Epizootics in harbour seals (*Phoca vitulina*): clinical aspects

Ursula Siebert¹, Frances Gulland², Timm Harder³, Thierry Jauniaux⁴, Henrike Seibel¹, Peter Wohlsein⁵ and Wolfgang Baumgärtner⁵

¹Forschungs- und Technologiezentrum Westküste, Christian-Albrechts-Universität zu Kiel, Werfstr. 6, D-25761 Bäsum, Germany
²Veterinary Science Department, Center for Marine Animal Health and The Marine Mammal Center, 1065 Fort Cronkhite, Sausalito, CA, 94965, U.S.A.
³O.I.E. und Nationales Referenzlabor für Aviare Influenza, Institut für Virusdiagnostik, Friedrich-Loeffler-Institut, Boddenblick 5a, D-17493 Greifswald-Insel Riem, Germany
⁴Department of Pathology, Veterinary College, University of Liege, B-4000 Liege, Belgium
⁵Institut für Pathologie, Tierärztliche Hochschule Hannover, Bünteweg 17, D-30559 Hannover, Germany

ABSTRACT

Epizoo tic diseases causing considerable mortality in harbour seal populations have mainly been reported from the waters of the United States and Europe. Such die-offs were largely attributable to viral infections. Several hundred individuals died from respiratory infections caused by Influenza A viruses at the coast of New England, USA, in 1979, 1980 and 1982. More than 53,000 harbour seals were killed in European waters by Phocine Distemper Virus (PDV), a morbillivirus, in two outbreaks in 1988 and 2002. For several other epizootics of smaller scale in the waters of the Atlantic and Pacific coast of the USA and, most recently, in Danish and Swedish waters in 2007 the causes remain unclear, although characteristic respiratory symptoms and interstitial pneumonia suspicious of viral etiology were detected as well as occasionally bacterial infections caused by *Erysipelothrix rhusiopathiae* and *Pseudomonas aeruginosa*. Mass mortalities caused by biotoxins, direct human interactions or changes in oceanographic conditions have so far not been described for harbour seals. However, high organochlorine loads detected in European harbour seal populations and suspected to impede immune functions, were considered an aggravating factor in the 1988 morbillivirus epizootic. Establishing supranational stranding networks is a key prerequisite for the detection of future unusual die-offs in marine mammals. Detailed post-mortem investigations of all organ systems are essential for targeted etiological studies towards the causes of mass mortalities in seals.

INTRODUCTION

Mass mortalities in harbour seals (*Phoca vitulina*) have exclusively been reported in the United States and European waters (Table 1). The seeming concentration of such die-offs on the northern Atlantic coast may be a consequence of supranational stranding networks operating in these areas and having capabilities to detect and diagnose seal mortality. Infectious diseases were determined to be the cause in some of the die-offs while in some cases no cause could be elucidated. The number of animals killed varied widely among the different outbreaks (Table 1). In this chapter, clinical aspects and epidemiology of different epizootics are reviewed.

VIRAL DISEASES

**Phocine distemper die-offs in 1988 and 2002 in European waters**

In 1988 and 2002 two large-scale epizootics occurred among harbour seals in European waters. Both epizootics started at the small Danish island of Anholt located in the Kattegat linking the Baltic and the North Seas. These outbreaks resulted in the deaths of more than 23,000 harbour seals in 1988 and 30,000 in 2002. In both years the cause of mortality was identified as Phocine Distemper Virus (PDV) (Kennedy 1990, Jensen et al. 2002, Müller et al. 2004). Details on the different geographical and chronological patterns of dispersal as well as population aspects are described elsewhere (Härkönen et al. 2006, Seibel et al. 2007, Härkönen and Isakson 2010).

### Table 1. Mass mortalities affecting harbour seal populations.

<table>
<thead>
<tr>
<th>Year</th>
<th>Geographic location</th>
<th>Reported number of dead animals</th>
<th>Etiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1978-80</td>
<td>Cape Cod, Massachusetts, U.S.</td>
<td>400</td>
<td>Influenza A virus</td>
</tr>
<tr>
<td>1982</td>
<td>European waters</td>
<td>23,000</td>
<td>Phocine Distemper virus</td>
</tr>
<tr>
<td>1991</td>
<td>New Jersey, U.S.</td>
<td>34</td>
<td>Unknown</td>
</tr>
<tr>
<td>1992</td>
<td>Oregon, Washington, U.S.</td>
<td>29</td>
<td><em>Erysipelothrix rhusiopathiae</em> and orthopoxvirus present</td>
</tr>
<tr>
<td>1992</td>
<td>Maine, Massachusetts U.S.</td>
<td>24</td>
<td>Unknown</td>
</tr>
<tr>
<td>1997</td>
<td>California, U.S.</td>
<td>90</td>
<td>Unknown, <em>Morbillivirus</em> and/or Influenza virus suspected</td>
</tr>
<tr>
<td>1998</td>
<td>Belgian and French North Sea</td>
<td>11</td>
<td>Unknown, <em>Pseudomonas aeruginosa</em> associated with pneumonia virus suspected</td>
</tr>
<tr>
<td>2000</td>
<td>California, U.S.</td>
<td>26</td>
<td><em>Morbillivirus</em></td>
</tr>
<tr>
<td>2002</td>
<td>European waters</td>
<td>30,000</td>
<td>Unknown, viral pneumonia suspected</td>
</tr>
<tr>
<td>2003-2004</td>
<td>Gulf of Maine, U.S.</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>2007</td>
<td>Danish and Swedish waters of the Kattegat and Skagerrak</td>
<td>285</td>
<td>Unknown</td>
</tr>
</tbody>
</table>
The virus family Paramyxoviridae comprises 2 subfamilies, Paramyxovirinae with the 3 genera Respirovirus, Rubulavirus and Morbillivirus, and Pneumovirinae with the genera Pneumovirus and Metapneumovirus as well as unclassified paramyxoviruses. They include some of the most important pathogens of humans and animals. The morphologically distinguishing features for viruses of the subfamily Paramyxovirinae are the size and shape of the nucleocapsids (diameter of 18 nm, 1 µm in length, a pitch of 5.5 nm). They have a left-handed helical symmetry. The biological criteria are: (a) antigenic cross-reactivity between members of a genus and (b) the presence (Respirovirus and Rubulavirus) or absence (Morbillivirus) of neuraminidase activity (www.ncbi.nlm.nih.gov/ICTVdb/ictv/index.html).

Morbillivirus infections can cause significant mortalities in humans and animals (Barrett 1999, Baumgartner et al. 2003). Measles virus is responsible for up to two million childhood deaths in humans annually in the developing world, while Paste des petits ruminants viruses cause severe epizootics in domestic and wild ruminants in areas of the world where it remains endemic (Barrett 1999). Rinderpest virus causing a highly contagious disease associated with massive mortality in domestic and wild ruminants has been eradicated globally due to successful vaccination campaigns in the last decade. Canine Distemper Virus (CDV) causes a fatal disease in many species of carnivores. New morbilliviruses with potentially important ecological consequences for marine mammals have been discovered over the past 15 years: Phocine Distemper Virus (PDV) in seals, cetacean morbilliviruses (CMV), including porpoise morbillivirus (PMV), and dolphin morbillivirus (DMV) detected in dolphins, whales and porpoises (Kennedy 1998, Wohlsen et al. 2007b). PDV and CDV are most closely related among the morbilliviruses. Arctic seals may have been infected by CDV several hundreds or thousands of years ago from terrestrial carnivores such as wolves, foxes, dogs or polar bears, after which the virus may have evolved into PDV (Barrett 1999). PMV and DMV constitute a separate branch in the phylogenetic tree, more distant from the presumed ancestral progenitor virus (Rima et al. 1992, Blikenkrone-Moller et al. 1994, Barrett et al. 1995, Müller et al. 2000, Wohlsen et al. 2007a). Samples taken from affected harbour and harp seals in Ireland, Denmark and the Netherlands in 1988 indicated that PDV was genetically identical in the northern European region. Samples obtained from harp seals from Ireland, Denmark and the Netherlands during the 1988 PDV epizootic indicated that the virus was genetically identical in these areas (Visser et al. 1990, Barrett et al. 1992). A comparison of the PDVs causing the 2002 and 1988 outbreaks showed that both strains were at 97% identical in the P gene sequence (Jensen et al. 2002, Müller et al. 2004).

Clinical manifestations of PDV in harbour seals include fever, ocularonasal discharge, conjunctivitis, ophthalmia, keratitis, coughing, dyspnoea, diarrhoea, abortion, increased buoyancy due to the emphysema and the inability to dive (Kennedy et al. 1989, Bergman et al. 1990, Kennedy 1998). The incubation time of experimental PDV morbillivirus infection in harbour seals is at least 5 to 12 days (Harder et al. 1990). In dogs, depending on the immune status of the animals, the virus CDV can cause an abortive, subclinical or severe systemic, often lethal infection (Krapkowska et al. 1985). The virus is shed in all excretions from infected animals and is transmitted through direct contact and inhalation. Systemic spread is achieved through infected macrophages and lymphocytes transporting the virus to permissive epithelial, mesenchymal and neuroectodermal tissues (Caswell and Williams 2007). The predominant pathological finding in PDV-infected seals is an interstitial and purulent pneumonia with marked pulmonary alveolar and interstitial emphysema (Kennedy et al. 1989, Bergman et al. 1990, Kennedy 1998, Jamiaux et al. 2001). Bordetella bronchiseptica is the most common bacterium isolated from animals with secondary bacterial infections (Baker and Ross 1992, Jensen et al. 2002, Müller et al. 2002) but less common bacteria including Mycoplasma spp. were also found (Kennedy 1998). Common patho-histological findings are lymphocytic depletion in lymphoid tissues and a non-suppurative encephalitis (Kennedy et al. 1989, Bergman et al. 1990, Kennedy 1998, Jamiaux et al. 2001). Cytoplasmic and intranuclear viral inclusion bodies are present in various organs.
including lung, liver, kidneys, pancreas, intestine and brain (Kennedy et al. 1989, Bergman et al. 1990, Kennedy 1998). Experimentally neutralising serum antibodies were apparent in seals 16 days post infection (Harder et al. 1990).

A main hypothesis for the source of the 1988 epizootic is that arctic harp seals (Phoca groenlandica) acted as both a reservoir and the primary vector for PDV (Dietz et al. 1989, Henderson et al. 1992, Markussen and Have 1992). Harp seals were not documented around the Anholt Island in Denmark, the site where both epizootics in the North Sea started but were seen in the vicinity of harbour seal haulout sites in the North Sea (Heide-Jørgensen and Härkönen 1992). In addition, unusual presence of harp seals was reported in 1987 on the European coasts around the North Sea and adjacent waters, and they may have transferred the disease to harbour seals (Goodhart 1988, McGoutry 1988). Over the years following the 1988 epizootic, there were no signs that PDV had continued to circulate among European harbour seals (Jensen et al. 2002, Thompson et al. 2002, Müller et al. 2004, Siebert et al. 2007), except for some cases in 1998 on the Belgian and northern French coast (Jauniaux et al. 2001), and the new outbreak in 2002 was most likely the result of another introduction of the virus from an unknown source. As the disease again started at Anholt Island, this haulout site requires closer scrutiny. Its most striking feature is that harbour seals haul out in large numbers (500-1000) on a limited area of sandy beaches on the eastern end of the island. This locality is shared with grey seals (Halichoerus grypus), which only occur in low numbers (5-10 animals). Harbour and grey seals haul out very close together, and are not separated in groups as seen in most other localities where the grey seal numbers are larger. Consequently, the grey seals at Anholt are potential candidates for transferring PDV to harbour seals. Grey seals in general have larger activity ranges than harbour seals. It is still puzzling why the epizootic started at Anholt and not e.g. in the western Dutch Wadden Sea, where more than 1,100 grey seals are present and over 150 pups are born annually (Reijnders et al. 1995). Mixed colonies of grey and harbour seals are also common here.

Environmental factors must be considered for their possible influence on variation in mortality during the PDV-epizootics. The role of organochlorine (OC) contamination, through impeding immune system function, was considered a predisposing factor in the 1988 epizootic, although a causal relation could not be firmly established (Hall et al. 1992, Reijnders and Aguilar 2002). Novel compounds of concern, such as organotins and brominated flame retardants (e.g. polybrominated diphenyl ethers), may pose a new threat to the immune fitness of seals. However, data on tissue concentrations of these compounds in seals in the North Sea area are scarce (Boon et al. 2002, Hall et al. 2003, Law et al. 2003). Moreover, information on the immuno-toxicological impact of these compounds on marine mammals is currently lacking. Therefore, no conclusions about the role of these compounds in the resistance of seals to PDV or other infectious diseases can be drawn at this time. Finally, another factor of predisposition is associated with the host species, since harbour seals within European waters are highly susceptible while other species, such as harp and grey seals, are more resilient (Härkönen et al. 2006). Morbillivirus seropositive harp seals have been reported in 1985 without associated disease (Dietz et al. 1989), while all tested harbour seals were negative before the first epizootics (naive population). This confirmed that harp seals could be considered a vector of the virus to the naive harbour seal population, predisposing it for the severe 1988 epizootics (Stuen et al. 1994).

**Influenza A, Cape Cod 1979-80, 1982**

From 1979 to 1980 and again in 1982, more than 450 harbour seals were found dead on the coast of Cape Cod, Massachusetts, US. Investigations revealed that Influenza A virus was responsible for the disease (Geraci et al. 1982, Hinshaw et al. 1984).

Influenza viruses belong to the family Orthomyxoviridae. These viruses are pleomorphic, enveloped and have a size of 80 to 120 nm in diameter. The nucleocapsids have helical symmetry. The genome of Influenza A viruses consists of 8 segments of single...
stranded RNA of negative polarity coding for at least 11 proteins (www.ncbi.nlm.nih.gov/ICTVdb/Ictv/index.htm). Influenza A viruses have an extraordinary broad host spectrum and have been isolated from birds (where they have their natural reservoir), to horses, pigs, cats, dogs, humans and marine mammals (Vahlenkamp and Harder 2006).

Influenza A viruses have also been isolated from harbour seals. An influenza A virus of subtype H7N7 (A/Seal/Mass/1/80), was present in harbour seals dying at the New England Coast in 1980 (Geraci et al. 1982). Mycoplasma spp. were isolated concurrently from these animals. Another influenza A virus, subtype H4N5 (A/Seal/Mass/133/82), was obtained from harbour seals from neighbouring locations between June 1982 and March 1983 (Hinshaw et al. 1984). In winter 1991-1992 two additional isolates of subtypes H4N6 and H3N3 (A/Seals/MA/3807/91, A/Seal/MA/3810/91, A/Seal/MA/3911/92) were obtained from these populations (Callan et al. 1995). All influenza A viruses that were isolated from seals were antigenically and genetically most closely related to avian influenza viruses, suggesting frequent spill-overs from pelagic birds (Kennedy-Stoskopf 2001). No evidence for stable circulating seal-adapted lineages of influenza A viruses have been obtained so far. The incubation period of the influenza virus infections in naturally infected seals seems to be 3 days or less (Webster et al. 1981, Geraci et al. 1984, Hinshaw et al. 1984). According to experimental inoculation trials, all isolates from harbour seals were able to replicate in ferrets, cats, pigs and phocid seals, including harbour, ringed and harp seals. (Webster et al. 1981, Geraci et al. 1984, Hinshaw et al. 1984).

Clinical signs of natural influenza infection were similar to those described in seals with PDV. These included dyspnoea, lethargy, blood-stained nasal discharge and subcutaneous emphysema. Pulmonary lesions were predominantly characterised by necrotising bronchitis and bronchiolitis, and hemorrhagic alveolitis (Geraci et al. 1982, Hinshaw et al. 1984).

So far, Influenza A virus infections associated with mortality in harbour seals have been found exclusively on the east coast of the United States. However, Influenza B virus (B/Seal/Netherlands/1/99), previously thought to be exclusively a human pathogen, was isolated from the respiratory tract of a single juvenile harbour seal from the Dutch Wadden Sea with clinical signs, which recovered from the infection (Osterhaus et al. 2000).

UNKNOWN CAUSES OF MORTALITIES

Mass mortality in Danish and Swedish waters of the Kattegat and Skagerrak in 2007

The most recent outbreak of disease leading to increased mortality of harbour seals in Europe began in 2007, again on the small Danish island of Anholt on 15 June 2007 (Härkönen et al. 2008). Clinical signs of diseased seals and gross pathological findings were similar to those observed in 1988 and 2002. It was initially believed that PDV was the cause of the third die-off.

From Anholt the disease spread to other major harbour seal colonies in the Kattegat and Skagerrak over the next months, and single carcasses where found until the end of 2007. In the Danish part of the Kattegat a total of 163 dead seals were found washed ashore. The majority of these animals were pups born in the same year (149 individuals, standard length (length from nose to tail when the seal is lying with its belly up) <100 cm Härkönen et al. 2008), 10 were subadults (standard length 101-139 cm), and 4 were adults (standard length >140 cm). The first diseased seals in Swedish colonies were observed on 9 July. By 22 September 2007, a total of 122 carcasses had been collected along the Swedish coast, of which 97 were pups born in that year, 12 subadults and 13 adults (Härkönen et al. 2008). Clinical observations included a dorsally misshaped silhouette with an intermittent hump formation in the shoulder region, and restricted movement. In the final stage, animals showed respiratory distress and coughing up blood. The results of post-mortem examinations (complete necropsy of 16 seals and investigations of the respiratory tract and spleen of 85 additional...
seals) revealed emphysema of the lung and mediastinum (Häkön en et al. 2008). Preliminary histopathological findings in 4 of the seals displayed multifocal acute catarrhal bronchitis, chronic interstitial pneumonia, severe atelectasis, moderate follicular hyperplasia and acute lymphocytolysis.

Bacteriological investigations of the lung and spleen of 3 harbour seals revealed untyped Gram-negative, biochemically inactive rods. Attempts to amplify PDV-specific RNA in lung tissues by conventional PCR, targeting the nucleocapsid and phosphoprotein genes and real-time PCR assays for PDV targeting the haemagglutinin protein gene, were negative. It was suggested that the epizootic most likely was caused by an unknown virus. As harbour porpoises found on the Swedish coast in the same year showed similar pathological findings, a cross-species infection could not be ruled out (Häkön en et al. 2008).

Mass-mortalities with unknown causes reported from the United States
Several smaller mortality events occurred on the coasts of the United States in the past 17 years (Gulland and Hall 2007). During each event between 24 and 34 harbour seals were found dead on both the west and the east coast.

In most cases pneumonia was found and a viral cause, such as morbillivirus or Influenza virus, was suspected from the histological lesions and the epidemiology but could not be proven. Different infectious agents were isolated from individuals found dead in New Jersey in 1991, such as Erysipelothrix rhusiopathiae and Orthopoxvirus. In harbour seals found dead on the coast of California in 1997, Pseudomonas aeruginosa was isolated, associated with pneumonia (Gulland and Hall 2007). The roles of these bacteria in causing mortality remain unclear.

SUMMARY

The published data shows that die-offs of harbour seals are recurrent events but are mainly reported from the waters of the United States and Europe. The causes of the epizootics so far have been mainly viral infections. For some events the source of infection remains unclear, but bacterial and viral infections were suspected. In other marine mammalian species, causes of mass mortalities have been biotoxins, parasites, human interactions, oil spills and changes in oceanographic conditions (Geraci et al. 1999, Gulland and Hall 2007).

An indirect contributing role on epizootics by anthropogenic activities, such as chemical pollution, has been shown in different studies (Ross 2002). Mortality rates vary strongly between causes, years and regions. However, it is clear that as a basis for the detection and understanding of epizootics a good monitoring programme with detailed investigations is needed. This programme would allow early detection of mortality rates above normal fluctuations, abnormal cause of death as well as isolation, identification and characterization of infectious agents, such as parasites, bacteria and viruses. Established systems can exclude or identify new as well as known causes for mass mortalities.
REFERENCES


Harbour seals in the North Atlantic and the Baltic


