REVIEW ARTICLE

Marine mammal brucellosis: a new dimension to an old zoonosis

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Brucellosis is an important zoonotic animal disease, transmissible to man. *Brucella* research recently has been marked with the discovery of a number of novel species and hosts therein. Isolation of newer *Brucella*-like bacteria in recent years from marine mammals became a significant new development. These bacteria were shown to cause a wide variety of reproductive disorders, including abortion and meningoencephalitis among marine mammals. Three human cases with naturally acquired infection and one case of laboratory-acquired infection by *Brucella* strains of marine origin have put these novel marine brucellae in the same category of zoonoses of concern.

Keywords: Brucellosis, marine mammals, reproductive disorders, zoonotic diseases.

Background

BRUCELLOSIS is an economically important disease affecting animals and man. It ranks among the major zoonotic diseases. Brucellosis, which remains an under-reported and neglected disease, causes significant medical, veterinary and socio-economic problems. The economic impact of the disease on the animal industry is reflected by reduced production and high costs towards its management due to reproductive disorders (abortion, stillbirth and sterility), reduced milk, meat and wool yield, poor health of animals, loss of progenies, vaccination, testing, segregation and slaughter of infected animals. Dairy cattle, goat, sheep and swine are the main species involved among the domestic animals. The disease has also been reported in recent years from wild terrestrial and marine mammals. Cross-infections between different species add to the complexity of the disease[1].

Around the globe, more than 500,000 human infections are reported annually[2]. Human brucellosis, with its historical background in the Mediterranean countries, is known by various names – Malta fever, Mediterranean fever, Gibraltar fever and rock fever. The disease in humans in modern times is more popularly known as undulant fever on account of the undulating nature of febrile reaction in clinical cases[3]. The understanding of pathogenic mechanisms, severity and progression of the infection, treatment responses, vaccine and rapid diagnostic tests and development of improved treatment regimens against brucellosis in man and animals is still posing challenges[3].

Due to their high infectivity and fastidious nature, *Brucella* spp. are a major potential bioterrorism threat and the Centers for Disease Control and Prevention, USA has classified them as category-B agents[4]. Lack of sufficient knowledge about the disease among physicians, under diagnosis or misdiagnosis and absence of effective disease management strategies are attributed to the spread of this disease among human populations[3]. Cases of human brucellosis are often misdiagnosed as typhoid and tuberculosis[3]. Long duration of expensive treatment decreases its efficacy in controlling the disease in humans. Therefore, the World Health Organization (WHO) has delineated the development of effective human vaccine-mediated control and eradication programme as a major cornerstone for the management of human brucellosis[5].

Introduction

Brucellosis was first reported in Malta by Marston in 1859. In 1887, David Bruce isolated the causative organism from the spleens of the fatally infected soldiers in Malta and the bacterium was named as *Micrococcous melitensis*. Wright and Smith (1897) were the first to describe a serological diagnostic test for *M. melitensis* in man and animals, which indicated the zoonotic potential of the disease. Zammit (1905) isolated the bacterium from goats. He further concluded that goats are the natural reservoirs for *M. melitensis* and consumption of raw milk and cheese infects man. In 1920, Meyer and Shaw proposed a new generic name, *Brucella*, for the organism[1,2].

The eco-epidemiological significance of *Brucella* species lies in its host propensity and hosts for all these species of *Brucella* are different terrestrial mammals. The classical strains of *Brucella*, namely *B. melitensis*, *B. abortus*, *B. suis*, *B. ovis*, *B. neotomae* and *B. canis* are

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associated with specific animal species. Among the smooth strains, *B. melitensis* chiefly infects goats and sheep, with some reports in cattle and buffaloes; *B. abortus* infects mainly cattle and buffalo. Primary host of *B. suis* is swine and that of *B. neotomae* is desert wood-rat. *B. canis* and *B. ovis* are the two rough strains causing infection, particularly in dogs and rams respectively. Among brucellae, *B. melitensis* is the most zoonotic followed by *B. suis*, *B. abortus* and *B. canis*. These strains can also infect animals other than their preferred hosts[3].

Brucellosis is a re-emerging zoonosis with continuously evolving epidemiology because of the emergence of novel *Brucella* strains. It has established itself in new hosts and ecological niches. During 1990s, new *Brucella* species were reported from the marine mammals and were initially named as *B. maris*[6,7]. Later, *B. microti* was isolated from common voles and red foxes and also identified as soil contaminant in Central Europe[8]. Two novel strains, *B. inopinata* and another similar to *B. inopinata*, have been isolated from a human breast implant and from a patient with chronic lung disease[9]. The natural reservoirs and ecology of these two strains remain unknown. A bacterial strain with *Brucella*-like characteristics but distinct from the currently described species has been reported from two baboons with stillbirth[10]. Recently, two atypical Brucella strains were isolated from wild red foxes (*Vulpes vulpes*) in eastern Austria[11]. These isolates had negative nitrate reductase and negative oxidase reactions which are atypical to genus *Brucella*. However, on the basis of serology and molecular analysis, it has been suggested that both strains possibly represent a novel *Brucella* species.

The present review article focuses on the etiology, epidemiology and diagnosis of brucellosis in marine mammals and its zoonotic implications. The marine *Brucella* isolates may act as etiological agents for various reproductive disorders in sea mammals (cetaceans and pinnipeds) leading to abortion and stillbirth, and can be of concern for the existence of threatened marine mammal species. Marine *Brucella* strains represent a zoonotic threat; however, the pathogenicity of these microorganisms to humans is yet to be clearly established. Sea mammals can also introduce brucellosis to new hosts and new areas, as their movement is independent of political and geographical boundaries.

Etiology and epidemiology of marine brucellosis

Isolation of *Brucella* spp. from marine mammals was reported for the first time in 1994 from stranded common seals (*Phoca vitulina*), harbour porpoises (*Phocoena phocoena*) and dolphins (*Delphinus delphis*) around the coast of Scotland[6]. In the same year the organism was also recovered from the aborted foetus of a bottlenose dolphin (*Tursiops truncates*) in California[7]. This new strain, initially designated as *Brucella maris*, was further divided into biovar 1 (isolated from seals and otters), biovar 2 (from cetaceans) and biovar 3 (from Californian bottlenose dolphin)[12]. Marine mammalian *Brucella* isolates have a host preference for either the order Cetacea (whales, dolphins and porpoises) or Pinnipedia (seals, sea lions and walruses), with the exception of one isolate which was recovered from sea otter (family Mustilidae and order Carnivora). The strains isolated from seals are different compared to those isolated from cetaceans[13]. Later on, two new species names were proposed, i.e. *B. cetaceae* for cetacean isolates and *B. pinnipediae* for pinniped isolates instead of *B. maris*[14]. *Bergey's Manual of Systematic Bacteriology* had described three novel *Brucella* species from marine mammals as *B. phocae* (seals), *B. phoecoenae* (porpoises) and *B. delphini* (dolphins)[15]. In addition, other nomenclatures based on various genetic and molecular techniques have been proposed to classify the marine mammal *Brucella* isolates[16–19]. Pacific cetacean *Brucella* isolates are quite distinct from European marine mammal isolates. Therefore it is believed that Pacific isolates may constitute a separate marine mammal species or subspecies[19]. Marine *Brucella* isolates have distinct genetic and phenotypic characteristics compared to terrestrial mammal isolates[20].

Brucella infection appears to be widespread among the sea mammals. A large number of marine mammals have been found to be sero-positive to *Brucella* antibodies around the world[13,21–26]. The serological evidences of marine brucellosis are documented more from the northern hemisphere than the southern hemisphere[17,21]. Among the various species of the sea mammals, Atlantic white-sided dolphin (*Lagenorhynchus acutus*) is found to be most commonly associated with *Brucella* infections, whereas *Stenella coeruleoalba*, the striped dolphin is reported to be a highly susceptible host and may act as a reservoir of *Brucella* infection[13,23,27]. Presence of anti-*Brucella* antibodies was detected more in Antarctic fur seals (*Arctocephalus gazella*) compared to other species of marine mammals tested in Antarctica[28]. Recently, a high (57%) sero-prevalence has been reported among Australian fur seals (*Arctocephalus pusillus doriferus*)[26].

Transmission of marine brucellosis

The transmission of marine brucellosis is poorly understood. The route of infection and marine mammal reservoirs and animal-to-animal transmission remain uncertain. Gregarious nature of some of the sea mammals is believed to aid in the transmission of brucellosis among the sea mammals[21,29]. *Brucella*-like organisms have also been detected in the lungworms; *Pseudalius inflexus*, lung worm of Pacific harbor seal (*P. vitulina richardsii*) and *Halocercus* spp., lung worm of bottle

nose dolphin[30–33]. This suggests a potential role of the lungworms in transmission of the disease among marine mammals[30–34]. Brucellae have also been isolated from longstanding cestodes (*Phyllobothrium delphini*) from bottlenose dolphin[33]. It may also be possible that species down the marine food chain may act as a common source of infection to different species of marine mammals[35]. Among terrestrial animals, brucellae are transmitted through exposure to infected placenta, birth fluids and vaginal secretions, venereal route, milk and through *in utero* transmission[3]. In marine mammals also, isolations of *Brucella* have been made from milk and mammary glands, reproductive organs, placenta, umbilical cord, foetal tissues, aborted foetus and secretions of pregnant sea mammals. Therefore, marine mammal *Brucella* isolates also have tropism for placenta and foetal tissues as in *Brucella*-infected terrestrial animals. The vertical transmission of the *Brucella*-infection has been recorded and possibility of the horizontal transmission among sea mammals cannot be denied. Further, isolation of the organism from the reproductive organs suggests the possible sexual transmission of the organism and/or sterility as sequelae to infection, similar to those reported in terrestrial animals[7,23,33,34].

Disease caused by marine mammal *Brucella* isolates

Disease in marine mammals

Brucella spp. have been reported from both apparently healthy and symptomatic animals[36,37]. The symptoms or clinical syndrome for brucellosis in the sea mammals are not clearly documented. Systematic brucellosis appears to be common in marine mammals, but it is rarely associated with pathological changes[38]. Brucellae have been isolated from a wide variety of tissues and from reproductive organs of both the sexes and also from the aborted foetuses and placentas[13]. *Brucella* spp. in marine mammals have been associated with various pathological expressions such as subcutaneous lesions, abscesses, hyperplastic lymph nodes, congested mammary glands, splenic and hepatic necrosis, necrotizing thrombo embolic pneumonia or meningitis/meningoencephalitis and abnormal joints and testes, epididymitis and abortions[13,21,34]. Placentitis and abortions are reported in the captive bottlenose dolphins and wild Atlantic white-sided dolphin[39]. Atlantic white-sided dolphins were found to have *Brucella* lesions mainly consisting of hepatic and splenic coagulative necrosis, splenomegaly, congested lungs, lymphadenitis, mastitis and possible abortions. *Brucella* organisms in marine mammals were also found to be associated with oesophageal ulceration and necrosis[13]. *Brucella* has also been reported as a secondary pathogen among stressed porpoises, seals and dolphin[13]. The main pathological findings recorded in porpoise (*P. phocoena*) were blubber abscession, spinal discospondlitis and splenic necrosis[13].

Brucella can also act as an opportunistic pathogen in marine mammals with poor state of nutrition or those suffering from some other disease or parasitism[13]. *Brucella* as a main etiological agent can cause death due to hepatic abscess, peritonitis and epididymitis in marine mammals[16,23]. Nervous form of the disease resulting in meningoencephalitis is seen in striped dolphins only[23,38,40]. Neurobrucellosis is evident by the inability to maintain buoyancy, ophisthtonus, tremors and seizures[23]. Animals suffering from nervous form of the disease had hyperemic meninges, congested brains and altered cerebrospinal fluid[23]. *Brucella* organisms were also isolated from adult female harbour porpoises with occluded bile duct and from the lungs and kidneys of malnourished pups of grey seal, *Halicherus grypus*[41]. The pregnant animals can develop placental abscesses due to *Brucella* infection[23]. Abnormal testes and caseated and calcified uterus were recorded as the main pathological findings among *Brucella* sero-positive common mink whales (*Balaenoptera acutorostrata*) and Bryde's whales (*Balaenoptera edeni*)[42,43].

Disease in other animals

The *Brucella* organisms are known to cross the species barrier causing disease in animals other than their preferred host[3,44]. Studies indicate that *Brucella* spp. isolated from marine mammals can also cause disease in terrestrial animals. The disease was induced in cattle, sheep and piglets through experimental inoculations with *Brucella* strains isolated from marine mammals. Marine *Brucella* species was re-isolated from the aborted cows showing histopathological changes and from various organs of unaborted animals with 100% sero-conversion[45]. Sheep inoculated with an isolate of seal origin developed a transient low level of anti-*Brucella* antibodies and the microorganism was also isolated from the one of the aborted ewes and its foetus[46]. The organisms were re-isolated from lymph nodes of experimentally infected pigs. Low and transient antibody titres were detected in culture-negative, experimentally infected pigs[47].

Antibodies against *Brucella* have also been detected in polar bears (*Ursus maritimus*) from Svalbard and the Barents Sea. The ringed seals (*Phoca hispida*), an important prey species for the Svalbard polar bears and harp seals (*Phoca groenlandica*) from the same geographical areas were also sero-positive to *Brucella* antibodies, suggesting possible transmission of brucellosis from prey to predator[48]. All these studies suggest that the disease occurring in sea mammals can be transmitted to domestic animals and wildlife residing in the nearby coastal areas.

Zoonotic implications of marine brucellosis

Human brucellosis is essentially an occupational disease. *Brucella* infections in humans occur due to direct or indirect contact with infected animals and/or their discharges, and contaminated animal products[3]. Consumption of contaminated animal products such as milk and meat products is major source of infection in man[3]. Person-to-person transmission is rare, though it may occur through sexual contact[49,50], tissue transfer, e.g. bone marrow and blood transfusion[51,52] and breastfeeding of infants[52]. In addition, laboratory-acquired *Brucella* infections due to accidental ingestion or inhalation, mucosal or skin exposure to infected tissue specimens or cultures of virulent or attenuated *Brucella* strains are major health hazards. The aerosols generated during the manipulation of *Brucella* cultures are the commonest source of laboratory infection[53–55]. Accidental exposures to animal vaccines can cause disease in handlers[56].

Transmission of brucellosis from marine mammals to man is not as extensively reported as *Brucella* infections in marine animals. Interestingly, to date four human cases with *Brucella* infections have been reported presumably of marine mammal origin[57]. Three of these cases were acquired through natural infection by marine origin *Brucella* – one case of spinal osteomyelitis from a patient in New Zealand[58] and two cases of neurobrucellosis from Peruvian patients[59]. Another case of laboratory-acquired infection has also been reported[55]. Cases of zoonotic marine brucellosis reported from Peru had serious central nervous system disease with intra cerebral granuloma[59]. In both cases there was no direct contact with the marine mammals. The patients had history of consumption of queso fresco (soft cheese) and raw shell fish ceviche (citrus-marinated seafood) respectively. One had frequently swum in the Pacific Ocean, whereas the other seldom visited the sea coast. However, the mode of transmission in these cases remains questionable because of the history of regular consumption of unpasteurized cheese[59]. In New Zealand, marine mammal-type *Brucella* strain was isolated from a patient with no direct exposure to marine mammals, but who had a history of regular fishing, contact with uncooked bait and consumption of raw snapper[58]. Isolates from Peruvian patients were similar to *B. pinnipidae* (seal strain), whereas the isolate reported from New Zealand was closely related to a *Brucella* sp. originating from a bottlenose dolphin (*T. truncatus*) in the United States and common seals (*P. vitulina*). All these cases can be seen as the early warning signs of an emerging zoonosis[58,59].

In general, incubation period of brucellosis in man could extend from one week to six months or more. It depends upon virulence of the infecting strain, size of the inoculum and resistance of the host. Among terrestrial strains, *B. melitensis* is associated with acute infection, whereas infections with other species are usually subacute and prolonged. Acute form of the disease is characterized by intermittent fever (38–41°C), which remains normal during the early part of the day and rises during the evening. Fever is associated with chills, shivering, malaise, nausea, extreme fatigue, inappetence and loss of body weight. After reaching a peak, the fever subsides rapidly with profuse sweating. Brucellosis also causes enlargement of the liver, spleen, superficial lymph nodes and abscess formation in the visceral organs. About 10% of the patients can develop bone and joint complications such as paravertebral abscess, spondylitis and reactive arthritis[3]. Nervous form of disease is seen in less than 5% of patients[60]. Neurobrucellosis, more commonly associated with *B. melitensis* infection, results in meningitis and meningioencephalitis[61]. Many cases diagnosed as tuberculous meningitis are in fact the those of neurobrucellosis[62].

Thrombophlebitis and endocarditis are the most frequent cardiovascular complications of brucellosis. Endocarditis is incriminated for a high proportion of mortality in brucellosis. Brucellar endocarditis can also be secondary to chronic rheumatic heart disease[63,64]. Epididymoorchitis is the commonest genitourinary complication in males and in pregnant women abortions may occur[65]. Many pregnant women suffering from the acute disease have also carried for the full term without any treatment[61]. In addition, *Brucella* organisms were also reported to cause pneumonia and colitis in human beings[65,66].

Infection with marine *Brucella* strains causes a range of symptoms, including fever, rigours, headaches, lassitude, sinusitis and lumbar spinal tenderness (spinal osteomyelitis). Nervous symptoms include headaches, nausea, vomiting, periorbital pain, periodic generalized tonic–clonic seizures and progressive deterioration in vision. The relative zoonotic potential of marine mammalian isolates is yet to be clearly established[55,58,59].

Diagnosis and classification of marine *Brucella* strains

The symptomatic diagnosis of the brucellosis in marine mammals cannot be made as no clinical syndrome has been established in the marine mammals. *Brucella* organisms have been isolated from both normal as well as symptomatic/diseased animals. Brucellosis can be diagnosed by host preference, serological and molecular techniques, or isolation of the organisms from the affected animal.

Isolation of the organisms from marine mammals

The majority of isolations of *Brucella* organism from sea mammals were made from dead animals. The organisms had been isolated from male and female reproductive organs, mammary glands, brain, spinal cord abscesses, diseased atlanto-occipital joint, lungs, spleen liver,

kidneys, cerebrospinal fluid, joints, foetal tissues, milk, secretion of pregnant animals, purulent blubber abscesses and a variety of lymph nodes. Isolations had also been made from blood cultures collected from heart and lungworms of sea mammals. The tissues with or without the gross or microscopic changes can provide positive *Brucella* cultures. The oral, nasal, tracheal, vaginal and anal swabs and faeces can also be collected for isolation of *Brucella* from live marine mammals[13,21–23,27,28,31,41,43]. Lungs are the primary organs for isolation of *Brucella* from the sea mammals[13,21,39].

Brucellae are Gram-negative, aerobic and non-motile cocco-bacilli. The organism is about 0.5–0.7 μm in diameter and 0.6–1.5 μm in length. It is a fastidious organism and has specific requirements for the growth[21,67,68].

Primary isolation of brucellae[21,57,68] may take 4–5 days and 10% CO_2 and a temperature of 37°C. Majority of *Brucella* isolations of organisms from sea mammals are done on Farell's medium, followed by Columbia sheep blood agar, *Brucella* agar with *Brucella* selective supplement and 1.4% crystal violet and brain heart infusion agar with 5 g of yeast extract. Cetacean isolates generally become visible within 4 days of inoculation on this medium. However, isolates from seals may fail to grow or take 7–10 days to grow. The samples should also be simultaneously incubated on certain non-selective media such as serum dextrose agar or blood agar. Most isolates from the pinnipeds are capnophilic, whereas those isolated from cetaceans can grow well without CO_2. It is also recommended that cultures should be discarded as negative only after 14 days of incubation[13,21,27,29,41,43,67].

Marine mammal *Brucella* isolates have smooth colony appearance with entire margins and are raised, convex and shiny. These appear as honey coloured and translucent when examined by transmitted light. Brucellae are acid-fast in modified Ziehl–Neilson's staining and show agglutination with *B. abortus* antisera[13]. *Brucella* species can be differentiated through sero-typing, phage-typing, dye sensitivity, CO_2 requirement, H_2S production and other metabolic properties[67]. Sea mammal *Brucella* strains can be differentiated from the other six *Brucella* species of terrestrial origin through a substrate-specific tetrazolium reduction test and phenotypic characters[12,69]. Similar procedures are applied to isolate marine *Brucella* strains from infected human beings[55]. The importance of direct isolation of the organism from the suspected human cases is stressed since prolonged or chronic illness, unknown host factors, symptom-based medication and low immunogenicity of the marine *Brucella* strains may result in low or absence of immune responses.

Brucella isolates from marine mammals have been subdivided into three different biovars on the basis of their CO_2 requirement, metabolic activity on galactose and dominant antigen and animal host[12]. On the basis of differences in host rage, genomic variations and carbohydrate metabolism (L-arabinose, D-galactose and D-xylose), new names – *B. ceti* and *B. pinnipedialis* were proposed for isolates from cetaceans and seals respectively[16].

Serological methods

Serological tests play a crucial role in the brucellosis surveillance programmes. A number of serological tests are in use to detect *Brucella* antibodies or agglutinins in man and animals. Each test has its own advantages and limitations in terms of sensitivity and specificity. The smooth lipopolysaccharide (S-LPS) is the immunodominant antigen in the *Brucella* cells. The antibodies against the S-LPS of *Brucella* spp. cross-react with S-LPS of other bacteria such as *Yersinia enterocolitica* O9, *Escherichia coli* O157 and *Salmonella* Urbana resulting in false-positive agglutination reactions or misdiagnosis. Moreover, absence of agglutinins does not exclude brucellosis, as many cases have been recorded in which a positive blood culture was obtained despite negative agglutination reactions. It is, therefore, desirable that a battery of serodiagnostic tests should be applied to screen a given population to detect as many reactors as possible[70].

The anti-*Brucella* antibodies have been detected in a number of marine mammal species. Serological tests, based upon *B. abortus* antigen, used for marine mammal brucellosis diagnosis are similar to those being used to diagnose brucellosis in terrestrial animals. These include Rose Bengal plate test, serum tube agglutination antigen (STAT)/tube agglutination test, ethylenediaminetetraacetic acid modified STAT, complement fixation test, card agglutination test, buffered acid plate antigens, rivanol test, enzyme-linked immunosorbent assays (ELISA), florescence polarization assays (FPA) and immunoblotting. Sero-prevalence of brucellosis varies with the species of the animals, number of animals screened, territory or area, number and type of tests employed for screening[24,71–79]. Threshold values or interpretation of these tests are the same as used in the diagnosis of brucellosis in terrestrial animals. Validation of these tests in terms of the specificity and sensitivity is required for diagnosis of brucellosis in various species of sea animals[80]. A consensus for determining a positive result often requires that a marine mammal serum sample tests positive on multiple serological tests[81]. It is likely that the serologic test which uses antigen from a marine mammal isolate may be more sensitive than those from terrestrial animals. LPS and protein antigen determinants may be sufficiently different to affect antigen–antibody affinity among different species of marine *Brucella* isolates[82].

Competitive ELISA (C-ELISA) and FPA were found appropriate as diagnostic screening tests for detection of *Brucella* antibodies in marine mammals[75]. An indirect ELISA using terrestrial *B. abortus* and *B. melitensis* LPS as antigen has been developed for testing odontocete serum[83]. A capture ELISA (cELISA) using whole-cell antigen from a harbour seal (*P. vitulina*) marine *Brucella*

sp. isolate was reported to have high sensitivity but lower specificity with cetacean sera. However, specificity and sensitivity were both reduced when the same test was applied on pinniped sera. The marine-origin cELISA was a more sensitive assay than the classical *B. abortus*-based tests for detecting anti-*Brucella* antibodies in both cetacean and pinniped species[84].

Molecular methods

Molecular or the genomic methods of diagnosis and differentiation of *Brucella* species are more useful than serology or culture isolations because of serological cross-reactions and fastidious nature of this zoonotic bacterium. Molecular analysis has confirmed the genetic distinctiveness of marine strains from the terrestrial strains[14,85–88]. DNA–DNA hybridization shows that the *Brucella* strains isolated from marine mammals have more than 77% DNA relatedness and belong to the monospecific genus *Brucella*. On the basis of ribotyping (*Hin*dIII rDNA restriction patterns), marine isolates were classified as a separate subgroup of the genus *Brucella*[86]. Occurrence of an IS711 element downstream of the *bp26* gene is a feature specific to the marine mammal *Brucella* isolates[87]. Infrequent restriction site polymerase chain reaction (IRS-PCR) targeting IS711 was able to identify *B. cetaceae* and *B. pinnipediae* separately[87]. The marine mammal isolates were shown to contain a higher number of IS711 copies compared to terrestrial mammal isolates and at least one specific IS711 copy was detected in all the marine isolates[89–91]. The omp2 locus containing two gene copies, *omp2a* and *omp2b*, coding for porin proteins is useful in molecular typing and identification of *Brucella*[14]. Isolates from dolphins and porpoises carry two *omp2b* gene copies instead of one copy each of *omp2a* and one *omp2b* gene or two similar *omp2a* gene copies reported from earlier recognized *Brucella* species. *omp2b* gene is a specific marker for grouping the marine mammal *Brucella* isolates[14]. The divergence found between their omp2b and omp2a nucleotide sequences indicates that marine isolates form a more heterogeneous group than isolates from terrestrial mammals. Brucellae isolated from diverse marine mammal species comprise more than one species, and at least two new species, *B. pinnipediae* and *B. cetaceae*. These two species are compatible with the classical classification criteria based on host preference and DNA polymorphism at the omp2 locus[14].

The variable number of tandem repeats (VNTR) typing and multilocus sequence analysis differentiated the marine mammal brucellae into three major genetic groups. One group contains isolates predominantly found in pinnipeds (seals) and were previously categorized under species *B. pinnipediae*. *B. cetaceae* isolates fall into two distinct groups that appear to have different preferred cetacean hosts (porpoises and dolphins). Interestingly, these two groups appear less closely related to each other than either group is to *B. pinnipediae* isolates[92]. On the basis of IRS-PCR, PCR-restriction fragment length polymorphism (RFLP) of outer membrane protein (omp) genes and IS711 fingerprint profile isolates originating from cetaceans and grouped under species *B. ceti*, fall into two clusters. These correspond to isolates with either dolphins or porpoises as their preferred host. Isolates originating predominantly from seals, and referring to *B. pinnipedialis*, cluster separately and can be further subdivided, with isolates from hooded seals comprising a distinct group[17]. Macrorestriction has identified subgroups within the pinniped and cetacean isolates and a 62 kb fragment was found only in pinniped isolates, except hooded seal isolates[93]. Strain typing on the basis of multiple locus VNTR analysis comprising 16 loci (MLVA-16), *B. ceti* group was subdivided into a cluster each of dolphins, mink whales and porpoises. Isolates from dolphins were further sub-divided into two subclusters. Similarly, the *B. pinnipedialis* group was identified to have three sub-clusters, one composed exclusively of isolates from hooded seals (*Cystophora cristata*) and the two others comprising other seal species isolates[20]. Multilocus sequence typing, classified marine mammal isolates into five groups from strain type (ST) 23 to ST27. The closely related ST24 and ST25 were composed of the pinniped isolates, forming the cluster C. ST26 was exclusively composed of dolphin isolates and formed the cluster A. The other cetacean isolates fell into cluster B (ST23) and consisted of strains isolated from porpoises and dolphins. ST27 was represented by only one isolate from an aborted bottlenose dolphin foetus originating from the Western coast of the United States[57,92].

A consensus on the nomenclature of *Brucella* has been hard to come by. The International Committee on Systematic Bacteriology, Subcommittee on the Taxonomy of *Brucella* in 1994, observed the absence of a clear definition for the concept of species and biovars within the genus and agreed on the need to revisit the definition of *Brucella*[94]. Later, taxonomy of *Brucella* has been reapproved to six *Brucella* nomenspecies. The previous *Brucella* taxonomy was based on > 90% DNA–DNA hybridization identity among brucellae[95], and *B. melitensis* was recommended as a single species with 18 biovars and five nomenspecies – *B. abortus*, *B. suis*, *B. ovis*, *B. neotomae* and *B. canis*. However, this has now been changed to the pre-1986 position[94].

Control and prevention of marine brucellosis

Brucellosis in marine mammals is an emerging zoonotic disease. Although marine *Brucella* strains were recognized recently, the studies conducted till date indicate that the disease is probably endemic in marine mammals. It has already been indicated that these strains might affect the reproductive activities in these animals, which is particularly a concern in threatened or naïve species.

Brucella organisms have been isolated from a newborn Maui dolphin (*Cephalorhynchus hectori maui*). These are considered to be the rarest marine dolphins, with only around 100 animals in the world[21]. The commonest mode of infection to human beings seems to be eating of raw or undercooked seafood. Marine mammals can shed brucellae actively and isolations have been made from faeces of *Brucella*-positive habour seal[96]. Direct contaminations of these kind pose direct threat to occupationally exposed human beings as well as other healthy sea mammals[96]. In view of the vast areas of inhabitation and routes of migration of the marine mammals, brucellosis can be easily and efficiently introduced to new hosts and newer regions and such spread of the disease will be difficult to control.

Brucella strains are susceptible to a wide range of antibiotics *in vitro*, but fewer antibiotics have proved effective during the treatment of the disease. WHO recommends 600–900 mg rifampicin and 200 mg doxycycline as a single dose for a minimum of 6 weeks[48]. Human cases of brucellosis with marine brucellosis have been treated successfully with combinations of rifampin and tetracycline (8 weeks)[59]; rifampin, doxycycline, gentamicin (one week) followed by trimethoprim-sulfamethoxazole (one year)[59] and ceftriaxone (6 weeks)[58]. Laboratory-acquired marine *Brucella* strain infection was successfully treated with a combination of rifampin and doxycycline (6 weeks)[55].

Indian perspective

The presence of brucellosis in India was established in the previous century and since then the disease has been reported from all over the country[97]. Brucellosis is considered to be one of the important but neglected diseases in India[98]. The reported incidence of human brucellosis in endemic areas has been reported from < 0.01 to > 200 per 100,000 population. Data on incidence and economic cost of brucellosis in India are not available and it is believed that the actual level of disease in the population may be much higher, given the level in domestic animal populations. The Indian Ocean Cetacean Sanctuary was established by the International Whaling Commission for supporting cetacean research in India. Forty species of cetaceans have been recorded from the Indian Ocean region and 25 species are represented in the Indian waters. Of these 25 species, many marine mammalian species are either endangered, vulnerable or information on them is insufficient. However, marine mammal research is in its infancy in India. The lack of research programmes, with focused attention on marine mammal biology research in India has been responsible for lack of experts on marine mammals. As there is poor or no research collaborations between veterinary microbiologists and marine mammal experts and biologists, any attempt to profile the disease status of marine mammals is also lacking. It is important that the groups involved in marine mammal conservation research in India should forge institutional linkages with veterinary research institutions to properly utilize the samples collected at the time of strandings or accidental entanglements[99].

Recommendations and future strategies

Marine brucellosis has been considered as an emerging hazard for persons occupationally exposed to infected tissues from marine mammals[100]. Pollution of coastal marine waters with human and domesticated animal faecal material has increased due to increase in human population, industrialization, urbanization and international transportation. This eventually leads scientists to believe that marine mammals and avian species may harbour these pathogens and become vectors of zoonotic infections. In order to study and understand such infections, future veterinarians must be trained in such fields of knowledge like marine mammal veterinary sciences and marine farming[101]. It is important to note that the modes transmission of brucellosis from marine mammals to man is still questionable. But reports on isolation of marine mammal brucellae indicate that people eating raw or uncooked food and those involved in recreational activities such as swimming are at higher risk of acquiring the infection[57–59]. In spite of marine mammal diversity of Indian seas being represented by around 30 recorded species, which form one-fourth of the world's marine mammals and almost 8% of all mammalian fauna recorded in India[102], studies on marine mammals are limited. However, as so far the capacity building for education and research is concerned, it is recommended to be based on multidisciplinary approaches by cross-linkages among institutions and systems so that it is sustainable and effective[103]. It has been proposed that support for member states for strengthening animal disease surveillance systems, including those involving aquatic animals, needs to be given[104]. The identification of bacterial diseases afflicting our marine mammal biodiversity along our seacoasts is important from both sea-mammal and human health perspectives. For this, apart from capacity building and training for improving the quality of the veterinary services and appropriate diagnostic laboratories on the basis of adopted standards of the OIE, bringing appropriate veterinary legislation and animal health policies, is also important[105]. We also need to understand the epidemiology of marine brucellosis, for which a baseline seroprevalence epidemiological survey for assessing the real situation of marine brucellosis needs to be undertaken.

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