A comprehensive meta-analysis of Brucella *infections in aquatic mammals*

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Summary
The presence of *Brucella* infections was documented in a large number of aquatic mammals, affecting wild animals living in oceans, seas, lakes and rivers within both northern and southern hemispheres. Through meta-regression analysis, this study provides a comprehensive view of the prevalence of *Brucella* spp. in aquatic mammals, identifying risk subgroups as well as most common sampling and testing methods. *Brucella ceti* and *Brucella pinnipedialis* represent the main marine *Brucella* spp., with documented enzootic potential, for which information on standardized diagnostic methods for the implementation of efficient screening and monitoring programs is needed. A total of 71 articles investigating the occurrence of brucellosis in aquatic mammals since 1987, have met the inclusion criteria and have been included in this study. The prevalence of brucellosis in males (30.42%) was significantly higher than females (18.59%). The family of *Delphinidae* was the most studied among aquatic mammals with a total prevalence of 39.66%. Our meta-regression analysis showed a strong and significant association between the prevalence of *Brucella* spp. in mammals and water temperature (C = 0.02, p value = 0.003), while no significant correlation was found with water salinity (C = - 0.09; p value = 0.10). At least 130 species of aquatic mammals have been identified as potential hosts for *Brucella* spp. There is no systematic veterinary inspection and global or local requirements for the monitoring of brucellosis in aquatic mammals. The association of brucellosis prevalence and water temperature warrants further studies to assess the potential direct and indirect impacts of climate change on brucellosis in aquatic mammals. This study would help to determine the basis of adaptive management strategies in order to control enzootic brucellosis in wild aquatic mammals.

Introduction

Brucellosis is a widespread zoonotic infection caused by *Brucella* spp., Gram-negative facultative intracellular pathogens, often leading to abortion or reproductive disorders in domestic and wild mammals (Miller *et al.* 1999, Rhyan *et al.* 2001). The genus *Brucella* includes several species that were classified with respect to phenotypic characteristics, pathogenicity and host preference including *Brucella melitensis* (goats and sheep), *Brucella abortus* (cattle), *Brucella canis* (dog), *Brucella ovis* (sheep), *Brucella neotomae* (desert woodrat) and *Brucella suis* (swine). Thanks to the help of whole genome sequencing (WGS) and modern molecular typing methods, a number of new species, mostly isolated from wildlife, such as *Brucella ceti* in cetaceans (dolphin, porpoise, and whale species) and *Brucella pinnipedialis* in pinnipeds (various seal species) along with several terrestrial species including *Brucella microti* (common vole and red foxes, soil, and marsh frogs), *Brucella inopinata* (human), *Brucella papionis* (baboons) and *Brucella vulpis* (red foxes)(Cloeckaert *et al.* 2020, Godfroid *et al.* 2011) have been identified. Human infections are mainly caused by *B. melitensis*, *B. abortus, B. canis, B. suis* (Whatmore 2009), and *B. inopinata* (Scholz *et al.*

2010), but marine *Brucella* species (i.e. *B. ceti* and *B. pinnipedialis*) (Dawson *et al.* 2008b, McDonald *et al.* 2006) are also responsible for severe bacterial infections in humans (Dadar *et al.* 2019c, Maquart *et al.* 2009b, Sohn *et al.* 2003).

The pioneer brucellosis investigations on marine mammals date back to 1994, leading to the isolation of *Brucella* sp. from the aborted fetus of an Atlantic bottlenose dolphin (*Tursiops truncatus*) held in captivity in California, United States (USA) (Ewalt *et al.* 1994). In the same year, the presence of *Brucella* infections was reported in the carcasses of a common dolphin (*Delphinus delphis*), a harbour porpoise (*Phocoena phocoena*) and a harbour seal (*Phoca vitulina*), stranded along the coast of Scotland (Ross *et al.* 1994). The *Brucella* strains isolated from marine mammals were first known as *B. maris* (Jahans *et al.* 1997). Next investigations using DNA polymorphism at the *omp2* locus led to the dissociation of at least two different *Brucella* species, one affecting pinnipeds (*Brucella pinnipediae*) and another affecting cetaceans (*Brucella delphinidae*) (Cloeckaert *et al.* 2001). In 2007, the name of these species were changed to *B. pinnipedialis* and *B. ceti*, respectively (Foster *et al.* 2007). Due to the heterogeneity observed in molecular genotyping, these two marine *Brucella* species are divided into several subgroups (Alava *et al.* 2019, Bourg *et al.* 2007, Bricker *et al.* 2000, Bricker *et al.* 2003, Whatmore *et al.* 2007, Whatmore *et al.* 2008).

At present, 130 species of aquatic mammals living in rivers, lakes, seas and oceans have been identified as potential hosts for *Brucella* spp. Among them, 36 species belonging to pinnipeds including the *Phocidae* (true seals), *Odobenidae* (walrus) and *Otariidae* (fur seals, sea lions), and 86 cetacean species in the suborders Mysticeti and Odontoceti, comprising porpoises, whales and dolphins were infected by *Brucella* spp. Besides, manatees (*Trichechus* spp.), sea otters (*Enhydra lutris*), polar bears (*Ursus maritimus*), dugongs (*Dugong dugon*), and marine otters (*Lutra felina*) are other aquatic mammals susceptible to *Brucella* sp. infections (Jefferson *et al.* 2011). Aquatic mammals appeared to be affected by brucellosis at different extents, for example, there are no reports of seropositivity or isolation of *Brucella* spp. in dugongs, manatees, or river dolphins (Moreno *et al.* 2012). Among the *Brucella* seropositive species, 9 pinniped and 33 cetacean species are consumed by humans worldwide (Hernández-Mora *et al.* 2013). The people of at least 114 countries have close and frequent contact with marine mammals due to the consumption of meat and other products (Robards and Reeves 2011). Until now, 3 cases of naturally acquired infection with *Brucella* spp. originating from marine mammals have been reported (Godfroid *et al.* 2011, McDonald *et al.* 2006, Whatmore *et al.* 2008). Furthermore, there have been two reports of natural *Brucella* infection in fish (1 with *B. melitensis* and 1 with a new *Brucella* species) (Eisenberg *et al.* 2017, Wael *et al.* 2010) and several reports of *Brucella inopinata*-like infections in amphibians (frog) (Eisenberg *et al.* 2012). These sporadic reports on fish or amphibians will not be further discussed in this paper which focuses particularly on the widespread *Brucella* infections among aquatic mammals.

Mass mortality events (MMEs) due to epizootics (mainly viral diseases) have increased significantly over the last 30 years, and have been associated with environmental variables, such as season and abnormal sea surface temperature (SST). In addition, such MMEs occur more frequently in semiaquatic species (pinnipeds) compared to obligate ocean dwellers (cetaceans) (Sanderson and Alexander 2020). MMEs due to *Brucella* infections have never been described. Importantly, *Brucella* infections are very different in cetaceans and pinnipeds. Brucellosis in cetaceans is a disease, comparable to the disease seen in wild and domesticated terrestrial mammals, whereas no significant pathology or reproduction failure has been seen in true seals. In addition, there are huge knowledge gaps in eared seals (Nymo *et al.* 2018). For hooded seals, it has been suggested that the infection is likely to be acquired from the environment while feeding (Nymo *et al.* 2013). Therefore, indirect effects of climate change linked to modifications of habitat (ice cover, hall out sites) and food availability are likely to have an impact on the emergence of infectious diseases like brucellosis, particularly in seals (Larsen *et al.* 2018, Sanderson and Alexander 2020).

The present meta-analysis aimed at synthesizing reported data regarding *Brucella* infections in aquatic mammals in order to determine risk subgroups and potential reservoirs of this zoonotic disease. This would help to determine the basis of prevention, control, and management strategies in order to predict and limit the risks of enzootic brucellosis in wild aquatic mammals.

Methods

Search strategy and selection criteria

This systematic review and meta-analysis followed the Cochrane protocols (Higgins and Green 2011) and the study selection process was based on the PRISMA protocols (Figure 1) (Liberati *et al.* 2009). A literature search was performed among public scientific databases including PubMed, Scopus and Cochrane databases to retrieve articles reporting *Brucella* infection in aquatic animal population from 1 January 1983 to 2 February 2020. The following keywords were used in search engines: "*B. ceti*" OR

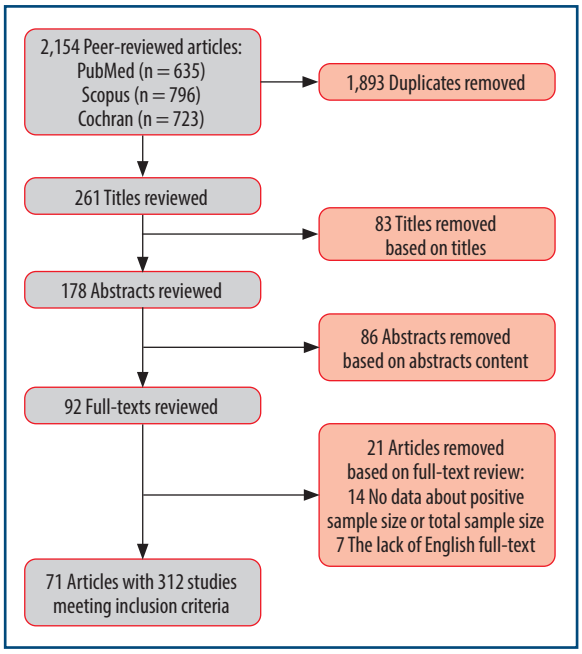

Figure 1. *The PRISMA flow chart of retrieved articles from different databases.*

"Wild" OR "aquatic mammals" AND "prevalence" OR "occurrence" OR "seroprevalence" OR "incidence" AND "Brucella" OR "brucellosis" OR "*B. abortus*" OR "*Brucella*" OR "*B. pinnipedialis*" OR "*Brucella* spp." OR "pinnipeds". The inclusion criteria for articles were 1: cross-sectional studies; 2: accessible full text in English; 3: studies carried out on aquatic mammals and 4: studies reporting both positive and total sample sizes and/or prevalence of *Brucella* spp. in aquatic mammals. The reference list of retrieved articles was further reviewed to obtain more related articles. Workshops, books and thesis have been excluded due to the lack of peer review (Fakhri *et al.* 2019).

Data extraction

The following data were extracted from all relevant articles: the year of the study, country, data of the study, animal family, trophic level (carnivorous, herbivorous, omnivorous), positive sample size, total sample size, detection method, clinical signs, sample kind, *Brucella* species, live or dead conditions of sampled animals, sex, animal family and the sampling location were extracted.

Meta-analysis of data

A Der Simoniane Laird random effects model was used to estimate the pooled prevalence of *Brucella* sp. infections (ratio of positive samples to the total sample size) in aquatic mammals (Dadar *et al.* 2020a). Pooled prevalence of *Brucella* sp. infections following 95% confidence interval (CI) for each study was estimated via Metaprop command (Freeman Tukey double arcsine transformation) (Dadar *et al.* 2020b, Rostami *et al.* 2020). We evaluated the statistical significance at the 5% level and presented Wald p-values and the corresponding 95% CIs for the impact of independent linear and categorical items. The pooled prevalence of *Brucella* spp. was estimated in different subgroups including countries, clinical signs, sample kind, bacteria species, live and dead condition, sex, method of detection, animal family, and location of sampling subgroups. In meta-analysis studies, heterogeneity is the variation in outcome studies (Petitti 2001). Cochran's Q analysis and I^2 statistic are used to detect heterogeneity among studies. Cochran's Q presented as the weighted sum of squared differences between study outcomes (Higgins and Thompson 2002). In addition, I^2 statistic is the percentage of variation outcomes of studies resulting from heterogeneity (Higgins 2008).

To detect the heterogeneity of studies, we used Chi-squared test and I^2 index. The I^2 values higher than 50% indicate significant heterogeneity (Higgins and Thompson 2002, Rostami *et al.* 2019). We employed random effect model (REM) for I^2 indexes higher than 50%, while fixed effect model (FEM) was used if I^2 was lower than 5%. All analyses were conducted with Stata software (v.13 Stata Corp., College Station, TX, USA).

Results

Distribution of studies and prevalence trends over time

As depicted in Figure 1, a total of 71 articles investigating the occurrence of brucellosis in aquatic mammals have been reported since 1987 and the highest annual number (n = 14) peaked in 1999. Since then, the annual number of articles on the occurrence of brucellosis in wildlife decreased but regained an upward trend in 2007 (n = 10), reaching 12 articles in 2010 and 11 articles in 2016. The results of meta-regression analyses of all the retained studies showed that the prevalence of *Brucella* spp. in aquatic mammals significantly increased over time (Coefficient = 0.41 and p value < 0.001). There is a significant association between the Human Development Index Ranking (HDI) of countries and the frequency of studies investigating the prevalence of brucellosis in aquatic mammals (C = 0.45, p value < 0.001). Most reports of *Brucella* infections in aquatic mammals were related to the family of Delphinidae (n = 131) with a prevalence of 39.66% (Table I). Extensive studies were carried out on *Delphinidae* in the Atlantic Ocean shores (Figure 2 and 3) of the Americas and Europe (n = 87) followed by (in decreasing order) Mediterranean Sea (n = 16), Pacific

Table I. *Statistical and meta-regression analyses regarding the prevalence of* Brucella *infections (%) in wild aquatic mammals according to the following subgroups: microbial species, living conditions, sex, trophic level and animal family.* Predicted effect size (ES) is indicated.

Subgroups	Study	N study	ES	Lower	Upper	Weight
***Brucella* Species**	*Brucella* sp.	229	15.00	11.27	19.01	72.21
	Brucella abortus	1	1.28	0.03	6.94	0.48
	Brucella pinnipediae	36	25.88	18.51	33.87	14.71
	Brucella melitensis	5	9.04	4.66	14.60	2.42
	Brucella ceti	36	90.26	68.76	100.00	9.28
	Not mentioned	5	2.01	0.00	33.31	0.91
Sex	Female	78	18.59	11.40	26.64	23.40
	Male	107	30.42	21.77	39.59	30.00
	Not mentioned	127	18.38	13.85	23.25	46.60
Trophic level	Carnivorous	300	22.29	18.54	26.20	96.40
	Omnivorous	12	0.00	0.00	3.57	3.60
Animal family	Phocidae	103	18.88	14.25	23.87	39.81
	Odobenidae	1	2.94	0.96	6.73	0.49
	Delphinidae	131	39.66	31.09	48.47	35.73
	Phocoenidae	15	27.21	5.61	54.36	5.00
	Balaenopteridae	15	9.88	2.47	19.94	5.05
	Mustelidae	7	0.00	0.00	12.75	1.96
	Monodontidae	3	38.69	0.00	100.00	0.85
	Ziphiidae	5	69.86	0.00	100.00	1.02
	Clariidae	4	11.50	8.28	15.15	1.94
	Otariidae	8	2.10	0.00	13.46	3.02
	Physeteridae	4	3.84	0.00	44.94	0.82
	Trichechidae	5	0.00	0.00	4.62	1.28
	Dasyatidae	1	100.00	2.50	100.00	0.13
	Pontoporiidae	4	2.50	0.35	5.92	1.82
	Balaenidae	2	0.00	0.00	44.44	0.37
	Kogiidae	4	4.22	0.00	63.68	0.72

Ocean shores (n = 12), North Sea (n = 4), Adriatic Sea (n = 4), Indian Ocean (n = 3), Sea of Japan (n = 3) and Black Sea (n = 2).

Geographical distribution of studies on aquatic mammals

The rank order of waters in which the studies were carried out (Figure 2) was Atlantic Ocean (161) > North Sea (37) > Pacific Ocean (32) > Mediterranean Sea (21) > Baltic Sea (9)> Norwegian Sea (7) > Sea of Japan (7) ~ Alaskan waters (7) > Bering Sea (5) > Nile river (4) ~ Adriatic Sea (4) > Indian Ocean (3) > Caspian Sea (2) ~ Lake Baikal (2) ~ Kara Sea (2) ~ Black Sea (2) ~ Okhotsk Sea (1) ~ Barents Sea (1) ~ Bali Sea (1).

Most studied wild aquatic mammals

Most research articles dealing with *Brucella* infections in aquatic mammals (Table I) showed that the family *Delphinidae* was extensively investigated with the highest numbers of studies (n = 131) followed by *Phocidae* (n =103), *Phocoenidae* (n = 15), and *Balaenopteridae* (n = 15). The family *Delphinidae* was the most studied among aquatic mammals for brucellosis over the three last decades with a total prevalence of 39.66% between 1987 and 2018. The highest number of studies were performed on marine mammals of the Atlantic Ocean (n = 161) (Figure 3).

Sampling methods and prevalence rates according to the type of samples

The overall pooled prevalence of *Brucella* spp. was estimated around 21%, (95% CI: 17.57-24.99%) in all tested aquatic mammals. In most studies, aquatic mammals were sampled after being found dead in the shores (in 168 out of 312 studies) or captured alive (in 141 out of 312 studies). The prevalence rate of brucellosis in stranded aquatic mammals was higher (32.25%) when compared to live captured (12.56%) animals (Table II). The biological specimens sampled from aquatic mammals for *Brucella* sp. detection mainly comprised visceral organs (169 studies), blood samples (119 studies) and lymph nodes (7 studies), while other samples such as aborted fetus, subcutaneous lesion, placenta, superficial punch biopsies of the skin, testis

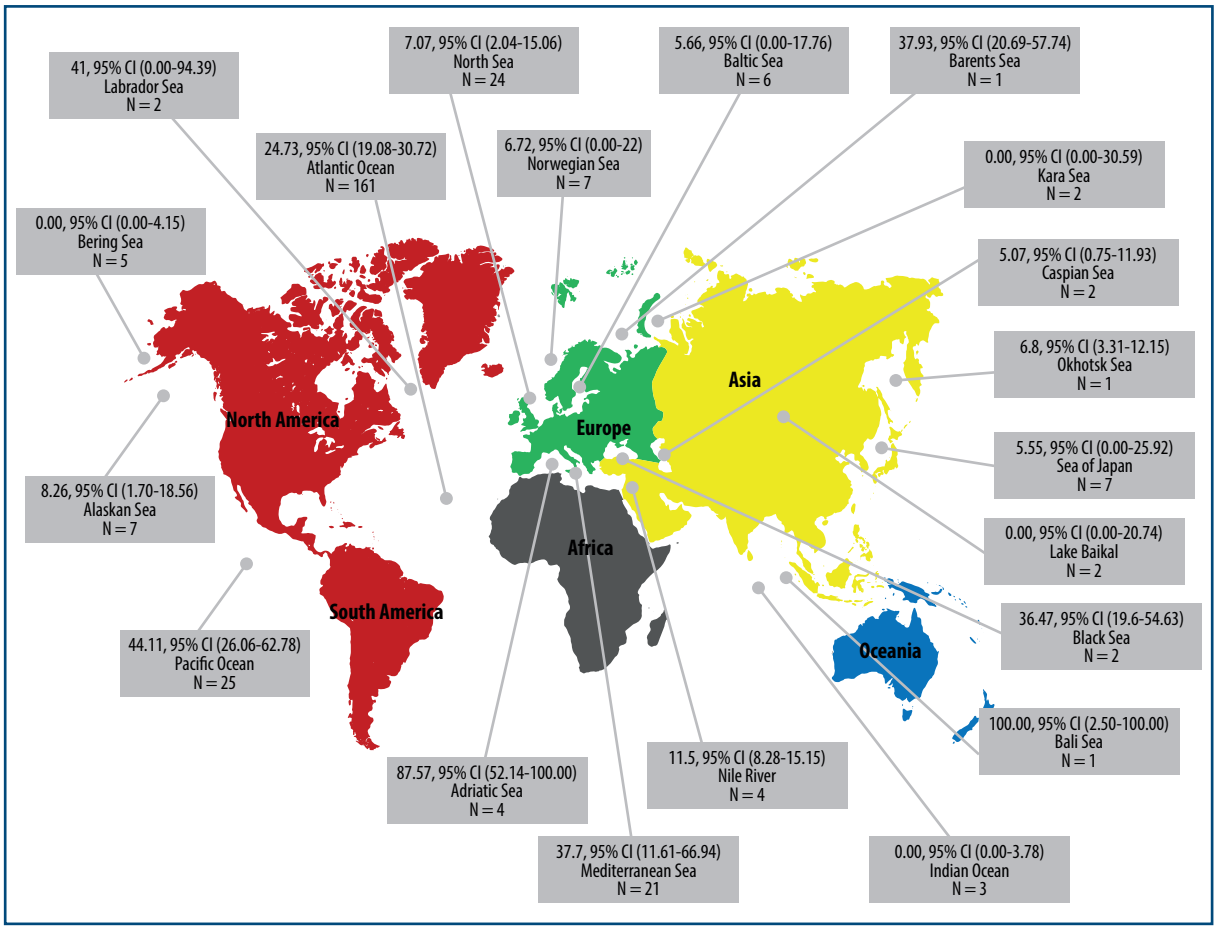

Figure 2. *Geographic distribution of studies and pooled prevalence rates along with 95% confidence intervals (CI) for* Brucella *spp. infections of tested aquatic mammals related to each area.*

	Balaenidae	Balaenopteridae	Physeteridae	Ziphiidae	Kogiidae	Monodontidae	Clariidae	Dasyatide	Mustelidae	Odobenidae	Otariidae	Phocidae	Delphinidae	Phocoenidae	Pontoporiidae	Trichechidae
Atlantic Ocean	2	3	2	4	3					1		44	87	6	4	6
North Sea		3		1					1			24	4	6		
Mediterranean Sea		1	1									1	16	2		
Norwegian Sea		4										3				
Labrador Sea												2				
Pacific Ocean		3	1		1				3		1	11	12			
Caspian Sea												2				
Lake Baikal												2				
Kara Sea												2				
Bering Sea						2			3							
Sea of Japan		1										1	3	1		
Black Sea													2			
Okhotsk Sea						1										
Nile River							4									
Alaskan Sea											1	6				
Indian Ocean													3			
Barents Sea												1				
Baltic Sea												9				
Adriatic Sea													4			
Bali Sea								1								

Figure 3. *Heat map showing the number of studies for each animal species in different aquatic environments worldwide.*

tissue, cerebrospinal fluid, liver, and rectal swabs were occasionally used (Table II). Interestingly, aborted fetuses and lymph nodes showed a higher prevalence rate of *Brucella* infection reaching 100% and 83.71%, respectively.

Methods used for diagnosing brucellosis in aquatic mammals

The main methods applied for the diagnostic of brucellosis in aquatic mammals (Table III) were based on direct diagnostic tests (182 studies) such as culture (n = 85), immunohistochemistry (n = 13), polymerase chain reaction (PCR, n = 69), restriction fragment length polymorphism of PCR products (PCR-RFLP, n = 7) and real-time PCR (n = 8). A total of 128 studies used indirect diagnostic tests such as enzyme-like immunosorbent assay (ELISA, n = 98), complement fixation test (CFT, n = 7), rose Bengal test (RBT, n = 8), tube agglutination test (TAT, n = 11), buffered acidified plate antigen test (BAPA, n = 2), fluorescence polarization assay (FPA, n = 1) and Rivanol test (n = 1). Among these methods, the highest and lowest prevalence rates of positive samples were obtained using PCR-RFLP (100%) and Rivanol test (8.33%), respectively (Table III).

It is worth re-iterating that indirect tests measure the exposure to *Brucella* spp. (past and current), while direct tests document the presence of *Brucella* spp. at the time of sampling.

Table II. *Statistical and meta-regression analysis on the prevalence of* Brucella *infections (%) in wild aquatic animals based on animal conditions prior to sampling, the diagnostic method, the samples and the symptoms of infected animals.* ES indicates predicted effect sizes.

	Study	N study	ES	Lower	Upper	Weight
Methods	ELISA	98	16.04	11.08	21.51	36.25
	Culture	85	27.49	18.32	37.38	22.54
	Immunohistochemistry	13	14.52	2.08	32.34	3.99
	CFT	7	91.15	44.88	100.00	1.47
	PCR-RFLP	7	100.00	100.00	100.00	1.78
	BAPA	2	8.99	3.09	17.04	0.89
	TAT	11	18.70	2.49	41.31	3.61
	PCR	69	22.33	14.89	30.46	21.68
	FPA	1	11.59	7.12	17.50	0.49
	RBT	8	17.76	8.20	29.23	3.24
	Rivanol test	1	8.33	4.07	14.79	0.49
	Real-time PCR	8	15.19	4.50	29.05	3.12
	Not mentioned	2	0.00	0.00	30.59	0.46
Living conditions	Live animal	141	12.56	9.29	16.12	51.71
	Dead animal	168	35.25	27.93	42.82	47.23
	Not mentioned	3	24.66	0.10	63.25	1.06
Sample kinds	Blood	119	12.51	9.16	16.16	44.04
	Aborted fetus	2	100.00	43.03	100.00	0.32
	Visceral organ	169	30.76	23.64	38.22	48.55
	Lymph node	7	83.71	62.07	98.46	1.56
	Subcutaneous lesion	1	50.00	11.81	88.19	0.30
	Placenta	1	0.00	0.00	52.18	0.28
	Superficial punch	1	0.00	0.00	97.50	0.13
	Testis tissue	1	45.45	24.39	67.79	0.42
	Rectal swab	3	1.28	0.08	3.39	1.43
	Cerebrospinal fluid	5	99.33	89.09	100.00	1.51
	Not mentioned	290	19.42	15.79	23.25	93.51
Symptoms	Abortion	2	100.00	43.03	100.00	0.32
	Meningoencephalitis	12	84.12	38.60	100.00	3.11
	Reproductive disease	1	25.93	11.11	46.28	0.44
	Swimming problems	1	100.00	59.04	100.00	0.32
	Granulomatous lesion	2	0.82	0.00	2.55	0.97
	Liver abscesses	1	0.82	0.02	4.48	0.49
	Pulmonary parasitism	3	67.45	39.26	91.11	0.85

CFT = Complement fixation test; RBT = Rose Bengal test; BAPA = Buffered acidified plate antigen; TAT = Tube agglutination test; FPA = Fluorescence polarization assay.

Table III. *Meta-regression analysis on the prevalence of* Brucella *spp. (%) based on diagnosis approach, genotype and molecular typing subgroups. ES indicates effect sizes.*

Subgroups	Study	N study	ES	Lower	Upper	Weight
Diagnosis	Indirect	128	0.17	0.13	0.22	46.29
	Direct	182	0.27	0.21	0.33	53.25
	Not mentioned	2	0.00	0.00	0.31	0.46
Genotype	ST27	6	78.28	13.83	100.00	0.94
	ST23	1	0.00	0.00	97.50	0.13
	ST25	6	41.41	21.98	62.18	2.54
	ST26	6	100.00	99.96	100.00	1.07
	ST24	3	15.86	0.00	73.58	0.75
	Not mentioned	292	19.59	16.03	23.34	94.56
Molecular typing	*omp2*	13	64.60	33.21	91.42	4.31
	Bp26	1	100.00	2.50	100.00	0.13
	IS*711*	3	100.00	89.66	100.00	0.73
	MLVA	9	39.70	16.65	64.62	2.63
	omp25	3	100.00	99.79	100.00	0.74
	MLST	9	76.33	38.64	99.92	2.24
	WGS	1	100.00	2.50	100.00	0.13
	Not mentioned	273	16.24	12.95	19.74	89.09

omp2 and *omp25* = Outer membrane proteins 2 and 25, respectively; Bp26 = *Brucella* periplasmic protein; IS*711* = Insertion sequence 711; MLVA = Multiple Loci VNTR Analysis; MLST = Multilocus sequence typing; WGS = Whole genome sequencing.

Clinical signs and pathology associated with *Brucella* infection in aquatic mammals

Among the common clinical signs and pathology linked to *Brucella* infections, meningoencephalitis was reported in 12 studies, pulmonary parasitism in 3 studies, abortion in 2 studies, granulomatous lesion in 2 studies and other clinical signs such as swimming problems, reproductive disease as well as liver abscesses were observed in one study (Table II).

The relevance of water salinity and temperature to the incidence of *Brucella* infections

Our meta-regression analysis showed a strong and significant association between the prevalence of *Brucella* spp. in aquatic animals and water temperature (C = 0.02, p value = 0.003), while no significant correlation was found with water salinity (C = - 0.09; p value = 0.10) (Figure 4 A, B).

Most prevalent *Brucella* spp. in aquatic mammals

Brucella spp. were more prevalent in aquatic mammals belonging to *Dasyatidae* (100%) > *Ziphiidae* (69.86%) > *Delphinidae* (39.66%) > *Monodontidae* (38.69%) > *Phocoenidae* (27.21%) > *Phocidae* (18.88%) > *Clariidae* (11.5%) and *Balaenopteridae* (9.88%). A lower prevalence rate was observed in *Kogiidae* (4.22%) > *Physeteridae* (3.84%) > *Odobenidae* (2.94%) > *Pontoporiidae* (2.5%) and *Otariidae* (2.1%). No *Brucella* sp. was detected in *Mustelidae*, *Trichechidae*, and *Balaenidae*. Our analyses also revealed the higher prevalence of *B. ceti* (90.26%) and *B. pinnipediae* (25.88%) in tested aquatic mammals through 36 studies, while *B. melitensis* (9.04%) and *B. abortus* (1.28%) were solely reported in natural infections in 5 and 1 studies, respectively (Table I).

Overall prevalence rates according to gender and feeding conditions

The present meta-analysis showed significant difference between the prevalence of *Brucella* spp. in male (30.42 % among 1,831 samples) and female (18.59% in 1,336 samples) marine mammals. In most available studies (n = 127), the sex of the sampled animals was not mentioned (NM). The highest overall prevalence rate was observed in carnivorous animals (22.29%), while no positive sample was reported in omnivorous animals (Table I).

Discussion

Brucellosis is a widespread zoonotic infection which remains endemic in different parts of the world. The results of our meta-analysis on almost three decades of *Brucella* investigations highlight the fact that *Brucella* infections in aquatic mammals have been globally distributed and its overall pooled prevalence in infected populations reached 21%.

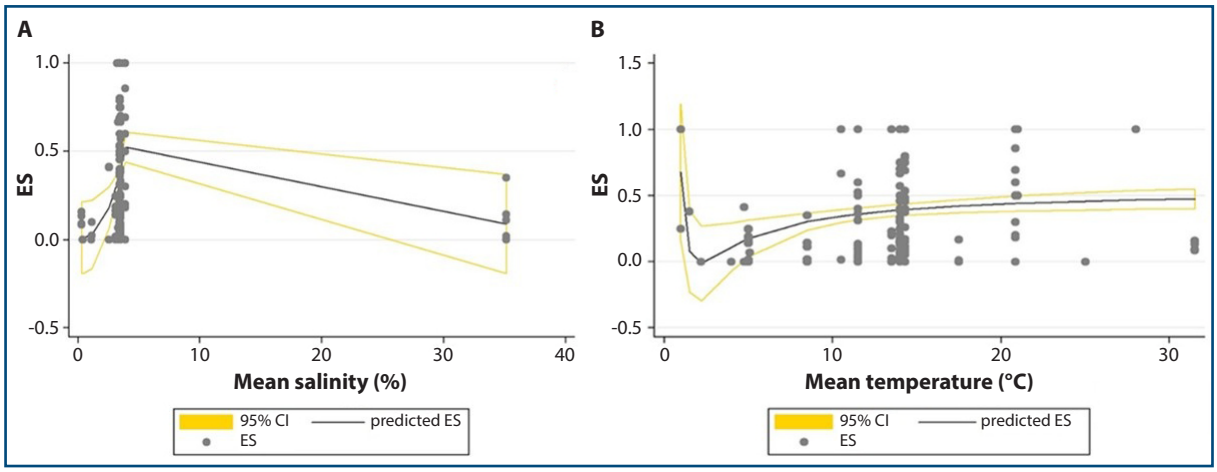

Figure 4. *Association of the prevalence of* Brucella *spp. in wild aquatic mammals with water salinity (***A***) and temperature (***B***).* ES and CI indicate the effect size and the confidence interval, respectively.

In most studies, aquatic mammals were sampled after being found dead on shores or captured alive. This indicates the critical role of the sampling and carcass recovery on aquatic animal disease. However, it was found that no surveillance system with a prescribed sample size and sampling strategy can address the range of situations experienced in aquatic environments (Cameron 2004). Our results also suggest that climate change may affect the contamination pathways, as a strong and significant positive association was found between the prevalence of brucellosis in aquatic mammals and water temperature while no significant correlation was found with water salinity. Such associations have been shown for MMEs occurring for epizootic outbreaks (Sanderson and Alexander 2020). Although climate change is an important issue, which may influence the development of zoonotic diseases, the data present in the literature do not allow drawing any conclusion about its effects on the prevalence of brucellosis in aquatic mammals. Undeniably, further studies are necessary to confirm the results obtained in this work. The present study is the first meta-analysis pointing out the effect of water temperature on the prevalence of brucellosis in aquatic animals opening up new avenues for future environmental studies in the field. Potential direct and indirect effects of climate change that may drive the emergence or re-emergence of brucellosis in marine mammals remain to be studied. The effective role of environment factors on the survival of *B. pinnipedialis* and *B. ceti* has not been investigated (Van Bressem *et al.* 2009). However, it has been shown that *Brucella* spp. isolated from terrestrial mammals can remain viable in freshwater and terrestrial environments for periods ranging from less than a day to > 8 months, depending on factors including temperature, exposure to sunlight, the presence of organic matter, and humidity (Cameron 1933, Coelho *et al.* 2015, Sammartino *et al.* 2006). How long *Brucella* spp. from aquatic mammals could survive in seawater is uncertain. The survival rate of *Brucella* at different temperatures would need to be considered. The indirect effect of water temperature on *B. pinnipedialis* infection in cod (*Gadus morhua*) has been documented experimentally: the survival of cod was dramatically reduced when kept at 6 °C compared to 15 °C (Larsen *et al.* 2018). The effect of climate change may therefore be more important in ectotherms, like fish, than in marine mammals that are able to maintain their body temperature independently from water temperature.

The highest number of studies on brucellosis of aquatic mammals was carried out in Brazil on Atlantic Ocean (n = 72). The family of *Delphinidae* was the most sampled aquatic species (131 studies) and showed the highest prevalence of *Brucella* infection estimated around 40%. At present, 53 species of aquatic mammals were reported as *Brucella* seropositive and in 18 of these species, *B. ceti* or *B. pinnipedialis* were identified using bacterial isolation or through polymerase chain reaction analysis (Hernández-Mora *et al.* 2013). A variety of serological tests has been used for the diagnosis of brucellosis in aquatic mammals including ELISA, CFT and TAT (Alekseev *et al.* 2007, Alekseev *et al.* 2009, Foster *et al.* 2018, Jepson *et al.* 1997, Moreno *et al.* 2012, Ohishi *et al.* 2003). Moreover, an indirect ELISA for odontocetes (Hernández-Mora *et al.* 2013) and a competitive ELISA for pinnipeds and cetaceans (Meegan *et al.* 2010) have been improved for indirect diagnostic of *Brucella* infections in aquatic mammals. The replacement of TAT with other more specific and sensitive screening serological tests has been recommended by the WOAH for the brucellosis screening in livestock (Greiner *et al.* 2009, Ragan *et al.* 2013). The CFT method has been gradually replaced by the indirect ELISA and, more recently, by the FPA

methods, although the majority of these serological methods should be standardized and validated for their efficient use in aquatic mammals (Godfroid *et al.* 2010).

Our results revealed that ELISA (n = 98) and culture (n = 85) were the most commonly used methods for the diagnosis of brucellosis in aquatic mammals. The highest record of bacterial isolation was cultured from visceral organs of dead animals such as lymph nodes, lung, spleen, liver, small intestine, kidney, brain, fetus, placenta, feces and subcutaneous lesions.

Among marine *Brucella* species, *B. pinnipedialis* has mainly been isolated from *Phocidae* or earless seals (32 studies) and was seldomly reported in *Phocoenidae* (1 study) *Delphinidae* (1 study), *Kogiidae* (1 study) and *Mustelidae* (1 study). The *Brucella* isolations from phocids have been achieved in seven true seal species: the hooded seal (*Cystophora cristata*), ringed seal (*Pusa hispida*), harp seal (*Pagophilus groenlandicus*), grey seal (*Halichoerus grypus*), Pacific harbour seal (*Phoca vitulina richardii*), bearded seal (*Erignathus barbatus*) and common seal (*Phoca vitulina*). The majority of these isolates have been obtained from animals sampled in the North Atlantic Ocean and North Sea. A number of the above-mentioned earless seals such as the ringed seal and the harp seal have been commercially important for their hides or oil, thereby increasing the risks of direct contact with humans (Hunt *et al.* 2008). Among *Otariidae* or eared seals, infections with terrestrial *Brucella* spp. (Ávalos-Téllez *et al.* 2014) and marine ST27 strains (Whatmore *et al.* 2017) were reported in California sea lions (*Zalophus californianus*).

Over the three past decades, *B. ceti* has been isolated from several families including *Balaenopteridae*, *Delphinidae* and *Phocoenidae* in the Atlantic Ocean, Mediterranean Sea and North Sea. *B. ceti* has been recovered from samples collected from bottlenose dolphins (*Tursiops truncates*), harbour porpoises (*Phocoena phocoena*), Atlantic white-sided dolphins (*Lagenorhynchus acutus*), white-beaked dolphins (*Lagenorhynchus albirostris*), common dolphins (*Delphinus delphis*), striped dolphins (*Stenella coeruleoalba*), minke whales (*Balaenoptera acutorostrata*), Sowerby's beaked whales (*Mesoplodon bidens*), long-finned pilot whales (*Globicephala melas*), as well as Clymene dolphins (*Stenella clymene*).

Clustering analysis on marine mammals (Table III) led to the identification of one cluster with five sequence types (STs) as ST23 in the majority of porpoises (75%), ST24 and ST25 comprising most seal isolates (80%), ST26 exclusively in dolphins and ST27 (the sole zoonotic ST identified so far) in bottlenose dolphins (Cvetnić *et al.* 2016, Duvnjak *et al.* 2017, Whatmore *et al.* 2008), California sea lions (*Zalophus californianus*) (Whatmore *et al.* 2017), minke whales (*Balaneoptera acuturostrata*), Hector's dolphins (*Cephalorhynchus hectori*) (Buckle *et al.* 2017) and Steller sea lions (*Eumetopias jubatus*) (Esquible *et al.* 2019). The recovery of gene fragments specific for ST23, ST24, ST25, ST26 and ST27 in positive samples has been achieved through multiplex real-time PCR (targeting *IS711*-specific chromosomal locations for *Brucella*), DNA polymorphism at the *omp2*, *bp26* and *omp25* locus, Multiple Loci VNTR Analysis (MLVA), Multi locus sequence analysis (MLSA) and whole genome sequencing (WGS). These different molecular assays appeared to be reliable approaches for the identification of *B. ceti* and *B. pinnipedialis*. Our meta-analyses note the presence of ST23 in harbor porpoises (1 study); ST24 in harbor seals, bearded seals and killer whales (3 studies); ST25 in harbor seals (6 studies); ST26 in long-finned pilot whales, Sowerby's beaked whales and striped dolphins (6 studies) and ST27 in Hectors dolphins, bottlenose dolphins, California sea lions, minke whales and Steller sea lions (6 studies). The genome-based characterization of marine *Brucella* strains represents promising tools considering the increasing availability of genome sequences as well as the limitations of the band-based methods (Nymo *et al.* 2011). This could explain a growing tendency to shift from band-based to sequence based methods; namely WGS, MLVA, MLSA, core genome multilocus sequence typing (cgMLST) and SNP-typing techniques (Bricker *et al.* 2003, Janowicz *et al.* 2018, Nymo *et al.* 2011, Wu *et al.* 2014, Wu *et al.* 2017). Sequence-based approaches generate considerable data which can be easily stored electronically. This allows the implementation of international genetic databases, thereby facilitating the development of international cooperation. Furthermore, epidemiological investigations supported by phylogeny analysis are possible by choosing appropriate genetic markers (Dadar *et al.* 2019b, Duvnjak *et al.* 2017, Whatmore 2009).

One of the important findings of our statistical analysis is the significant difference observed in the prevalence of *Brucella* infections between male and female aquatic mammals. The prevalence of brucellosis in males (30.42%) was significantly higher than females (18.59%). This is in contradiction with data obtained in terrestrial animals showing quite similar prevalence rates between male and female animals (Lulu *et al.* 1988, Samaha *et al.* 2009). Although brucellosis in aquatic mammals affects both males and females, sex susceptibility has not been fully reported. In cetaceans, *Brucella* spp. have been reported in the female and male reproductive organs, fetal fluids, placenta, fetal organs, mammary gland, sites of clinical localization and lymph nodes (González-Barrientos *et al.* 2010). As opposed to

cetaceans, in hooded seals, seropositivity decreased with age and *B. pinnipedialis* could not be isolated from females reaching reproduction age (Nymo *et al.* 2013). Age and sex are thus important variables that may have different effects on the brucellosis status of cetaceans compared to pinnipeds. Therefore, further studies need to take into account host species besides sex and age as brucellosis explanatory variables. The main clinical feature caused by brucellosis in terrestrial mammals is infertility and abortion (Dadar *et al.* 2019a, Miller *et al.* 1999, Rhyan *et al.* 2001). This is also the case in cetaceans, where high abortion rates and reproductive disease were reported in bottlenose dolphins (*Tursiops truncates*) and Hector's Dolphins (*Cephalorhynchus hectori*), respectively. Abortion has been reported in both captive (Ewalt *et al.* 1994) and free-ranging cetaceans (Miller *et al.* 1999). Of importance, abortion has not so far been described in seals. Among cetaceans, orchitis and epididymitis were frequently observed in infected males of toothed whales (Ohishi *et al.* 2003, Ohishi *et al.* 2004, Ohishi *et al.* 2008) as well as among Bryde's whales and harbor porpoises (Dawson *et al.* 2008a, Foster *et al.* 2002, Maquart *et al.* 2009a, Ohishi *et al.* 2008).

Furthermore, meningoencephalitis and arthritis are other important clinical signs reported in 12 studies performed on striped dolphins (*Stenella coeruleoalba*) and short-beaked common dolphins (*Delphinus delphis*) infected by *B. ceti* (González-Barrientos *et al.* 2010). Gross pathology has not been described in seals. The impact of *B. ceti* infection on reproductive failure in free-ranging cetaceans stresses the need for further cross-disciplinary investigations on cetacean brucellosis (Alba *et al.* 2013, Cloeckaert *et al.* 2001, Davison *et al.* 2015, Goertz *et al.* 2011, González-Barrientos *et al.* 2010, González *et al.* 2002, Hernández-Mora *et al.* 2013).

To conclude, *B. pinnipedialis* and *B. ceti* are smooth-type *Brucella* infecting a large number of aquatic mammals around the world. However, there is no systematic veterinary inspection and global or local requirements for the monitoring of brucellosis in aquatic mammals, nor specific requirements for their harvest and the processing of their meat and derived products. Therefore, international standards for the diagnostic and characterization of *Brucella* spp. infecting aquatic mammals are needed and could significantly assist the efforts to detect and prevent the zoonotic threat of ST27 for humans as well as its possible transmission to other aquatic and terrestrial animals.

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