjugates (iELISA), western blot (WB), dot blot (DB), immunofluoresence (IF) and complement fixation (CF). Indirect ELISA's rely on species-specific reagents that are not commercially available. This limitation of the lack of polyclonal or monoclonal antibodies to many wildlife species immunoglobulins, can be partly overcome by the use of either Protein A or Protein G conjugates (Nielsen et al., 2004). Other techniques like competitive ELISA's or the Fluorescent Polarization assay that do not rely on species-specific reagents and have been proven useful in marine mammals (Nielsen et al., 2005). In cetaceans, a broad cross reaction among immunoglobulins of different *Odontoceti* families has been documented, allowing the use of antiserum raised against one species of dolphin as general reagent for detecting antibodies against different species of this suborder (Hernandez-Mora et al., 2009).

Marine Mammal *Brucella* spp. infections in Livestock and Fish

An experimental inoculation of three pregnant cattle with a *Brucella* spp. isolates from a Pacific harbour seal resulted in two of the animals aborting. This study indicated that marine mammal *Brucella* spp. is capable of producing antibodies and abortion in cattle but is less pathogenic than *B. abortus* (Rhyan et al., 2001). Another experimental investigation demonstrated colonisation, limited establishment of infection, transmission, and low pathogenicity of the three marine mammal *Brucella* spp. strains for sheep (Perrett et al., 2004). Lastly, ten weaned piglets were challenged by the oral and ocular routes with a human *Brucella* spp. strain (02/611), isolated from a patient with spinal osteomyelitis (McDonald et al., 2006) and is closely related to a *Brucella* spp. originating from a bottlenose dolphin from the United States ((Sohn et al., 2003;McDonald et al., 2006;Whatmore et al., 2008). Low and transient antibody titres were only detected in three pigs, two of which were culture negative. *Brucella* spp. strain 02/611 does not seem to replicate readily in pigs and thus it is unlikely that pigs are maintenance hosts for these *Brucella* spp (Bingham et al., 2008).

Brucella spp. was not known to infect poikilotherms until recently. Nile catfish (*Clarias gariepinus*) has been experimentally infected with *B. melitensis* biovar 3. The fish seroconverted and *B. melitensis* was isolated from internal organs, but the bacterium was not transferred to non-infected sentinel fishes (Salem and Mohsen, 1997). Nile catfish were shown to be seropositive for *Brucella* spp. by Rose Bengal and the Rivanol tests. Further, *B. melitensis* biovar 3 was cultured from skin swabs and PCR confirmed the identity of the

bacterium (El-Tras et al., 2010). These findings suggest that fish are susceptible to *Brucella* spp. infection and thus may also be susceptible to marine *B. ceti* and *B. pinnipedialis*. If infection with marine mammal *Brucella* spp. is proven to occur in fish, this would have a tremendous economic impact on the fish industry and significant veterinary public health implications given the potential zoonotic concern of these *Brucella* species. This clearly warrants further investigation.

Zoonotic Considerations

Today, brucellosis in humans is mainly occupational (abattoir, animal industry, hunters and health workers). Symptoms like undulant fever, tiredness, night sweats, headaches and chills may drag on as long as three months before the illness becomes so severe and debilitating as to require medical attention (Godfroid et al., 2005).

Zoonotic concerns regarding marine mammal strains were initially raised following the recovery of a cetacean strain of *Brucella* spp. from a laboratory worker at the Central Veterinary Laboratory, Weybridge who had sero-converted after suffering from headaches, lassitude and severe sinusitis (Brew et al., 1999). People at risk of zoonotic transfer of marine mammal brucellosis are individuals in traditional communities where products from whales and seals are still an important part of the diet. Also people with only occasional consumption of marine mammal meat, people handling stranded marine mammals, whale and seal hunters, people handling products from marine mammals, people in contact with raw products from the ocean, veterinary meat inspectors and researchers could be exposed. Because of the unspecific and varied symptoms of human brucellosis and the very recent awareness of the existence of marine mammal brucellosis transfer from marine mammals to humans could pass unrecognized.

In April 2003, the first report of community-acquired human infections with marine mammal-associated *Brucella* spp. was published. The authors described the identification of these strains in two patients with neurobrucellosis and intracerebral granulomas. Despite a more than 15-year separation, these cases have similarities: both patients were from Peru and denied significant exposure to marine mammals (Sohn et al., 2003). In 2006, the isolation and characterization of a marine *Brucella* from a New Zealand patient was reported (McDonald et al., 2006). It was suggested that all three reported cases of natural human infection associated with *Brucella* spp. from marine mammals were associated with ST27 (Whatmore et al., 2008). Unfortunately the natural host of ST27 (first isolated from a captive dolphin in the USA) has

not been identified although there is molecular evidence of the presence of this genotype in minke whale from the Northern Pacific (Ohishi et al., 2004).

Norwegians have a long tradition of consumption of meat from harp seals, hooded seals and minke whales all to be found infected with *Brucella* spp. In spite of this, brucellosis has not been reported in humans at risk (whale- and seal-hunters, veterinarians controlling the meat, other marine mammal meat handlers or consumers). Marine mammal *Brucella* spp. isolates were tested for their ability to infect human and murine macrophage cells. The study showed that some *B. ceti* and *B. pinnipedialis* isolates were found to be virulent in these models of infection whereas other isolates were not. In fact, all the *B. pinnipedialis* isolated from hooded seals did not demonstrate ability to infect human and murine macrophage cells (Maquart et al., 2009b) which may be an explanation for the absence of records of human infection with hooded seal *B. pinnipedialis*.

Significance and Implications for Conservation

Several of the cetacean and seal species diagnosed with brucellosis are listed in the IUCN Red list of threatened species (The World Conservation Union, http://www.iucnredlist.org/search). In spite of this, the level of endemism of cetacean species in the Central American littorals generally is not estimated for their protection or epidemiological surveillance. In this sense, it would be desirable that future conservation and management efforts would initiate on whales and dolphin species that occupy neritic waters, where human activities are most intense and more likely to affect their populations, and promote the spreading of infectious diseases. Indeed, practices such as littoral pollution, microorganism contamination, fishing and hunting, that jeopardize the food resources of the cetaceans, may promote malnutrition, competition and clustering of different species in reduced areas where food is available. These phenomena could favour the number of susceptible animals and increase the transmission of brucellosis within and between species of cetaceans. It has been shown that the pup production of the Greenland Sea hooded seal decreased substantially since the 1950s and stabilized at a low level since the 1970s, despite reduced hunting. Population fertility is one important parameter that varies in response to environmental changes, but other factors, like infections, may also be contributing factors. Although it is not known if *B. pinnipedialis* induced abortion in hooded seals despite their high prevalence, its importance in reproductive failure should be investigated. Perhaps some *B. ceti* and *B. pinnipedialis* strains are well adapted to some marine mammals which could

serve as the primary reservoir hosts. This may be de case of porpoises, in which *Brucella* antibodies are relatively frequent, but pathology limited to a few cases. On the contrary, some cetaceans such as striped dolphins may be highly susceptible to brucellosis, as demonstrated by the number of fatal cases recorded in different latitudes of the world. Alternatively, some strains of *B. ceti* and *B. pinnipedialis* are more virulent than others, as suggested by some limited experiments *in vitro* replication of several marine mammal *Brucella* strains (Maquart et al., 2009b). In any case, these conjectures remain open questions, until more *Brucella* related pathologies are documented in cetaceans and pinnipeds.

Lastly, there are only very scarce data on the transmission of *Brucella* spp. in marine mammals and the role of fish as reservoirs has not been investigated.

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