Microparasites and their potential impact on the population dynamics of small cetaceans from South America: a brief review

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ABSTRACT

We briefly review the pathology, epidemiology and molecular biology of cetacean viruses (including morbilli, papilloma and pox) and *Brucella* spp. encountered in South America. Antibodies against cetacean morbillivirus were detected (by iELISAs and virus neutralisation tests) in SE Pacific and SW Atlantic delphinids. Morbilliviruses are possibly enzootic in *Lagenorhynchus obscurus* and offshore *Tursiops truncatus* from Peru and in *Lagenodelphis hosei* from Brazil and Argentina, but no morbillivirus antibodies were found in inshore small cetaceans. Papillomaviruses cause genital warts in at least three odontocete species in Peru. Two *Phocoena spinipinnis* papillomaviruses (PsPVs) were found in warts of Burmeister's porpoises; one (PsPV-1) was cloned and characterized. Half of porpoises had developed genital warts, while in 10% of males the lesions were sufficiently numerous and severe to at least hamper, if not impede, copulation. High titers of cowpox virus neutralising antibodies were detected in Peruvian *D. capensis*, *T. truncatus*, *L. obscurus* and *P. spinipinnis* in 1993-1995. The high prevalence of orthopoxvirus neutralising antibodies with high titres indicates common infection by poxviruses antigenically related to cowpox virus, the probable causative agents of tattoo skin disease. Cetacean poxviruses may cause significant mortaliy among neonates and calves unprotected by maternal immunity. In Peru, *Brucella* spp. antibodies were detected (competitive ELISA) in *D. capensis*, *T. truncatus, L. obscurus* and *P. spinipinnis*. Brucellosis is likely enzootic in the latter two species, and may lead to orchitis and bone lesions in *L. obscurus* and *D. capensis*. The enzootic circulation of brucellae in *L. obscurus* and *P. spinipinnis* may constitute a measurable limiting factor among the environmental variables affecting population dynamics. Also, widespread *Brucella* spp. infection in several Peruvian odontocetes has public health implications (zoonosis), considering frequent manipulation of carcases and consumption of meat. Extrinsic anthropogenic factors may not only exacerbate the consequences of viral infections on the health of a particular individual, but also operate at the population level.

INTRODUCTION

Evidence is mounting that microparasites, including viruses, bacteria, and protozoans, may constrain the growth of wild animal populations (Anderson and May, 1979; Anderson, 1982; Fenner, 1983; Gulland, 1995). The intensity of this effect is a function of the heightened mortality rate and/or reduction in reproductive capacity. Thus, microparasites may increase the risk of extinction of small populations in combination with other factors (May, 1986; Thorne and Williams, 1988; Gulland, 1995; Raga *et al*., 1997). The potential of viruses to significantly influence population dynamics of cetaceans was mostly overlooked until the advent of morbillivirus mortalities in harbour porpoises *Phocoena phocoena* in the NE Atlantic in 1988-1990 and of a lethal epizootic of morbillivirus in striped dolphins *Stenella coeruleoalba* in the Mediterranean in 1990-1992 (Kennedy *et al*., 1988; Domingo *et al*., 1990; Van Bressem *et al*., 1991, 1993). These mortalities have stimulated research on microparasite infections in cetaceans and stressed the need to investigate their long-term consequence. Here we summarize current understanding on the presence of cetacean morbilliviruses,

Phocoena spinipinnis papillomaviruses and *Brucella* spp. in small cetaceans from South America (Van Bressem *et al*., 1998, 1999a, 2001a,b, 2007a,b).

CETACEAN MORBILLIVIRUSES

The dolphin and porpoise morbilliviruses (DMV and PMV) are strains of a recently recognized member of the genus *Morbillivirus* (family *Paramyxoviridae*, single, negative-strand RNA viruses) called cetacean morbillivirus (CeMV) (Barrett *et al*., 1993; Blixenkrone-Møller *et al.*, 1996). A morbillivirus detected in a female long-finned pilot whale *Globicephala melas* stranded in New Jersey and partially genetically characterized may represent a third strain of cetacean morbillivirus (Taubenberger *et al*., 2000). Other morbilliviruses and their respective natural hosts are measles virus (MV) in humans, rinderpest virus (RPV) and peste des petits ruminants virus (PPRV) in artiodactyls, and canine and phocine distemper viruses (CDV and PDV) in carnivores. DMV and PMV are more closely related to the ruminant morbilliviruses and MV than to the distemper viruses (Visser *et al*., 1993; Barrett *et al*., 1993; Blixenkrone-Møller *et al*., 1994; 1996; Haffar *et al*., 1999). All members of this genus require large populations of individuals (e.g. 300,000 for measles virus in humans) to be maintained endemically (Black, 1991) and all cause serious, often lethal, systemic diseases in their hosts.

Serum antibodies against dolphin morbillivirus were detected by indirect enzyme-linked-immunosorbent-assay (iELISA) in 6 of 30 (20%) dusky dolphins *Lagenorhynchus obscurus*, 1 of 8 long-beaked common dolphins *Delphinus capensis* and 3 of 6 offshore common bottlenose dolphins *Tursiops truncatus* caught off central Peru in 1993-1995 (Van Bressem *et al*., 1998). Porpoise morbillivirus and/or DMV neutralizing antibodies were detected in the sera of 2 *T. truncatus* and 3 *L. obscurus* that reacted positively with DMV antigen in the iELISA and also in the serum of a *D. capensis* which was negative in the iELISA. No convincing morbillivirus-specific antibody positive reactions were seen in the sera from 20 Burmeister's porpoises *P. spinipinnis* and 2 inshore *T. tuncatus* (Van Bressem *et al*., 1998). Three of 4 Fraser's dolphins *Lagenodelphis hosei* stranded in 1997 and 1999 in Argentina and Brazil had serum antibodies against DMV in the iELISA. All had DMV neutralizing antibodies. The two dolphins from Argentina were mature and estimated to be 18-20 years old. None of the 14 franciscanas *Pontoporia blainvillei*, 5 estuarine dolphins *Sotalia guianensis* or 6 Atlantic spotted dolphins *Stenella frontalis* incidentally caught in fisheries off Brazil in 1996-1999 were positive in DMV-iELISA tests (Van Bressem *et al*., 2001a).

These results strongly suggest that a virus closely related, or identical to, the cetacean morbilliviruses present in Atlantic waters infects several species of Delphinidae of the SE Pacific and SW Atlantic Oceans. Morbillliviruses require large populations of susceptible animals to persist enzootically since there is no carrier state and infection, if survived, confers lifelong immunity. Dusky, bottlenose, Fraser's and common dolphins are gregarious species forming large herds that can range from a few tens to several hundred individuals and often associate with other cetacean species (Frazer, 1976; Leatherwood and Reeves, 1983; Würsig and Würsig, 1980; Wells *et al*., 1980). These characteristics, and the apparent lack of cetacean morbillivirus species-specificity (Taubenberger *et al*., 1996) would enhance the chances of the virus remaining enzootic by effectively increasing the number of susceptible animals available to maintain the infection. The virus would have a significant negative impact on population density by causing mortalities among calves not protected by maternal immunity. The apparent absence of morbillivirus antibodies in small cetaceans that occupy an inshore habitat in Peru and Brazil may indicate that the virus does not circulate among them. If these populations are indeed naive to the infection, accidental introduction of the virus could cause an epizootic with high mortality, as occurred in inshore *T. truncatus* in the northern hemisphere (Lipscomb *et al*., 1994a; Kraft *et al*., 1995).

PAPILLOMAVIRUSES

Papillomaviruses (PVs) are double-stranded DNA viruses constituting the *Papillomaviridae* family. Their genome of about 8kb comprises an early (E) region encoding non-structural proteins, a late (L) region encoding two capsid proteins and a non-coding upstream regulatory region (URR) controlling viral replication and transcription (Howley and Lowy, 2001; Münger *et al*., 2004). PVs cause benign hyperproliferative epithelial lesions of the skin and mucosa (warts, papillomas and condylomas). High-risk PVs may induce invasive carcinomas (Lowy and Howley, 2001). A high prevalence of genital warts was reported in *L. obscurus*, *D. capensis*, *T. truncatus* and *P. spinipinnis* from Peru (Van Bressem *et al*., 1996). Group-specific papillomavirus (PV) antigen was detected in a genital wart from a male, immature *P. spinipinnis* (JAS-44) using antibodies to disrupted human PV type 1. Sequences from two distantly related PVs were detected by polymerase chain reaction (PCR) assays in warts from two porpoises (JAS-44 and mature female JAS-50), indicating the existence of two *P. spinipinnis* (Ps) PVs. The entire genome of PsPV1 was cloned and sequenced. It consists of 7,879 nucleotides and presents unusual features. It lacks an E7 open reading frame (ORF), a *bona fide* E5 ORF and an E8 ORF. It has a larger E6 ORF and two novel ORFs (E3 and L3). It is the sole member of the *Omikronpapillomavirus* genus (Van Bressem *et al*., 2007a). Primers derived from the sequence of PsPV-1 permitted the amplification of PV DNA in genital warts from a *T. truncatus* and *L. obscurus* from Peru (Cassonnet *et al*., 1998).

At least two PV genotypes are the aetiological agents of genital warts in *P. spinipinnis*, *T. truncatus* and *L. obscurus* from Peruvian waters. PsPV-1 is the first sequenced cetacean PV and the first genital PV isolated in mammals

belonging to another order than the Primates. The high prevalence (48.5%) of genital warts in 33 Burmeister's porpoises examined in 1993-1995 (Van Bressem *et al*., 1996) and the detection of PV sequences in 5 of 7 genital warts sampled in this study indicates that PV infection is frequent. Porpoises may become infected early in life as indicated by the isolation of PsPV-1 from a tumour in a sexually immature individual and the common presence of genital warts in this age class (Van Bressem *et al*., 1996). They could acquire PV infection maternally very early in infancy as described in humans (Antonsson *et al*., 2003; Rintala *et al*., 2005) or through sexual activities. A PV detected in a cutaneous wart from a harbour porpoise (Van Bressem *et al*., 1999b) likely represents another PV infecting Phocoenidae. Two of 20 male (10%) *P. spinipinnis* suffered genital lesions of sufficient severity that may impede, or at least, hamper copulation. PVs in some circumstances (especially if non-randomly distributed) may exert an indirect impact on the dynamics of this population (Van Bressem *et al*., 1999a).

POXVIRUSES

Poxviruses (family Poxviridae) are endemic in Peruvian populations of *L. obscurus*, *D. capensis*, *T. truncatus* and *P. spinipinnis* (Van Bressem and Van Waerebeek, 1996) and possibly infect Chilean dolphins *Cephalorhynchus eutropia* from southern Chile and *S. guianensis* from Sepetiba Bay, Brazil (Van Bressem *et al*., 2007b). Tattoo skin diseases (TSD) is characterized by irregular, gray, black or yellowish, stippled lesions known as "tattoos" that may occur on any part of the body but which show a preferential corporal distribution depending on the species (Van Bressem and Van Waerebeek, 1996). The virus is thought to induce humoral immunity that protect calves from the disease (Smith *et al*., 1983; Van Bressem and Van Waerebeek, 1996). The poxviruses affecting small cetaceans from Peru were revealed by electron microscopy but have not yet been isolated nor characterized. Other cetacean poxviruses were recently detected in captive and free-ranging dolphins by a PCR assay targeting the DNA polymerase and DNA topoisomerase genes of members of the subfamily *Chordopoxvirinae* (Bracht *et al*., 2006). They belong to a new genus of *Chordopoxvirinae*, but have a common, most immediate ancestor with terrestrial poxviruses of the genus *Orthopoxvirus* (Bracht *et al*., 2006). The latter genus includes, among others, the smallpox virus (now eradicated in humans), vaccinia virus (the smallpox vaccine of unknown origin), and cowpox virus, endemic in European wild rodents and accidentally infecting humans, cats and cattle (Hazel *et al*., 2000; Esposito *et al*., 2004).

Cowpox virus neutralising antibodies were detected in the sera of 6 *D. capensis,* 8 *T. truncatus* as well as in 17 of 27 (63%) *L. obscurus,* and in 14 of 17 (82.4%) *P. spinipinnis* taken off central Peru in 1993-1995. Among 12 positive dolphins and 7 *P. spinipinnis*, neutralising titres ranged from 40 to over 1600, and 50 to 200, respectively. The high prevalence of orthopoxvirus neutralising antibodies with high titres indicates that Peruvian small cetaceans are commonly infected by poxviruses antigenically related to cowpox virus. Till date, the only poxviruses microscopically encountered in these mammals are those causing the endemic and distinctive TSD disease (Van Bressem and Van Waerebeek, 1996) and it is highly likely that infection by these viruses elicited the neutralising antibodies detected in this study.

Cetacean poxviruses may have evolved to counteract the immune response and persist in skin cells which in turns would assure its maintenance in cetacean communities and populations, as suggested by the lesion size, presence of virus particles in most samples examined and the occurrence of high VN titres against cowpox virus in dolphins and porpoises with a high density of tattoo lesions (Van Bressem, 1997; Van Bressem *et al*., 1999a, 2006a). Clinical and epizootiological data do not indicate that poxvirus infection induces a high mortality rate when enzootic (Van Bressem and Van Waerebeek, 1996; Van Bressem *et al.*, 2003). However, it may kill neonates and calves without protective immunity and thus affects host population dynamics. Indeed, although more than one third of inferred sexually mature female *L. obscurus* (36.2%, Van Bressem and Van Waerebeek, unpublished data) and *P. spinipinnis* (35.7%; Van Bressem and Van Waerebeek, 1996) suffered tattoo lesions in the period 1993-1994, indicating that an appreciable percentage of these females (and thus their calves) were not immune against the virus, none of the calves and neonates examined showed skin lesions (Van Bressem and Van Waerebeek, 1996). The consequences of the introduction of cetacean poxviruses in a naive population are unknown. However, several poxviruses cause high mortalities in all age classes when first introduced in a population (Fenner, 1983). The epidemiology of TSD and poxviruses should be further studied in South American small cetaceans and virus characterization should be attempted.

BRUCELLAE

Brucellosis is a globally distributed zoonotic disease of mammals that causes inter alia diseases of the reproductive system and abortion. It is caused by Gram-negative, facultative intracellular bacteria of the genus *Brucella*. In the 1990s, previously unknown strains of *Brucella* were detected in free-ranging pinnipeds and cetaceans from the Americas, Europe, the Antarctic and western North Pacific as well as in captive bottlenose dolphins (Ewalt *et al*., 1994; Ross *et al*., 1994; Jahans *et al*., 1997; Clavareau *et al*., 1998; Miller *et al*., 1999; Bricker *et al*., 2000; Van Bressem *et al*., 2001; Foster *et al.*, 2002; Ohishi *et al*., 2003, 2004). On the basis of host preference and molecular characteristics, it was proposed that these brucellae belong to at least two new species: *Brucella cetaceae* for cetacean isolates and *Brucella pinnipediae* for pinniped isolates (Cloeckart *et al*., 2003). Disorders associated with the infection in cetaceans

include placentitis, orchitis, abortion, lung infection and non-suppurative meningoencephalitis (Miller *et al*., 1999, Gonzalez *et al*., 2002; Ohishi *et al*., 2004).

Brucella spp. antibodies were common in Peru, namely in 21 of 27 (77.8%) *L. obscurus*, 3 of 6 *D. capensis*, 1 of 2 inshore, 2 of 3 offshore *T. truncatus* and in 5 of 20 (25%) *P. spinipinnis* detected by competitive ELISAs. All males (n=10) and females (n=6) of the adult *L. obscurus* sub-sample were positive, while all male (n=6) and female (n=5) immature *P. spinipinnis* were negative. Significance of differences in prevalence (≤ 0.05) was verified with one-tailed Fisher's exact tests (Swinscow, 1981). Sexual variation in prevalence of *Brucella* antibodies in immature *L. obscurus* ($p= 0.8$) and adult porpoises ($p= 0.83$) was not significant and sexes were pooled. Prevalence of seropositive individuals was significantly higher in sexually mature *L. obscurus* ($p=0.0016$) and *P. spinipinnis* ($p= 0.008$) than in immature individuals. Variation between these species was also significant: both mature and immature dusky dolphins were more likely to be seropositive than, respectively, mature ($p=0.01$) and immature ($p=0.018$) *P. spinipinnis*. Among the *T. truncatus* and *D. capensis*, all but one positive animal (a male offshore *T. truncatus*) were immature, but few mature individuals were sampled in these species.

The high prevalence of *Brucella* sp. antibodies in *L. obscurus* (100% in adults) and *P. spinipinnis* suggests that the infection is enzootic in these species. The significantly higher prevalence in mature individuals suggests that cetacean brucellosis is predominantly a disease of sexually mature animals, as it is in terrestrial mammals (Quinn *et al*., 1994; Ferguson, 1997). Orchitis and other testicular pathologies as well as vertebral lesions were observed in Peruvian *L. obscurus* and *D. capensis* (Van Bressem *et al*., 2000; Van Bressem *et al*., 2006b). It is conceivable that the enzootic circulation of brucellae in Peruvian *L. obscurus* and *P. spinipinnis* may constitute a measurable limiting factor among the environmental variables affecting population growth. Widespread *Brucella* sp. infection in several odontocete species has clear public health implications for coastal communities in Peru. Although capture of small cetaceans and commercialisation of their meat was outlawed since 1990, dolphins and porpoises taken in gillnets are still widely handled, butchered and consumed in fishing towns without sanitary precautions or control (Van Waerebeek and Reyes, 1994; Van Waerebeek *et al*., 1999; Van Waerebeek and J.Alfaro, unpublished data). Whether or not the strains of *Brucella* infecting Peruvian dolphins and porpoises may be transmitted to humans and cause disease is unconfirmed, most members of the genus *Brucella*, including the marine ones, are pathogenic in man (Brew *et al*., 1999). Two *Brucella* spp. isolated from intracerebral granulomas in two Peruvians were closely related to *B. pinnipediae* (Sohn *et al*., 2003).

CONCLUSIONS

Viruses including pox, papilloma, morbilli, and herpes, as well as *Brucella* spp. are circulating in South American small cetaceans (Van Bressem *et al*., 1994, 1996, 1999, 2006a). All are known to be pathogenic and many are thought to have at least some impact on populations. Morbilliviruses due to their extreme virulence represent the greatest threat to these populations, especially when the viruses are not present enzootically. Extrinsic anthropogenic factors including fisheries interactions, pollution and habitat loss and degradation possibly may not only exacerbate the consequences of viral infections on the health of a particular individual, but also operate at the population level. By reducing the number of animals in a population, and hence the number of susceptible individuals, fisheries may prevent the establishment of enzootic morbillivirus infections and favour recurrent epizootics which will further deplete the population (Van Bressem *et al*., 1999a).

Poxviruses, papillomaviruses and brucellae also have the potential to exert a negative impact on the population dynamics of cetaceans, by increasing natural mortality and/or by negatively affecting reproduction. We recommend to systematically include serological, pathological and epidemiological studies of viral diseases in all advanced cetacean research programmes and to account for the potential impact of microparasites when constructing population dynamics models.

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