Summary of the zoonotic potential of marine mammal *Brucella* species	
Risk level estimate	Very Low
Level of confidence in the assessment of risk	Good
Actions(s)/Recommendations(s)	No actions required. Continue to monitor and review new evidence as it becomes available.
This risk assessment will be reviewed as and when new information / evidence becomes available.	

Date of this risk assessment:	**2 December 2013**
Reason for review:	**Updated UK animal data**
Expert contributors acknowledged:	**Geoff Foster, Scottish Agricultural College Veterinary Services; Paul Jepson, Institute of Zoology; Judy Stack, AHVLA National Reference Laboratory for Brucellosis**
Date of previous risk assessment:	**August 2008**
Date of initial risk assessment:	**August 2008**

Scientific Secretariat to the HAIRS Group

Overview of the situation	
Background information	This topic was initially referred to HAIRS in 2008 by the Veterinary Laboratories Agency (now the Animal Health and Veterinary Laboratories Agency, AHVLA) for guidance on the potential zoonotic risks to those who may come into contact with stranded marine mammals, and the course of action to be taken if the AHVLA or other agencies handling marine mammals (such as SAC Consulting Veterinary Services (SACCVS)) identify *Brucella* spp. in a marine mammal. *Brucella* species were first reported in marine mammals in the UK in 1994, when *Brucella*-like organisms were isolated from the internal organs of four common seals, two harbour porpoises and one common dolphin at SACCVS, Inverness. A letter was written to the *Veterinary Record* alerting those involved in work with marine mammals of the potential zoonotic risk[1]. Since this time *Brucella* spp have been isolated from a range of marine animals, and two new species of *Brucella* have been identified, and recently named based on their preferred host species; *Brucella ceti* (for strains with cetaceans as their preferred host) and *Brucella pinnipedialis* (for strains with pinnipeds (seals) as their preferred host). These species have a 77% DNA-DNA relatedness value with the six established members of the *Brucella* genus.[2] The HAIRS group was provided with a briefing paper from the AHVLA. Following the first identification of *Brucella s*pp. in animals from UK waters, a serological survey was carried out to ascertain the range of marine mammals which might have been exposed to the organisms. Serum was collected from 153 marine mammals stranded around the coasts of England and Wales between 1989 and 1995 and tested for brucella antibody by competitive and indirect ELISA. Positive titres were recorded for 6/62 (10%) grey seals, 1/12 (8%) common seals, 11/35 (31%) harbour porpoises, and 9/29 (31%) common dolphins. A study reported in 1996 described the isolation of *Brucella* spp. from 9 seals, 8 cetaceans and a European otter, all from around the Scottish coast, except for one harbour porpoise from NE England. Organisms were isolated from tissues including spleen, mammary gland, mandibular, sublumbar, gastric, external and internal iliac and colorectal lymph nodes, testes and blood.[3] A 2002 review of the literature on marine mammal brucellosis indicated that *Brucella* infection occurred in a wide range of species inhabiting a vast amount of the world's oceans. It was further stated that *Brucella* spp had been cultured from all the major body organs of infected animals including brains and various other sites including reproductive tissue, the lymphatic system and sub-cutaneous lesions.[4] A paper reported 3 cases of neurobrucellosis in live-stranded striped dolphins stranded in Scotland[5] and a further 9 cases due to *B. ceti* in striped dolphins in Costa Rica, which were found stranded in populated areas.[6] One animal was pregnant, and *B. ceti* was isolated from milk, fetal tissues and secretions from this dolphin. The authors commented that a number of people handled and touched all the infected dolphins, mainly as attempts to return them to the ocean.[6] Seroconversion has been reported in cattle experimentally infected with a *Brucella* sp. isolated from a Pacific harbour seal. Of 6 pregnant cattle exposed by intravenous injection or intraconjunctival inoculation, all seroconverted and 2 aborted, and brucellae were detected in the foetus and dams

	immediately after abortion. The authors considered that marine mammal strains of *Brucella* were capable of producing seroconversion and abortion in cattle but were less pathogenic than *B. abortus*.[7] An experimental study at AHVLA Weybridge infected sheep conjunctivally with *Brucella* strains isolated from a porpoise, seal and dolphin in Scotland.[8] This demonstrated colonisation, limited establishment of infection and low pathogenicity for the three marine mammal strains, although one strain was isolated from the placenta and amniotic fluid of an animal that lambed prematurely with moribund triplets, but not from the tissues of the aborted animals. Transient and low antibody titres were detected in 3 of 10 piglets challenged with a *B. ceti* strain isolated originally from a human in New Zealand. Only one of these three piglets was culture positive. No pathological changes were detected in any of the animals.[9] Marine mammal associated species of *Brucella* have been reported in humans on only four occasions. The first case was published in 1999, in a member of research laboratory staff who worked with strains of *Brucella* isolated from marine mammals in Scotland[10], and reported continuous headaches, lassitude and severe sinusitis. Blood tests were positive for *Brucella* antibodies by IgM and IgG ELISA and serum agglutination tests, and rising titres were documented. A *Brucella* species indistinguishable from strains of *Brucella* previously isolated from cetaceans was isolated after 6 weeks incubation. Naturally acquired human infections have been reported three times. A report published in 2003 described two cases of neurobrucellosis and intracerebral granulomas in Peruvian nationals, who were seen at hospitals in the USA. The first was a 26 year old man seen in 1985 with a 3 month history of symptoms including headaches, periorbital pain and seizures. The second was a 15 year old boy seen in 2001, with a 1 year history of headaches, nausea, vomiting and progressive deterioration in visual function. *Brucella* spp. were cultured from biopsy specimens from both patients and identified by PCR targeting the bp26 gene and sequencing of the omp2a gene. The isolates were most closely related to *B. pinnipedialis.* The second patient was serologically negative for brucella infection, but the first was positive. Both patients reported regular consumption of unpasteurised cow's or goat's cheese (queso fresco) and occasional consumption of raw shellfish (ceviche), but no other raw meats. Neither patient recalled contact with marine mammals, however the first patient had frequently swum in the Pacific Ocean.[11] The most recent case was in a 43 year old man from New Zealand, who presented in February 2002 with a 2 week history of spinal osteomyelitis, fever, rigors and lumbar spinal tenderness. The patient was serologically positive on tests using *B. abortus* antigens, and blood cultures grew an organism initially identified as *B. suis*, and later identified as closely related to *Brucella* spp. originating from a US bottlenose dolphin and common seals. The patient reported no consumption of unpasteurised milk or cheese, and no occupational exposure to meat or meat products, but had dressed two freshly killed pigs in December 2001. He reported no exposure to marine mammals, but was a regular fisherman and had been exposed to uncooked fish bait, including pilchards, bonito, squid and mullet, and consumed freshly caught snapper.[12]
Summary of discussion amongst HAIRS group	In 2008 it was felt that the main risk would be to those working with cetaceans, particularly sick cetaceans, and it was agreed that the risk assessment would be circulated to the appropriate VLA staff,

	and to colleagues working with cetaceans.
Initial conclusions of the HAIRS group	RA Level 2 – consider setting up surveillance of exposed groups. This was done informally as indicated above.
Update(s)	No further human cases have been documented in the literature to date (1/8/2013); however marine mammal cases continue to be documented worldwide. In GB, necropsies have been done on between 90-150 marine mammals per year at the SACCVS in Inverness and at AHVLA disease surveillance centres in England and Wales. *Brucella* spp are occasionally isolated - over the last 20 years, between 2 and 20 animals, both cetaceans and seals, have been culture positive per year. (Personal communication, Geoff Foster SAC; AHVLA VIDA data)

Risk assessment of zoonotic potential

QUESTION	OUTCOME	QUALITY OF EVIDENCE
Level 0: Animal disease: Pathogen identified?		
Brucella ceti and *Brucella pinnipedialis*	**Yes**	**Good**
Level 0: Does the microbiology/virology support the possibility of the organism causing human infection?		
Several *Brucella* species are known to cause human infection; *B. melitensis (*biovars 1-3), *B. abortus* (biovars 1-6 and 9), *B. suis* (biovars 1-5), and *B. canis*.[13] Brucellosis in humans is also known as undulant fever or Mediterranean fever. Clinical features are diverse and onset can be acute or insidious, with the main features being prolonged fever and debilitation, weight loss, sweating, headache, lethargy, anorexia and joint pains. Arthritis affecting the large joints and spinal osteomyelitis are common. Patients may have a dry cough or enlarged liver and spleen; neurological involvement and endocarditis are rare but important clinical presentations.[14] The case fatality rate of untreated brucellosis is about 2%, the majority due to endocarditis from *B. melitensis* infection.[9] As described above, only 4 human cases of infection with marine mammal strains of *Brucella* have been described in the literature. The naturally acquired infections were severe while the laboratory acquired infection was mild and uncomplicated. Brucella isolates from both marine mammals and humans have been further investigated for their ability to infect human macrophages.[15]. This study showed that there is variation in this measure of virulence, with some	**Yes**	**Good**

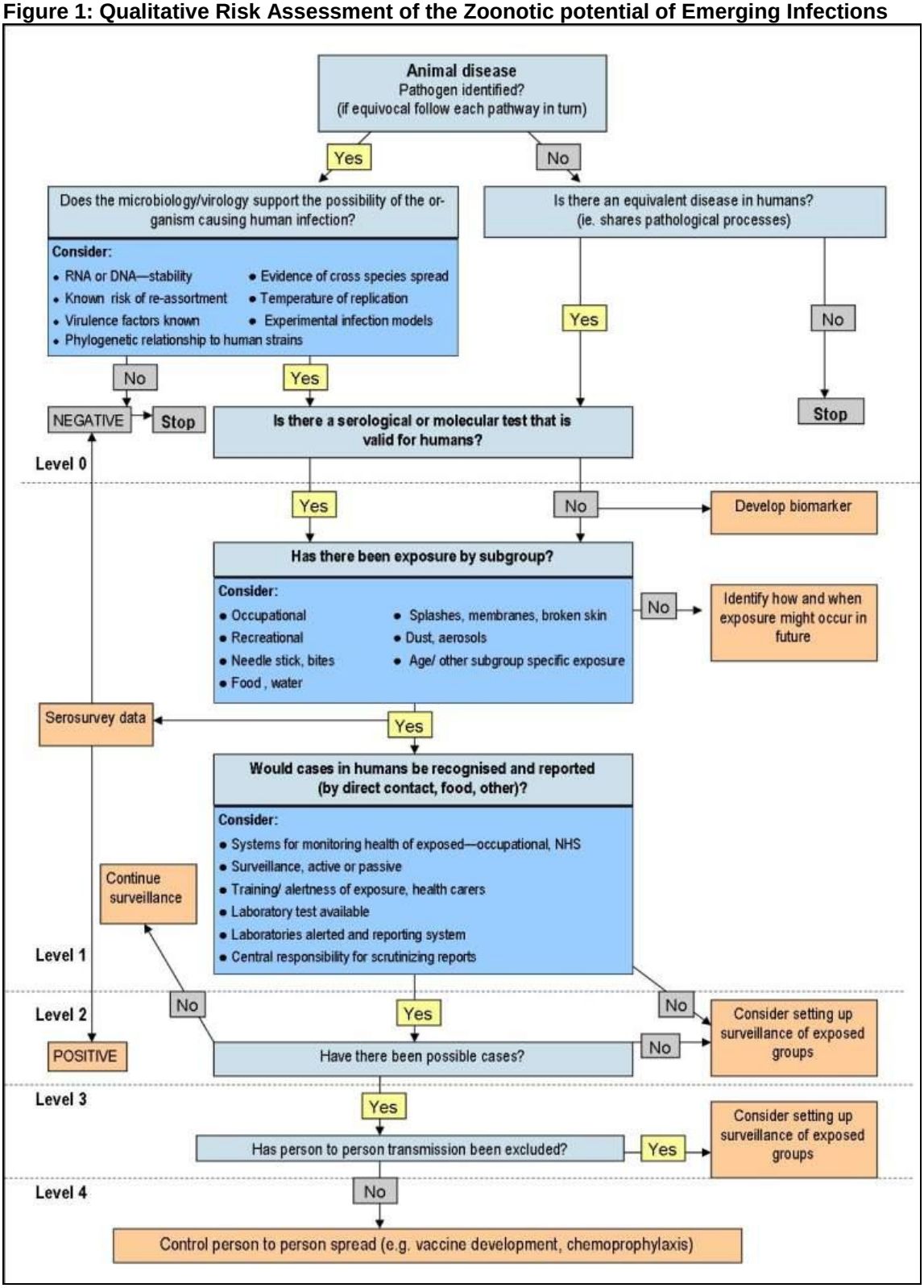
strains *B. pinnipedialis* unable to invade macrophages. These results suggest that there may be variation in the degree to which marine *Brucella* species can infect humans. The isolates from the three naturally acquired infections have also have been further characterised and found to be of a sequence type (ST27) thus far only associated with a Pacific dolphin.[16] Whether ST27 strains are more pathogenic for humans cannot be determined until more human isolates of marine brucellae are available for testing.		
Level 0: Is there a serological/molecular test that is valid for humans?		
Laboratory diagnosis of brucellosis is based on serology using agglutination tests, with antigens of *B. melitensis* and *B. abortus*, however serological responses do not completely distinguish between the two species. The test will cross react with *B. suis* infections but not with *B. canis* infection.[14] Modern commercial sero-diagnostic assays for *Brucella* spp. typically use purified lipopolysaccharide derived from the Gram-negative cell wall. Hence antibody responses are genus and not species specific. Killed *B. abortus* is the usual LPS source.[17] In England and Wales serological testing for brucella infection is carried out at the Brucella Reference unit in Liverpool.[18] The laboratory screens sera using a CE marker total brucella antibody assay and specific IgG/IgM enzyme immunoassays. Samples which test positive on screening undergo further testing with in-house micro-agglutination and complement fixation assays. If acute brucellosis is suggested the laboratory will discuss clinical management and risk assessment with the referring laboratory. Cultures suspected to be *Brucella* sp. are sent to the AHVLA in Weybridge. Of the four human cases described above, three were serologically positive for brucella infection. This suggests that the marine species of *Brucella* cross react with the antigens used for serological testing in humans. The organisms can also be isolated on culture and may be identified as marine mammal strains by specific PCR testing and molecular analyses.[19],[20]	**Yes**	**Good**
Level 0: Is there an equivalent disease in humans?		
Human brucellosis occurs worldwide due to infection with other *Brucella* species, particularly *B. melitensis* and *B. abortus.* However, not all *Brucella* species are zoonotic.	**Yes**	**Good**
Level 1: Has there been exposure by subgroup?		
Humans in contact with cetaceans or seals could be exposed to these organisms. Situations such as strandings of marine mammals tend to attract members of the public, who are unlikely to be aware of the potential infection risks and may have contact with the animals while trying to refloat them. Staff working for marine rescue or conservation organisations may also be exposed to these organisms, however, they are more likely to be aware of the potential infection risks and take appropriate precautions. Comprehensive health and safety guidance is given to volunteers and staff working for organisations such as the British Divers Marine Life Rescue and the Cornwall Wildlife Trust. Some organisations require workers to sign a form to indicate compliance with the guidance. Those swimming with dolphins or seals could also be exposed.	**Yes**	**Good**

An open letter was posted on the website of the British Divers Marine Life Rescue in April 2008, providing information on the human health risks associated with contact with wild dolphins. The letter states that infections may be transmitted via inhalation of blowhole expirations, contact with faecal and urinary waste, infected wounds, and the mouth and teeth of the animal. Brucellosis is listed as a potential zoonosis that may be contracted from cetaceans, and pregnant women are highlighted as being at particular risk.[21] The main routes of transmission of non-marine brucellosis to humans are ingestion of unpasteurised milk or milk products; inhalation of aerosols from infected products of conception in animals, or contaminated dust and droplets; direct contact or inoculation via the conjunctiva, blood transfusion or organ transplantation.[14] Human cases have occurred without evidence of direct transmission[11,12]. In the absence of a direct association with marine mammals, alternative indirect routes of transmission such as the consumption of uncooked shellfish should be considered. While the complete life cycle of many parasites of marine mammals such as lungworms remains unknown, there is evidence of *Brucella* within the uterine tissues of nematodes that may in turn cycle through intermediate hosts.[22] [23] Direct contact with an infected marine mammal is unlikely to pose a high risk of transmission. There may be a theoretical risk of transmission from marine tissues if the handler has non-intact skin and does not wear gloves. This may occur at autopsy but is thought less likely when simply handling a marine mammal.[17] It is assumed that large numbers of people have potentially been exposed to infected marine mammals around the world, with strandings and unusual mortality events. Despite this, diagnosed cases in humans remain extremely rare (4 to date). Data from USA indicated that 25% of 107 stranded dolphins were positive for brucella.[24] Four people exposed to an infected harbour porpoise during necropsy were followed up in the USA.[25] Due to their exposure to aerosol generating procedures without respiratory protection, antibiotic prophylaxis was advised. None became unwell or seroconverted. Active surveillance is in place in the USA amongst those working with rescued or stranded animals, or conducting autopsies. Thus far no serological evidence of infection nor clinical illness has been identified in these persons.[26] In the UK, necropsies have been done on between 90-150 marine mammals per year at the SACCVS in Inverness and at AHVLA disease surveillance centres in England and Wales. *Brucella* spp are occasionally isolated - over the last 20 years 2 to 20 animals, both cetaceans and seals, were culture positive per year (~5-10% of animals examined). (Personal communication, Geoff Foster SAC; AHVLA VIDA data). All those exposed to marine mammal necropsies in the UK who have been tested have remained sero-negative. (Personal communication, Paul Jepson Institute of Zoology)		
Level 1: Would cases in humans be recognised and reported?		
Human cases of infection with marine *Brucella* spp. may be detected using standard serological tests, however they may not be specifically recognised as marine species unless further diagnostic work was carried out. The organisms are readily isolated on culture of marine mammal samples and would be expected to be no different from human samples. The three naturally acquired human cases recognised so far were all in patients with severe or chronic infection, 2 neurobrucellosis and 1 spinal osteomyelitis. Neurological involvement is a rare clinical presentation of brucellosis, and it may be that cases with milder clinical signs were not detected. The laboratory worker	**Possibly**	**Poor**

reported continuing headaches, lassitude and severe sinusitis, lasting for one week, with no further complications reported after treatment. The request form issued by the Brucella Reference Laboratory requires details on risk factors, this does not specifically mention exposure to marine mammals but does include occupational hazards. The only human case of marine mammal associated brucellosis in the UK occurred in a laboratory worker, and was detected using standard serological tests. However it is unclear whether cases without an obvious risk factor would be recognised.		
Level 2: Have there been possible cases?		
Between 2000 and 2010 an average of 20 human cases of acute brucellosis were identified each year in the UK.[27] With the exception of some cattle-associated *B. abortus* cases in Northern Ireland, all human cases in the UK were acquired abroad.[14] Four human cases of infection with marine mammal *Brucella* species have been reported in the literature. One of these occurred in the UK, in a research laboratory worker. Aside from this, no human cases of brucellosis due to marine mammal strains have been detected in the UK to date. The Brucella Reference Unit in Liverpool provides information and advice to clinicians on suspected cases of brucellosis. No queries have been received to date regarding suspected human cases linked to marine mammals.	**Yes**	**Poor**
Level 3: Has person to person transmission been excluded?		
Person-to-person spread of brucellosis is extremely rare, although mother to child transmission and sexual transmission have been documented.[28]	**Yes**	**Good**

Outcome: The level of zoonotic risk is **Very Low**.

Figure 1: Qualitative Risk Assessment of the Zoonotic potential of Emerging Infections



[1] Ross HM, Foster G, Reid RJ, Jahans KL, MacMillan AP. *Brucella* species infection in sea-mammals. *Veterinary Record* 1994;**134**:359.
[2] Foster G, Osterman BS, Godfroid J, Jacques I, Cloeckaert A. *Brucella ceti* sp. nov and *Brucella pinnipedialis* sp. nov for *Brucella* strains with cetaceans and seals as their preferred hosts. *Int J Syst Evol Microbiol*. 2007;**57**:2688-93.
[3] Foster G, Jahans KL, Reid RJ, Ross HM. Isolation of *Brucella* species from cetaceans, seals and an otters. *Veterinary Record* 1996;**138**:583-86.
[4] Foster G, MacMillan AP, Godfroid J, Howie F, Ross HM, Cloeckaert A, Reid RJ, Brew S, Patterson IAP. A review of *Brucella* sp. infection of sea mammals with particular emphasis on isolates from Scotland. *Vet Microbiol* 2002: **90:**563-580
[5] Gonzales L, Patterson IA, Reid RJ, Foster G, Barberan M, Blasco JM, Kennedy S, Howie FE, Godfroid J, MacMillan AP, Schock A, Buxton D. Chronic meningoencephalitis associated with *Brucella* sp. infection in live-stranded striped dolphins (*Stenella coeruleoalba*). *J Comp Path* 2002;**126:**147-152.
[6] Hernández-Mora G, González-Barrientos R, Morales J, Chaves-Olarte E, Guzmán-Verri C, Baquero-Calvo E *et al*. Neurobrucellosis in stranded dolphins, Costa Rica. *Emerg Infect Dis* 2008;**14(9)**:1430-1433.
[7] Rhyan JC, Gidlewski T, Ewalt DR, Hennager SG, Lambourne DM, Olsen SC. Seroconversion and abortion in cattle experimentally infected with *Brucella* sp. isolated from a Pacific harbour seal. *J Vet Diagn Invest*. 2001;**13(5)**:379-82.
[8] Perrett LL, Brew SD, Stack JA, MacMillan AP, Bashiruddin JB. Experimental assessment of the pathogenicity of *Brucella* strains from marine mammals for pregnant sheep. *Samll Rumin Res* 2004: **51:**221-228.
[9] Bingham J, Taylor TK Swingler JE, Meehan G, Middleton DJ, Mackereth GF, O'Keefe JS, Daniels PW. Infection trials in pigs with a human isolate of *Brucella* (isolate 02/611 'marine mammal type'). *New Zealand Vet J* **56:**10-14.
[10] Brew SD, Perret LL, Stack JA, MacMillan AP, Staunton NJ. Human exposure to *Brucella* recovered from a sea mammal. *Veterinary Record* 1999;**144(17)**:483
[11] Sohn A, Probert WS, Glaser CA, Gupta N, Bollen A, Wong JD et al. Human neurobrucellosis with intracerebral granuloma caused by a marine mammal *Brucella* spp. *Emerg Infect Dis* 2003;**9(4)**:485-8.
[12] McDonald WL, Jamaludin R, Mackereth G, Hansen M, Humphrey S, Short P *et al*. Characterization of a *Brucella* sp. strain as a marine-mammal type despite isolation from a patient with spinal osteomyelitis in New Zealand. *J Clin Microbiol* 2006;**44(12)**:4363-70.
[13] Glynn K, Dragon, D. Brucellosis. In: Heymann, DL *Control of Communicable Diseases Manual*. Washington: American Public Health Association. 2008;87-90.
[14] Health Protection Agency. Brucellosis: General information. Available online: http://www.hpa.org.uk/webw/HPAweb&HPAwebStandard/HPAweb_C/1195733811188?p=1191942171106
[15] Maquart M, Zygmunt MS, Cloeckaert A. Marine mammal *Brucella* isolates with different genomic characteristics display a differential response when infecting huma macrophages in culture. *Microbes Infect* 2009:11;361-366
[16] Whatmore AM, Dawson CE, Groussaud P et al. Marine mammal Brucella genotype associated with zoonotic infection. *Emerg Infec t Dis* 2008:14;517-8
[17] Richard Cooke, Brucella Reference Unit, personal communication.
[18] Health Protection Agency. Brucella Reference Unit. Available at: http://www.hpa.org.uk/webw/HPAweb&Page&HPAwebAutoListName/Page/1200660021750?p=1200660021750
[19] Nymo IH, Tryland M, Godfroid J. A review of *Brucella* infection in marine mammals with special emphasis on *Brucella pinnipedialis* in the hooded seal (*Cystophora cristata*). *Veterinary Research* 2011;42:93-106
[20] Guzman-Verri C, Gonzalez-Barrientos R, Hernandez-Mora G et al. *Brucella ceti* and brucellosis in cetaceans. *Frontiers Cellular Infect Microbiol*. 2012:2;Article 3. Available at
[21] Barnett J. British Divers Marine Life Rescue. Available at: http://www.wdcs.org/submissions_bin/Solitary_dolphin_statement_April08.pdf

[22] Garner, M. M., D. M. Lambourne, S. J. Jeffries, P. Briggs Hall., J. C. Rhyan, D. R. Ewalt, L. M. Polzin and N. E.Cheville. Evidence of *Brucella* infection in Parafilaroides lungworms in a Pacific harbour seal (Phoca vitulina richardsi). *Journal of Veterinary Diagnostic Investigation* 1997:9;298-303.

[23] Dawson CE, Perrett LL, Stubberfield EJ, Stack JA, Farrelly SSJ, Cooley WA, Davison NJ, Quinney S. Isolation and Characterisation of *Brucella* from the Lungworms of a Harbour porpoise (Phocoena Phocoena) Journal of Wldlife Disease, 2008;44(2):273-46.

[24] National Marine Fisheries Service. 2010-2013 Cetacean unusual mortality event in northern Gulf of Mexico. Accessed 6/8/2013, available at: http://www.nmfs.noaa.gov/pr/health/mmume/cetacean_gulfofmexico2010_brucella.htm

[25] MMWR Human Exposures to Marine *Brucella* Isolated from a Harbor Porpoise — Maine, 2012. 2012 / 61(25);461-463. Available at: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6125a2.htm

[26] Fauquier DA et al. Investigation of animal care workers exposed to marine mammal *Brucella* species. April 2013 IAAAM 44th Annual Conference Proceedings.

[27] UK Zoonoses Report for 2011. Available at: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/69638/pb13851-zoonoses-2011.pdf

[28] WHO Brucellosis in humans and animals. WHO 2006. Available at: http://www.who.int/csr/resources/publications/Brucellosis.pdf