SKIN DISEASES IN CETACEANS

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ABSTRACT

Micro-organisms that are known or suspected to cause skin diseases in cetaceans are briefly reviewed. Viruses belonging to four families i.e. *Caliciviridae*, *Herpesviridae*, *Papillomaviridae* and *Poxviridae* were detected by electron microscopy, histology and molecular techniques in vesicular skin lesions, black dots perceptible by the touch, warts and tattoos in several species of odontocetes and mysticetes. Herpesviruses, poxviruses and likely a cutaneous papillomavirus are cetacean specific. Among bacteria, *Dermatophilus* spp., *Erysipelothrix rhusiopathiae*, *Mycobacterium marinum*, *Pseudomonas* spp., *Streptococcus iniae* and *Vibrio spp.* were isolated from ulcerative dermatitis, pyogranulomatous dermatitis and panniculitis, diamond skin disease and slow-healing ulcers and abscesses. *Aeremonas* spp., *Mycobacterium marinum*, *Pseudomonas* spp. and *Vibrio* spp. are normally present in the marine environment while *Erysipelothrix rhusiopathiae* and *Streptococcus iniae* are fish pathogens that may also infect captive dolphins. Most seem to be opportunistic pathogens, exploiting some break-down in the host's defenses to initiate an infection. Selection of antibiotic-resistant bacteria through the prophylactic use of antibiotics in aquaculture is suggested to be a growing problem in South America and may account for the emergence of unusual cutaneous conditions. At least four groups of fungi i.e. *Candida albicans*, *Fusarium* spp., *Trichophyton* spp. and *Lacazia loboi* cause skin diseases. Candidiasis occurs predominantly in captive odontocetes. The lesions are often localized around the body orifices and may become extensive, granulating and ulcerated. Fusariosis is characterized by firm, erythematous, cutaneous nodules. *Trichophyton* spp. was isolated from widespread superficial nodules in an Atlantic *T. truncatus* kept in captivity in Japan. Lobomycosis or lacaziosis is distinguished by grayish, whitish to slightly pink, verrucuous lesions, often in pronounced relief that may ulcerate. While initially described only in *Tursiops truncatus* and *Sotalia guianensis* from the Americas, lobomycosis seems to be expanding to other continents. The role of ballast water in transporting fungi worldwide should be investigated. Finally, ciliated protozoans, likely *Kyaroikeus cetarius*, caused invasive dermatitis in small cetaceans from the USA and Korea. The aquatic environment of cetaceans is naturally home to bacteria and fungi but cetacean skin has several mechanisms to impede invasion. Chemical contaminants may affect natural skin barriers and depress the immune system. Wounds and specific viral infection (poxvirus, herpesvirus) may provide routes of entry.

INTRODUCTION

Skin diseases in cetaceans have been reported at least since the 1950s (Simpson *et al.*, 1958; Slijper, 1962; Greenwood *et al.*, 1974). Caldwell *et al.* (1975) provided a comprehensive description of lobomycosis in common bottlenose dolphins *Tursiops truncatus* from the Northwest Atlantic. Geraci *et al.* (1979) gave an insight into the clinical aspects and histopathology of tattoo skin disease (TSD) in captive and free-ranging *T. truncatus* and Atlantic white-sided dolphin *Lagenorhynchus acutus*. More recently the epidemiology and ecology of skin diseases including TSD, lobomycosis and lobomycosis-like disease (LLD) were investigated in some detail in free-ranging dolphins worldwide (Reif *et al*., 2006; Van Bressem and Van Waerebeek, 1996; Van Bressem *et al*., 2003a,b, 2007a, 2008a,b). Skin lesions were also recently reported in mysticetes (Pettis *et al*., 2004; Brownell *et al*., 2007; Bertelotti *et al*., 2008; Mouton *et al*., 2008). The role of natural and anthropogenic factors in the ecology of cutaneous illnesses of known and unknown aetiology has been thoroughly examined and the data gathered suggest that their emergence as well as their high prevalence in some populations is linked to a degraded environment, though water temperature and salinity may play a role too (Harzen and Brunnick, 1997; Wilson *et al*., 1999; Van Bressem *et al*., 2003a, 2007a, 2008a,b; Reif *et al*., 2006; Klaich *et al*., 2008). Skin lesions also often reflect the health status of cetaceans (Pettis *et al*., 2004; Van Bressem *et al*., 2008a).

Here we review the micro-organisms that are known or suspected to cause skin diseases in cetaceans.

VIRUSES

Caliciviruses

A strain of vesicular exanthema of swine virus (VESV), the cetacean calicivirus (CCV Tur-l) belongs to the genus *Vesivirus* of the family *Caliciviridae* (ICTV 2006). It was isolated from vesicular skin lesions that developed on a tattoo and old scars in two Atlantic bottlenose dolphins. The vesicles quickly eroded, leaving shallow ulcers in one of the dolphins (Smith *et al*., 1983). Though CCV Tur-l was isolated in the early 1980s, there are no published reports of further isolates and very little is known about its distribution and epidemiology. The role of vesiviruses in the aetiology of cutaneous vesicles observed in several cetaceans in South American coastal waters is suspected (Brownell *et al*., 2007; Van Bressem *et al*., 2007a; Flach *et al*., 2008).

Neutralizing antibodies to several other marine vesiviruses were detected by serology in mysticetes from the North Pacific (Smith and Latham, 1978, Smith and Boyt, 1990) and Alaska (Smith *et al*., 1987, O'Hara *et al*., 1998). California sea lions and one of their prey, the opaleye fish *Girella nigricans* (Dailey, 1970), may be primarily involved in the maintenance of these viruses in the North Pacific Ocean (Smith and Boyt, 1990). Transmission of the virus to other marine mammals is probably linked to contacts between species, migratory pathways and vectors (Smith *et al*., 1980a,b, Smith and Boyt, 1990). Metazoan parasites like *Zalophotrema* sp. and *Parafilaroides decorus* may act as mechanical vectors (Smith *et al*., 1980a,b).

Herpesviruses

Herpes-like virus particles were detected by electron microscopy (EM) in epithelial cells from skin lesions of free-ranging and captive beluga whales *Delphinapterus leucas* from the St. Lawrence estuary and the Churchill river, Canada (Martineau *et al*., 1988, Barr *et al*., 1989) and of two dusky dolphins caught off central Peru in 1991 (Fig. 1) (Van Bressem *et al*., 1994). The skin lesions in the belugas were paler than the normal skin, circular or elliptical and slightly in relief (Martineau *et al*., 1988, Barr *et al*., 1989). In the St. Lawrence estuary individual the marks showed a dark small center surrounded by a narrow ill-defined dark rim (Martineau *et al*., 1988). They were either numerous, generalized and up to 2cm in diameter (St. Lawrence beluga), or few, localized and up to 20-30 cm in diameter (Churchill river's individual). The infection did not seem to have affected the general body condition of the captive beluga (Barr *et al*., 1989). Histologically, the lesions were characterized by epithelial necrosis and intranuclear inclusions. Intracellular oedema with micro-vesicle formation in superficial epithelium was also observed (Martineau *et al*., 1988, Barr *et al*., 1989). In the dusky dolphins, black dots, perceptible by the touch, were present on the head and body (Fig. 1). The infection did not seem to be more than mildly pathogenic (Van Bressem *et al*., 1994). Herpesvirus-like lesions were also observed in Burmeister's porpoises *Phocoena spinipinnis* and long-beaked common dolphins *Delphinus capensis* from the central coast of Peru in 1991-1995 (Van Bressem *et al*., 2006; Van Bressem and Van Waerebeek, unpublished observations). In Peruvian small cetaceans they seem to mostly affect immature animals (Van Bressem *et al*., 1994, 2006; Van Bressem, Van Waerebeek, Garcia-Godos, unpublished observations).

Previously unknown members of the subfamily *Alphaherpesvirinae* were detected by polymerase chain reaction (PCR) in skin lesions from two Atlantic *T. truncatus*, closely resembling those described in the dusky dophins (Manire *et al*., 2006; Smolarek Benson *et al*., 2006). Though related to the simplexviruses of humans, they are cetacean-specific and probably co-evolved with their hosts for thousands of years (Smolarek Benson *et al*., 2006). Identical or very similar alphaherpesviruses may be responsible for both cutaneous and fatal systemic infections in Atlantic *T. truncatus* (Smolarek Benson *et al*., 2006). However, much remains to be learned on the ecology of these viruses as well as on their impact on survival.

Papillomaviruses

Intranuclear virus-like particles were described in hyperplastic epithelial lesions observed on the skin of a harbour porpoise *Phocoena phocoena* incidentally taken in the Bay of Fundy (Geraci *et al*., 1987) and of a captive killer whale (*Orcinus orca*) of Icelandic origin (Bossart *et al*., 1996). Papillomavirus infection was demonstrated by immunohistochemistry in cutaneous warts from a male *P. phocoena* (Fig. 2) stranded alive in Germany in December 1993 (Van Bressem *et al.*, 1999). Cutaneous warts are infrequently observed on the skin of North Atlantic harbour porpoises and were never seen in Peruvian small cetaceans in the period 1993-1994 though genital warts were highly prevalent in these species (Van Bressem *et al.*, 1996). The low prevalence of cutaneous PVs in small cetaceans may be explained by i) a high rate of unapparent or subclinical infections perhaps related to the high turnover of epidermis; ii) an early regression of the warts; iii) the development of a long lasting protective immunity; and iv) weak contagious nature of the disease (Van Bressem *et al.*, 1999). The impact on survival of cutaneous PVs is supposedly low or nihil.

Poxviruses
Newly recognized members of the family Poxviridae possibly belonging to a new genus of *Chordopoxvirinae*, caused tattoo skin disease (TSD) characterised by very typical, irregular, grey, black or yellowish, stippled lesions in cetaceans worldwide (Fig.3) (Bracht *et al.*, 2006, Pearce *et al.*, 2008). These viruses are cetacean-specific and likely sub-order and family-specific (Bracht *et al.*, 2006, Pearce *et al.*, 2008). The epidemiology and ecology of TSD has been thoroughly investigated. Results of a recent study suggest that the prevalence and severity of the disease is the highest in coastal species and populations living in a contaminated environment (Van Bressem *et al.*, 2003a, 2008a). Thus, TSD may be considered as a general health indicator for cetaceans and their habitat. Tattoos may provide a route of entry to other viruses, bacteria and fungi that may increase the severity of the lesions (Smith *et al.*, 1983; Frasca *et al.*, 1996; Flach *et al.*, 2008; Van Bressem *et al.*, 2008c).

BACTERIA

Several bacteria were isolated from skin lesions in cetaceans. Most are opportunistic, exploiting some break in the host defenses to initiate an infection. Some may have developed antibiotic-resistance due to the uncontrolled use of antibiotics in aquaculture in some countries (Cabello, 2004, 2006) that may render them more difficult to fight.

***Aeromonas* spp.**
A. hydrophila caused ulcerative dermatitis and pneumonia in a *T. truncatus* from North Carolina, USA in the 1970s (Cusick and Bullock, 1973). *Aeromonas* species thrive in the aquatic environment and have been recorded in the proximity of salmon farms in Chile (Mirand and Zemelman, 2002). In humans they may cause severe skin lesions including necrotizing fasciitis (Lehane and Rawlin, 2000; Hiransuthikul *et al.*, 2005; Tsai *et al.*, 2007). They are likely candidates in the aetiology of skin diseases in cetaceans from SA (Fig. 4).

Dermatophilosis
Dermatophilus spp. are actinomycetes of the family *Dermatophilaceae*. *Dermatophilus*-like bacteria were observed in slightly depressed, round cutaneous areas with a dull surface and pale grey colour in six belugas stranded along the shores of the St Lawrence estuary (Mikaelian *et al.*, 2001). The lesions measured 0.5-4 cm in diameter and, in five of the six cetaceans, were numerous, covering about 5% of the surface of the whole body. Attempts to isolate *D. congolensis* were unsuccessful, possibly because another species of *Dermatophilus* was involved (Mikaelian *et al.*, 2001). The morphology of *Dermatophilus* spp. is so unique that a strong presumptive diagnosis can be made on examination of stained smears alone (Quinn *et al.*, 1994).

Erysipelothrix rhusiopathiae
E. rhusiopathiae belongs to the family *Erysipelotrichaceae*. It may cause a sub-acute to chronic dermatological disease and septicemia in captive cetaceans (Geraci *et al.*, 1966). The skin disease is characterized by dermal infarction that results in sloughing of the epidermis. The formation of micro-infarcts may result in the rhomboid areas of cutaneous necrosis, known as diamond skin disease and characterized by grayish, raised, irregular in relief patches that may ulcerate (Geraci *et al.*, 1966). Animals with this form usually recover with timely antibiotic treatment. The septicemic form is usually acute or peracute, with the animal found moribund or dead. *E. rhusiopathiae* is a common contaminant of fishes. Ingestion of contaminated fish or injuries from the teeth of other cetaceans is presumed to be the route of infection for this disease.

Mycobacterium marinum
M. marinum belongs to the *Mycobacteriaceae* family and is ubiquitous to fresh and marine sediments. It is an opportunistic, infrequent pathogen of homeotherms, including humans and is usually acquired through contamination of wounds but aerosol transmission also occurs (Bowenkamp *et al.*, 2001). *M. marinum* was isolated from multicentric 1-3 cm diameter ulcerative skin lesions that developed on the thorax and abdomen and near the genital folds of a captive beluga. On cut section they were epidermal ulcers with cores of purulent material that extended deep into the blubber.

Histologically, the lesions consisted of pyogranulomatous dermatitis and panniculitis (Bowenkamp *et al*., 2001). There is no other report of skin disease related to this bacterium in cetaceans. However, it has been suggested that captive *T. truncatus* may harbour the bacteria asymptomatically and transmit the disease to humans through biting (Flowers, 1970).

***Pseudomonas* spp.**
Belonging to the family *Pseudomonadaceae*, *P. aeruginosa* is an opportunistic pathogen widely present in the terrestrial and fresh water environments as well as in the open ocean. It may be found in a biofilm, attached to some surface or substrate, or in a planktonic form, as a unicellular organism. It may cause disease and mortality in plants, humans and animals (Khan *et al*., 2006). Most *Pseudomonas* infections are both invasive and toxinogenic. The ultimate *Pseudomonas* infection may be seen as composed of three distinct stages: i) bacterial attachment and colonization; ii) local invasion; iii) disseminated systemic disease (Todar, 2008). *Pseudomonas aeruginosa* caused fatal bronchopneumonia and extensive dermatitis in an Atlantic *T. truncatus* caught off Florida in the 1970s (Diamond *et al*., 1979). The dolphin died 70 days after being caught after displaying dyspnea and anorexia. It had hard, round, and raised dermal nodules with necrotic centers all over its body surface. Similar skin lesions were observed in a female *L. obscurus* caught off Peru in 1994: it presented several nodules (some bleeding) on the back and tailstock (Van Bressem and Van Waerebeek, unpublished observations).

Staphylococcus delphini
A member of the Family *Streptococcaceae*, *S. delphini* was isolated from multiple suppurating skin lesions that responded well to antibiotic treatment in captive dolphins (Varaldo *et al*., 1988). *Staphylococcus* spp. have also been isolated from the respiratory tract and the genitals of a franciscana *Pontoporia blainvillei* and a southern right whale *Eubalaena australis* from Brazil, respectively (S. Siciliano, pers. observations).

Streptococcus iniae
Streptococcus iniae is a member of the family *Streptococcaceae*. It is a serious pathogen of fishes that causes high losses in farmed fishes (Agnew and Barnes, 2007). It has a zoonotic potential with human infection linked to fish manipulation. It triggers 'golf ball disease' in captive botos (*Inia geoffrensis*). The disorder is characterised by slow growing, multiple subcutaneous abscesses that eventually rupture (Bonar and Wagner, 2003). The disease is apparently linked to the fishes used to feed the dolphins and environmental conditions (Bonar *et al*., 2007). It may also occur in free-ranging *I. geoffrensis* from the Brazilian Amazon Basin (da Silva *et al*., 2008).

***Vibrio spp*.**
Members of the *Vibrionaceae* Family, *Vibrio* spp. thrive in the aquatic environment. Their presence and numbers are influenced by factors such as temperature, salinity and algal density. *V. damsela*, *V. alginolyticus*, *V. parahaemolyticus*, *V. vulnificus* and *V. fluviatilis* were isolated from slow-healing ulcers and abscesses of captive *T. truncatus* as well as from miscellaneous wounds sampled in free-ranging cetaceans from Brazil (Schroeder *et al*., 1985; Fujioka *et al*., 1988; Pereira *et al*., 2007). Traumata in coastal dolphins living in biologically contaminated environment likely provide a portal of entry to these bacteria.

FUNGI

At least four groups of fungi have been isolated from skin lesions in cetaceans. Recently reports of mycotic diseases in free-ranging cetaceans (Fig. 5) have dramatically increased worldwide (Reif et al., 20006; Van Bressem et al., 2007; Kiska and Van Bressem, 2008; Moreno *et al*., 2008; Mouton *et al*., 2008; Shirakihara *et al*., 2008; Siciliano *et al*., 2008; V. Peddemors, P. Le Noury and Van Bressem, unpublished data). *Lacazia loboi* seems to be the more widespread fungal pathogen in free-ranging cetaceans. However, further histological and EM studies are required to confirm this assumption.

Candidiasis
Belonging to the Order Saccharomycetales, *C. albicans* lives under normal circumstances in 80% of the human population with no harmful effects, although overgrowth results in candidiasis. Candidiasis is often observed in immunocompromised individuals. Candidiasis is relatively common in captive cetaceans and occurs secondary to stress, unbalanced water desinfection with chlorines, or indiscriminate antibiotic therapy. The lesions usually may be localized around the body orifices. However, *C. albicans* has also caused extensive, granulating and sometimes ulcerated skin lesions and oesophago-gastric ulcerations. Cases of disseminated candidiasis caused the death of *T. truncatus*, *P. phocoena* and a long-finned pilot whale (*Globicephala melas*) (Nakeeb *et al*., 1977; Dunn *et al*., 1982).

Fusariosis

Fusarium species are common soil saprophytes and plant pathogens. However, in recent years, they have been reported with increasing frequency as causes of opportunistic infections in humans and in animals, including reptiles, turtles, pinnipeds and dolphins (Frasca *et al.*, 1996; Cabañes *et al.*, 1997). *F. oxysporum*, *F. solani* and *F. verticillioides* are also serious emerging pathogens of humans with a broad resistance to the available anti-fungal drugs. They may cause lethal, invasive, fungal infections in immuno-compromised patients (Ortoneda *et al.*, 2004). *F. oxysporum* is broadly used in South America to destroy coca (*Erythroxylum coca*) plantations. *Fusarium* spp. caused raised, firm, erythematous, 2 to 5 mm cutaneous nodules that were most prominent on the head, trunk, and the caudal portion of an Atlantic white-sided dolphin and a pygmy sperm whale *Kogia breviceps* stranded along the northeast coast of the USA in 1991 (Frasca *et al.*, 1996) as well as in a in a captive beluga (Bowenkamp *et al.*, 2001). The yeast isolated from the *L. acutus* and *K. breviceps* appeared most like *F. oxysporum* (Frasca *et al.*, 1996).

Lobomycosis or lacaziosis

Lacazia loboi is an uncultivated pathogen that belongs with the other dimorphic fungal pathogens to the order Onygenales (Herr *et al.*, 2001). *L. loboi* naturally affects humans and cetaceans from the Americas causing lobomycosis (Caldwell *et al.*, 1975; Reif *et al.*, 2006; Van Bressem *et al.*, 2007a). *L. loboi* cells found in *T. truncatus* infected tissues are significantly smaller than those found in humans, suggesting that the organism may not be identical in the two hosts (Haubold *et al.*, 2000). Lobomycosis in dolphins is characterized by grayish, whitish to slightly pink, verrucuous lesions, often in pronounced relief that may ulcerate (Migaki *et al.*, 1971). Though the disease evolves slowly it may eventually lead to death (Simões-Lopes *et al.*, 1993;Van Bressem *et al.*, 2007a; Bermúdez-Villapol *et al.*, 2008). The clinical symptoms, histology and epidemiology of the disease are reviewed in detail in Reif *et al.*, 2006; Van Bressem *et al.*, 2007a; Moreno *et al.*, 2008; Paniz-Mondolfi *et al.*, 2008; Siciliano *et al.*, 2008. In South America, several cases highly reminiscent of lobomycosis were observed in free-ranging coastal *T. truncatus* and *S. guianensis* (Fig. 6). In the absence of a histological diagnosis, the disease was called lobomycosis-like disease (Van Bressem *et al.*, 2007a).

***Trichophyton* spp.**

Trichophyton spp. was isolated form widespread superficial nodules on the trunk of a captive Atlantic *T. truncatus* kept in captivity in Japan (Hoshina *et al.*, 1956).

PROTOZOANS

Dermatitis with invasive ciliates have been reported in *T. truncatus*, spotted dolphins *Stenella attenuata*, a common dolphin *Delphinus delphis*, a pygmy sperm whale, a Fraser's dolphin *Lagenodelphis hosei* and a killer whale *Orcinus orca* from the United States as well as in a "*Tursiops gilli*" kept in captivity in Seoul Grand Park, Korea (Schulman and Lipscomb, 1987; Choi *et al.*, 2003). The disease was characterized by discrete ulcers with subjacent dermal, and often subcutaneous, necrosis and inflammation in several *T. truncatus* that died during a morbillivirus epizootic along the Atlantic coast of the US in 1987-1988 (Schulman and Lipscomb, 1999). Prevalence of this condition was high (19%; N= 95) during the epizootic in comparison with other years, probably because of the immune-suppression caused by the viral infection. The ciliates observed in the skin lesions were morphologically identical to *Kyaroikeus cetarius* isolated from the blowhole of captive and free-ranging cetaceans (Sniezek *et al.*, 1995; Poynton *et al.*, 2001). They may be opportunistic invaders taking advantage of skin traumas (Schulman and Lipscomb, 1999). In the *T. gilli* the lesions initially developed on a traumatic skin injury on the neck area and then spread throughout the body surface (Choi *et al.*, 2003). The protozoans detected in the *T. gilli* were also morphologically similar to *K. cetarius* (Choi *et al.*, 2003). Protozoan-like organisms were also observed by transmission electron microscopy in rounded marks spread over the entire body surface of a male *D. capensis* and in small, dark, rounded marks, often punctured, present in an immature female *L. obscurus* (Van Bressem *et al.*, 2006; unpublished observations).

CONCLUSIONS

Commensal bacteria, fungi and micro-algae are naturally present in marine waters. The nanorough surface of the dolphin skin smoothed by a shear-resistant gel at the epidermal surface likely prevent their adhesion (Meyer *et al.*, 2008). The presence of the antibiotic, cationic enzyme lysozyme and antibiotic peptide group of Defensins in the epidermis may also serve as non-specific defense against these micro-organisms (Meyers and Seegers, 2004). Chemical contaminants may affect natural skin barriers while wounds and specific viral infection (poxvirus, herpesvirus) may provide routes of entry to opportunistic pathogens. By depressing the immune response of infected animals, morbilliviruses may favour protozoan infection (Schulman and Lipscomb, 1999).

The apparent emergence of skin diseases in cetaceans worldwide has generated an increased interest in these conditions and the role of environmental factors. Previously unrecognised cetacean viruses have been isolated, shedding more light into the phylogeny of several virus families. However, in a large number of cases the aetiology of these diseases is unknown, often because the affected cetaceans are free-ranging and sampling unrealistic. Photo-identification provides a good way of following the development of the illness, examine its general characteristics and study its epidemiology. When feasible, samples should be taken from stranded or by-caught specimens for isolation, electron microscopy and molecular studies. The remoteness of some study areas and lack of financial support sometimes prevents that such studies are undertaken.

The association between coastal waters that have been anthropogenically degraded and the health of its marine inhabitants has been clearly demonstrated in fishes years ago (Murchelano, 1990). Unless, drastic measures are taken to improve treatment of run-off waters, reduce chemical contamination, decontaminate water ballast and stop or at least reduce the heavily use prophylactic antibiotics in aquaculture, coastal environments will become inhabitable and unsuitable for human recreation purposes.

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FIGURES

Figure 1. Black dots, perceptible by the touch in a *Lagenorhynchus obscurus* by-caught in December 1993 off central Peru.

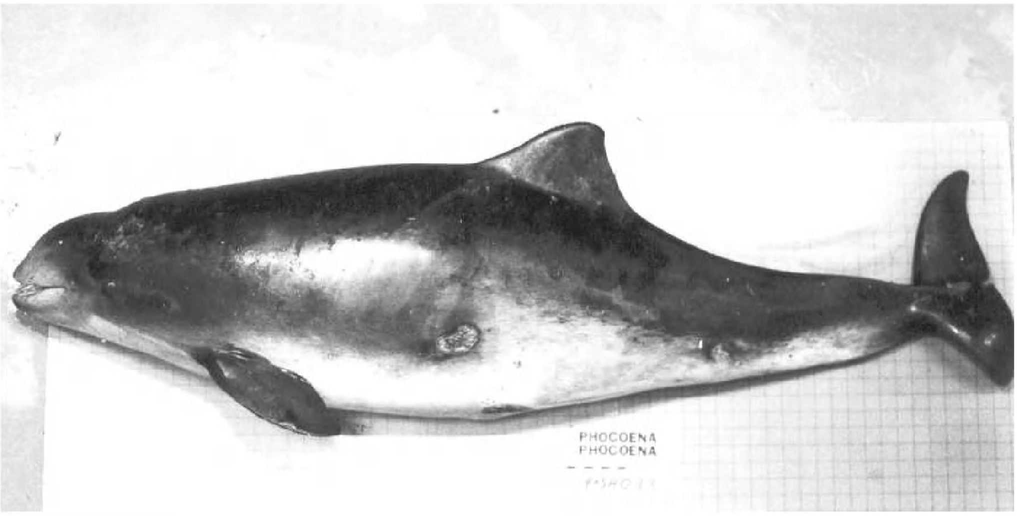

Figure 2. Papillomavirus skin infection in a *Phocoena phocoena* stranded in Germany in December 1993.

Figure 3. Tattoo lesions in a *Lagenorhynchus obscurus* by-caught in June 1994 off central Peru.

Figure 4. Whitish, velvety lesions in a *Tursiops truncatus* from the Bay of Paracas, Peru, in July 2006.

Figure 5. Mycotic disease in a *Tursiops aduncus* from Plettenberg Bay, South Africa in December 2004.

Figure 6. Lobomycosis-like disease in a *Sotalia guianensis* from the Paraná estuary, Brazil in February 2007.