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Review

Influenza Virus Infection of Marine Mammals

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Abstract: Interspecies transmission may play a key role in the evolution and ecology of influenza A viruses. The importance of marine mammals as hosts or carriers of potential zoonotic pathogens such as highly pathogenic H5 and H7 influenza viruses is not well understood. The fact that influenza viruses are some of the few zoonotic pathogens known to have caused infection in marine mammals, evidence for direct transmission of influenza A virus H7N7 subtype from seals to man, transmission of pandemic H1N1 influenza viruses to seals and also limited evidence for long-term persistence of influenza B viruses in seal populations without significant genetic change, makes monitoring of influenza viruses in marine mammal populations worth being performed. In addition, such monitoring studies could be a great tool to better understand the ecology of influenza viruses in nature.

Keywords: marine mammals, influenza viruses, zoonotic diseases

Introduction

Interspecies transmission of influenza viruses is an important event in the evolution and ecology of these viruses. Influenza A viruses (IAV) infect a variety of hosts, including domestic and wild birds, humans and marine mammals. Influenza B viruses, in contrast, have only been isolated from humans and in a rare event from a seal. Influenza viruses are among the few zoonotic pathogens known to have caused infections in seals and other marine mammals. Based on the close genetic relatedness of influenza viruses isolated from marine mammals and wild birds, it has been hypothesized that wild birds are the main source of influenza infection of marine mammals (Fig. 1). Contact between marine mammals and wild birds at hauling-out sites or when feeding on the same food resources, i.e. fish or krill species, can facilitate cross-species transmission of avian influenza viruses (AIV). The role of marine mammals as hosts or carriers of potential zoonotic pathogens such as highly pathogenic AIV is an issue which needs further analysed. Nevertheless, migration of seals and other marine mammals may play a key role in the spread of AIV from one continent to anothers.

Here, we describe findings of a literature review aimed at assessing the circulation of influenza virus A and B in marine mammals. Firstly, we present virological and serological findings by species; secondly, we highlight and describe episodes concerning the transmission of influenza

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viruses between sea mammals, humans and other primates; and eventually, we discuss the cellular receptor specificity of marine mammal influenza A and B viruses.

Influenza Infection in Marine Mammal Populations

Seals

The first reported occurrence of influenza in marine mammals dates back to December 1979, when marine biologists in Boston observed a sudden increase in the number of stranded and dead harbour seals (*Phoca vitulina*) in Cape Cod, Massachusetts, USA. In the following nine months, about 500 mainly juvenile specimens of harbour seals were found dead along the north-eastern coast of the United States due to an infection with an influenza A virus of the H7N7 subtype (Table 1; Webster et al. 1981a; Lang et al. 1981). This epizootic infection affected at least 20% of the local seal population and some animals developed severe acute haemorrhagic viral pneumonia (Geraci et al. 1982). Haemagglutination inhibition (HI) assays showed that the causative influenza virus was antigenically similar to A/Fowl Plague/Dutch/27 (H7N7) (Webster et al. 1981a). The haemagglutinin (HA) gene of the virus was later fully sequenced and phylogenetic analysis proved its association with avian H7 viruses (Kida et al. 1982; Naeve & Webster, 1983). It was later shown that, although the seal virus was genetically related to avian influenza viruses (AIV), it behaved biologically more like a mammalian strain, replicating to high titres in ferrets, cats and pigs (Webster et al. 1981a; Callan et al. 1995). By contrast, it replicated poorly and produced no clinical signs in avian species (chickens, ducks, turkeys and parakeets) and faecal shedding in these animals after experimental infection was not detected. This was probably due to its adaptation to mammalian hosts during replication in seals (Webster et al. 1981a). However, serosurveys in several seal populations (8 harbour and 9 grey seals sera from Iceland & 227 sera from northern fur seals collected between 1971 and 1980 from the Bering Sea, Pacific Ocean, and the Sea of Okhotsk) provided no apparent evidence for previous influenza infection (Webster et al. 1992), thus suggesting that the virus had probably been introduced only shortly before from the avian reservoir to the seal population. This widespread outbreak attracted public attention because it provided the first evidence that a completely avian influenza virus could transmit to a mammalian population and cause severe disease and mortality (Webster et al. 1992). In addition, the virus raised concern as a zoonotic threat since it caused purulent conjunctivitis in humans who handled the dead seals (Webster et al. 1981b).

The second report of influenza infection in marine mammals was related to an H4N5 influenza A virus, which was again associated with pneumonia in harbour seals and affected the same location in Massachusetts, from June 1982 to August 1983. Approximately 60 harbour seals died due to the infection. The causative virus was isolated from the lungs and brains of dead seals. Based on the HI assay results and due to the high replication of the virus in avian

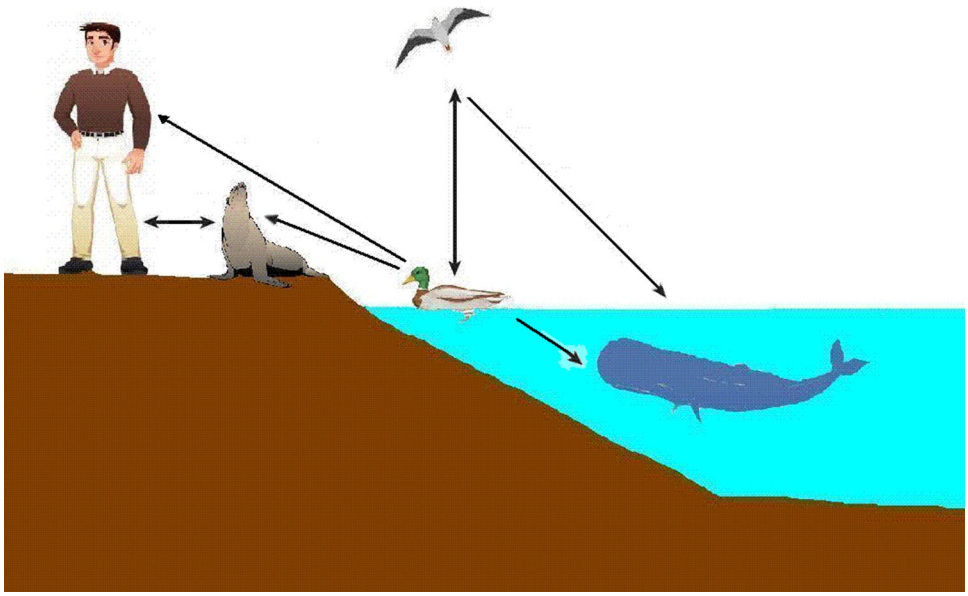

Figure 1. Inter-species transmission of influenza viruses between marine mammals and other species. Arrows indicate known transmission routes between each species based on reported cases, phylogenetic analysis and experimental studies.

Table 1. Summary of Influenza Virus Outbreaks/Isolations in Marine Mammals

Month/year	Location	Species	Mortality	Subtype	Isolated viruses	References
1975–1976	South Pacific	Minke whale	?	H1N3	A/whale/PO/19/76	Lvov et al. (1978)
Dec 1979–Oct 1980	USA, Cape code	Harbour seal	500–600	H7N7	A/seal/Massachusetts/80	Webster et al. (1981a)
Jun 1982–Aug 1983	USA, Cape code	Harbour seal	60	H4N5	A/Seal/MA/133/82	Hinshaw et al. (1984)
1984	USA, Maine	Long-finned pilot whale	120	H13N2/H13N9	A/whale/Maine/328/84 A/whale/Maine/1/84	Hinshaw et al. (1986)
Jan 1991	USA, Cape code	Harbour seal	?	H4N6	A/Seal/MA/3807/91 A/Seal/MA/3810/91	Callan et al. (1995)
Sep 1991–Apr 1992	USA, Cape code	Harbour seal	?	H3N3	A/Seal/MA/3911/92 A/Seal/MA/3984/92 A/Seal/MA/4007/92	Callan et al. (1995)
Apr 2010	USA,	Elephant seal	0	H1N1	A/Elephant seal/California/1/2010	Goldstein et al. (2013)
Sep 2011–Dec 2011	USA, New England coast	Harbour seal	162	H3N8	A/harbour seal/Mass/1/2011	Anthony et al. (2012)

hosts, it was concluded that the HA was antigenically and biologically similar to avian viruses (Hinshaw et al. 1984). This second outbreak focused the scientific attention on the role of marine mammals in the ecology and epidemiology of influenza viruses in nature (Webster et al. 1992).

In 1989, an extensive surveillance study along the New England coast was implemented and over 450 tissue samples from marine mammals were collected and tested within two years. As a result, in January 1991, two influenza viruses of the H4N6 subtype were isolated from the lung tissue of two dead harbour seals. In post-mortem examination, pathological lesions consistent with those described during previous outbreaks, such as acute interstitial and/or haemorrhagic pneumonia and subcutaneous emphysema were observed. Based on the neuraminidase variation from previous seal H4N5 viruses, the authors concluded that the two H4N6 isolates were biologically close to wild bird isolates (Callan et al. 1995). Although the evidence was insufficient, further researchers relied on this conclusion and emphasised the phylogenetic relatedness of the viruses to wild bird isolates (Ito et al. 1999; Osterhaus et al. 2000; Fouchier et al. 2001; Nielsen et al. 2001; Ohishi et al. 2006; Reperant et al. 2009; Arbiza et al. 2012).

A few months later, i.e. from September 1991 to April 1992, an increase in the number of stranded seals along Cape Cod was reported. Pathologic lesions, including acute interstitial pneumonia and subcutaneous emphysema, were observed in dead seals; and three influenza viruses of the H3N3 subtype were isolated from the lung tissue of three harbour seals (Callan et al. 1995). This was the first isolation of H3 subtype influenza viruses from seals and phylogenetic analysis demonstrated a close relationship to North American AIVs. Indeed, the increased number of stranded seals registered at Cape Cod between 1991 and 1992, could be associated with influenza infection; nevertheless, a severe epizootic of viral pneumonia in the seal population, such as in previous H7N7 (1979–1980) and H4N5 (1982–1983) outbreaks, was not observed (Callan et al. 1995).

In April 2010, nasal swabs were collected from 42 female free-ranging Northern elephant seals (*Mirounga angustirostris*) along California's central coast (Goldstein et al. 2013). Two swabs tested positive by matrix gene reverse transcription polymerase chain reaction (RT-PCR); and subsequently, AIVs were isolated from samples. The isolated viruses showed an homology greater than 99% to the pandemic influenza virus A/California/04/2009 (pdmH1N1) isolated from humans in 2009. Testing of serum samples

collected between early January–April 2010 showed serum conversion of the seals against H1N1 during this period, which indicated a recent introduction of the virus into the local seal population (Goldstein et al. 2013).

In an outbreak which hit the New England coast between September–December 2011, 162 harbour seals (*Phoca vitulina*) had died of pneumonia. The mortality rate was approximately four times higher than expected, if compared to the estimates from previous years; as well, an avian influenza virus of the H3N8 subtype was isolated from several tissues of five dead seals during the peak of the outbreak. The main pathological findings were pneumonia and ulcerations of the skin and oral mucosa. In the viral HA and PB2 proteins, the virus shared two mutations with mammalian H3N8 viruses (Anthony et al. 2012). The D701 N mutation in the viral PB2 protein is known to increase transmissibility and virulence of influenza viruses in mammals (de Jong et al. 2006); however, there is no clear evidence of transmission of the recently isolated H3N8 viruses to other mammalian species, including humans.

Whales

The first report of influenza A infection in whales was published in 1986 after H13 subtype influenza viruses had been isolated from a long-finned pilot whale (*Globicephala melas*) near Portland, Maine, USA. The whale was found in poor conditions in October 1984. After post-mortem examination, two different subtypes of influenza viruses (H13N2 and H13N9) were isolated from the hilar lymph nodes and lungs of the whale. Pathological findings included a large hilar lymph node (five times the normal size) and haemorrhagic lungs. The H13 whale viruses were shown to be closely related to the H13 influenza viruses circulating in that same period among seagulls (species not specified) in the USA (Hinshaw et al. 1986). It was supposed that these whale H13 influenza viruses had probably been related with two mass strandings of long-finned pilot whales along the New England coast (97 whales in October, 23 whales in November, 1984), although samples collected from 19 dead pilot whales were all influenza virus negative, probably due to extensive tissue autolysis (Hinshaw et al. 1986; Van Bressem et al. 1999; Waltzek et al. 2012). In an experimental study, both whale H13 viruses could not replicate in the lower intestine of ducks after oral inoculation, but moderately replicated after rectal inoculation (Hinshaw et al. 1986). This phenomenon was also shown earlier and may have reflected the susceptibility of H13 viruses (originated from gulls) to low pH of the crop and proventriculus of ducks (Hinshaw et al. 1982).

The only influenza A virus detected in marine mammals outside the USA territories belonged to subtype H1N3 (Lvov et al. 1978). The virus was isolated from the lung and liver of a striped whale (family *Balaenopteridae, reported by* Duignan, 2000) hunted in the South Pacific in 1975/76. In this study, lung and liver samples were collected from 72 whales; 13 strains of influenza viruses from lungs specimens, as well as one strain from one liver specimen were isolated (no detailed data on subtypes were published, Lvov et al. 1978).

Phylogenetic analysis was performed to make a comparison between the nucleoprotein (NP) sequence of three influenza viruses isolated from marine mammals (i.e. whale H1N3, whale H13N2, seal H7N7) and several wild bird AI viruses (including those isolated from a gull in the USA and from a tern of the Caspian Sea). The results indicated high genetic relatedness between marine mammal viruses and those reported from wild birds (Mandler et al. 1990). This could mean that introduction of influenza viruses from wild birds into marine mammal populations (Mandler et al. 1990) might have occurred on several occasions independently.

Influenza B Virus

In contrast to influenza A viruses, which have been isolated from many different species, influenza B viruses are human pathogens with no or unknown reservoirs in nature. The seal influenza B virus (B/Seal/Netherlands/1/99) was isolated in 1999 from a throat swab of a juvenile seal in a rehabilitation centre in the Netherlands. Phylogenetic analysis of the HA gene showed a close relation to viruses which had been circulating in humans between 1995 and 1996 (Osterhaus et al. 2000). This finding was surprising, as the HA protein genome of influenza B viruses displays a high mutation rate, and therefore, a substantial antigenic change in the human population over time. It was later concluded that the virus might have been introduced and maintained in the seal population without further evolution until 1999. Retrospective analyses of 580 sera samples from seals, collected before 1995, and of 391 sera samples, collected between 1995 and 1999, also supported this theory. Only 2% of sera samples collected after 1995 tested positive for antibodies to the influenza B virus, while none were detected among those collected before 1995. This

Table 2. Influenza Sero-Survey Characteristics of Marine Mammals and Results

Location	Year	Diagnostic test	Species	Number of samples	Positives	Reference
North Sea	1978–1988	NP-ELISA	Seal	757	3	de Boer et al. (1990)
			Seal & sea lion	338	43	
North Atlantic & Barents Sea	1992–1993	NP-ELISA	Harp seal	183	33	Stuen et al. (1994)
			Hooded seal	100	8	
Alaska	1998	AGID	Harbour seal	131	0	Danner et al. (1998)
			Walrus	54	0	
			Ringed seal	32	1	
			Steller sea lion	27	0	
			Ribbon seal	14	0	
			Spotted seal	9	0	
			Bearded seal	8	0	
Antarctica	1985–1986	EIA	Weddell seal	233	0	Austin and Webster (1993)
Arctic Canada	1984–1998	cELISA	Beluga whale	418	5	Nielsen et al. (2001)
			Ringed seal	903	23	
			Walrus	210	0	
			Narwhal	76	0	
			Bowhead whale	4	0	
Caspian Sea	1993–2000	indirect ELISA	Caspian seal	77	28	Ohishi et al. (2002)
Western North Pacific and Antarctic regions	2000–2003	Indirect ELISA	Common minke whale	179	7	Ohishi et al. (2006)
			Dall's porpoise	34	2	
			Bryde's whale	93	0	
			Antarctic minke Whale	104	0	
			Sperm whale	14	0	
			Beluga	4	0	
			Melon-headed whale	5	0	
			Pygmy sperm whale	2	0	
			Risso's dolphin	1	0	
			Short-finned pilot whale	1	0	
Alaska	1994–1996	AGID	Pacific walrus	38	8	Calle et al. (2002, 2008)
			Bearded seal	3	0	
Hokkaido, Japan	1998–2005		Kuril Harbour seal	322	15	Fujii et al. (2007)
Uruguay	2004	HI	Fur seal	37	10	Blanc et al. (2009)
Australia	2007–2009	cELISA	Australian fur seal	125	0	Lynch et al. (2011)
Netherlands	2002–2012	HI (influenza B)	Harbour seal	548	6	Bodewes et al. (2013)
			Grey seal	67	4	

suggests that the virus had been introduced in the seal population from a human source around 1995, although it never really spread extensively in the seal population (Osterhaus et al. 2000; Fouchier et al. 2001). Nonetheless, the seal might be a wild species able to carry influenza B viruses and facilitate their further evolution or transmission to humans.

A further sero-surveillance study implemented between 2002–2012 on harbour and grey seals living in the Dutch coastal waters detected positive serum samples only in 2010 (9/21 samples tested positive) (Bodewes et al. 2013).

Serological Testing of Marine Mammals for Orthomyxovirus Infections

Several serological studies have been carried out to estimate the prevalence of influenza infections among marine mammals. Different methods such as HI, indirect enzyme-linked immunosorbent assay (ELISA) and competitive ELISA were used. Each method had its intrinsic sensitivity and specificity, which in most cases never validated for marine mammal serum samples; however, the characteristics of sero-survey and results are included in Table 2.

In an extensive serological study to evaluate a NP-ELISA assay, 3 out of 757 seal serum samples from the North Sea and 43 out of 338 seal and sea lion samples from the Bering Sea tested positive (species were not specified). The samples were collected between 1978–1988 (de Boer et al. 1990).

The serological screening of 183 harp seals (*Pagophilus groenlandicus*) and of 100 hooded seals (*Cystophora cristata*) from the North Atlantic Ocean and the Barents Sea, carried out between 1991–1992 using an NP-ELISA revealed a prevalence of 18% (33) and 8% (8) influenza positive samples, respectively (Stuen et al. 1994). Although the observed variation had probably been due to an expected variation from sampling rather from a biological effect, the authors concluded that one main reason for the different prevalence found in these two seal species could be their social behaviour. Harp seals are known to stay in groups in water and land and form large groups at parturition and moulting. Thus, influenza aerosol transmission between these animals could be easier compared to what happens with hooded seals, a solitary species which does not aggregate in large groups and which reaches no more than two or three animals during pupping and moulting (Stuen et al. 1994).

Danner et al. tested the serum samples of 272 marine mammals in Alaska, including 131 harbour seals (*Phoca vitulina)*, 54 walruses (*Odobenus rosmarus*), 32 ringed seals (*Pusa hispida*), 27 Steller sea lions (*Eumetopias jubatus*), 14 ribbon seals (*Histriophoca fasciata*), 9 spotted seals (*Phoca largha*) and 8 bearded seals (*Erignathus barbatus*). They used an agar gel immunodiffusion (AGID) assay detecting AIV NP, and found only one positive ringed seal among them (Danner et al. 1998). Since no comparative assays were performed to validate the method, it is not clear whether the low detection rate was caused by the low sensitivity of the method, which is known to predominantly detect IgM antibodies, or if it reflected the low prevalence of influenza infection.

Analysis of 233 serum samples collected from Weddell seals (*Leptonychotes weddelliifrom*) in 1985–1986 from Antarctica revealed negative results using an indirect enzyme immunoassay (EIA). Plates were coated with antigen prepared from disrupted purified A/duck/Bavaria/2/77 (H1N1) influenza virus (Austin & Webster, 1993).

Another sero-survey was conducted in 24 locations throughout Arctic Canada, using 1611 serum samples collected from five species of marine mammals from 1984 to 1998 (Nielsen et al. 2001). Five out of 418 (1.2%) beluga whales (*Delphinapterus leucas*) and 23 out of 903 (2.5%) ringed seals (*Pusa hispida*) were serologically positive. None of the 210 walruses (*Odobenus rosmarus rosmarus*), 76 narwhals (*Monodon monoceros*) and 4 bowhead whales (*Balaena mysticetus*) had detectable anti-influenza A antibodies. A competitive ELISA using monoclonal antibody against influenza A NP was used in this study. (Nielsen et al. 2001).

In the Caspian Sea, 28 out of 77 serum samples collected from Caspian seals (*Pusa caspica*) from 1993 to 2000 tested positive by an indirect ELISA assay, using peroxidise-conjugated Protein A for antibody detection (Ohishi et al. 2002; Ohishi 2002). A further study, which used an HI assay, found antibodies against the H3 influenza A virus subtype in these samples, and also in those collected from the ringed seals (*Pusa hispida*) of Arctic Russia and from the Baikal seals (Pusa sibirica) of Lake Baikal (Ohishi et al. 2004).

In a recent study, 7 out of 179 common minke whales (*Balaenoptera acutorostrata*) and 2 out of 34 Dall's porpoise (*Phocoenoides dalli*) were found serologically positive using an in-house indirect ELISA assay, while none of 93 Bryde's whales (*Balaenoptera edeni*), 104 Antarctic minke whales

(*Balaenoptera bonaerensis*), 14 sperm whales (*Physeter macrocephalus*), 4 belugas (*Delphinapterus leucas*), 5 melonheaded whales (*Peponocephala electra*), 2 pygmy sperm whales (*Kogia breviceps*), one Risso's dolphin (*Grampus griseus*), and one short-finned pilot whale (*Globicephala macrorhynchus*) were positive using ELISA and Western blot analysis. The serum samples were collected from the western North Pacific and Antarctic regions (Ohishi et al. 2006).

In a general viral and bacterial serological screening study, serum or heparinised plasma samples were obtained between 1994 and 1996 from Pacific walruses (*Odobenus rosmarus divergens*) and bearded seals (*Erignathus barbatus*) from St. Lawrence Island and Round Island, Alaska. Eight out of 38 walrus serum samples reacted positive using AGID assay. The positive samples had also antibodies specific for hemagglutinin subtype H10 using HI assay (Calle et al. 2002). However, none of the 3 seal serum samples showed positive results using AGID assay (Calle et al. 2008).

In another study, serum samples from 322 Kuril Harbour seals (*Phoca vitulina stejnegeri*) were collected between 1998 and 2005 in 3 different areas of Hokkaido, Japan. Although the distance between the two islands is around 170 km, positive seals (3–12% of samples) were found every year in one area only. The authors suggested that this may have been due to the territorial behaviour of each population, since mixing and contact was not common between them (Fujii et al. 2007).

Serum samples were collected from 37 fur seal (*Arctocephalus australis*) in September 2004 in Uruguay and were tested against influenza A and B viruses. Using an HI test, 10 and 17 serum samples had titres $\geq$80 against a human H1N1 strain and against influenza B viruses, respectively (Blanc et al. 2009). A highly sensitive cELISA revealed negative results for 125 serum samples collected from adult Australian fur seals (*Arctocephalus pusillus doriferus*) between 2007 and 2009 in Australia (Lynch et al. 2011).

Transmission of Influenza Viruses Among Seals, Humans and Primates

During the 1979–1980 influenza H7N7 outbreak in harbour seals in the northeast of the USA, purulent conjunctivitis was observed in four people who had been involved in autopsies of dead seals, but no virus isolation was attempted in that period. However, during subsequent experimental infections of harbour seals using the identical virus, an infected animal sneezed on an investigator's face, who then developed severe conjunctivitis. Two days post infection, the influenza A (H7N7) virus was isolated from the swab sample of the conjunctival membrane (Webster et al. 1981b). The authors reported the virus as being antigenically very close to A/FPV/Dutch/27 (H7N7), which had been responsible for a case of human influenza virus infection following an accidental laboratory exposure in 1977. This virus had caused keratoconjunctivitis and the individual case of human infection had been confirmed by virus isolation (Taylor and Turner, 1977).

The seal influenza H7N7 virus proved to have the potential to cause conjunctivitis in humans, but did not spread further. Affected people recovered without complications, and antibodies to the virus were not detectable in the serum of infected individuals (Webster et al. 1981b). In an animal experiment, the seal influenza virus had replicated in the lungs and nasopharynx of squirrel monkeys after intratracheal administration, and induced symptoms almost similar to those of a human influenza A virus infection. The H7N7 virus was recovered from the spleen, liver, muscles, and lungs of a monkey died of pneumonia, thus, indicating its capability for systemic spread in primates (Murphy et al. 1983).

Isolation of H1N1 influenza viruses from healthy seals in 2010 provided evidence for cross transmission of influenza viruses from humans to marine mammals. Replication of isolated virus (A/Elephant seal/California/1/2010) in canine kidney cells was similar to reference strains of pdmH1N1, but replication in human epithelial respiratory cells was not as efficient as reference strains, indicating that these isolates may be elephant seal adapted viruses (Goldstein et al. 2013).

Cellular Receptor Specificity of Marine Mammal Influenza Viruses

The presence of influenza virus-specific cellular receptors in the intestinal and respiratory tracts of animals determines their susceptibility to the influenza infection; as well as potential host range. The receptor specificity of influenza A and B viruses from marine mammals, wild birds and humans has been evaluated in two studies (Ito et al. 1999; Ramis et al. 2012). Ito et al. (1999) demonstrated that two influenza A viruses, A/seal/Mass/1/80 (H7N7) and A/whale/ME/328/84 (H13N9) agglutinated horse and pig

erythrocytes, which abundantly harbour sialic acid α2,3-galactose (SAα2,3 Gal) on their surface, while they could not bind to human transferrin, containing only SAα2,6 Gal. These findings showed that both seal and whale viruses seemed to recognise only bird specific receptors, thus suggesting the likely avian origin of those viruses (Ito et al. 1999).

The wild type seal H7N7 virus was shown to replicate poorly in Madin–Darby Canine Kidney (MDCK), as well as in chicken embryo cells (CEC) in the absence of trypsin. Nevertheless, following several passages of the virus in these cell cultures and subsequent genomic changes, it was adapted to grow in the absence of exogenous proteases. The changes were determined by the cell type used for adaptation (Li et al. 1990). CEC-adapted virus variants developed multiple mutations in the cleavage site, i.e. insertion of multiple monobasic amino acids in this site. Thus, the virus gained the potential of cleavability by ubiquitous proteases after passages in chicken originated cells. Not only were the variant viruses highly pathogenic for chickens, but also for mammalian species such as mice, ferrets and rats (Scheiblauer et al. 1995).

In a recent study, in vitro binding of several avian and seal influenza A viruses to the respiratory tract of four marine mammals species was evaluated. One human influenza B virus was also included in this study. Based on virus strain and host species, extensive diversity in the pattern of attachment of these viruses was observed (Ramis et al. 2012). In this study, a moderate attachment of several avian influenza A viruses to the trachea and bronchi of harbour seals was reported, while no attachment to the respiratory organs of harbour porpoises (*Phocoena phocoena*) and bottlenose dolphins (*Tursiops truncatus*) was observed. Although no infections or outbreaks of AIVs in grey seals (*Halichoerus grypus*) have been reported to date, a moderate viral binding to the trachea and bronchi of grey seals was observed (Ramis et al. 2012). Therefore, it could be concluded that the in vitro study of the virus attachment to the cell membranes is not sufficient to explain the natural infection; furthermore, grey seals should be more extensively monitored for influenza infections.

The influenza B virus showed similar in vitro binding patterns to the respiratory tract of the four marine mammal species: moderate attachment to the respiratory tract of seals and no attachment to the trachea and bronchi of cetaceans. It was concluded that this lack of attachment is consistent with the absence of reported influenza B virus infection cases in cetaceans species (Ramis et al. 2012). No reports exist on influenza A or B virus infections in dolphin species, despite their close contact to wild birds and human societies.

Conclusions

Despite the fact that several viral and bacterial zoonotic pathogens can either infect marine mammals, cause clinical disease or transform them to recovered carriers, comprehensive knowledge on the presence of potential zoonotic pathogens in marine mammals meat and products is not available, and the risk of human infection due to the handling of their carcases is not well understood (Hunt et al. 2008; Tryland et al. 2011).

Infection of seals and other marine mammals with influenza A and B viruses have been reported on several occasions. Many constraints and high costs of monitoring marine mammals have made it difficult to determine the frequency of influenza infections in these species, and even to evaluate many cases of individual or mass mortalities. Nevertheless, data from several outbreaks and also serological evidence show that this is not a rare event, and probably the real prevalence has been underestimated. The current knowledge about influenza infections of marine mammals, including antigenic and phylogenetic analyses of isolated viruses, suggests that direct transmission from wild birds is the most probable route of introduction of influenza A viruses into the marine mammals population (Hinshaw et al. 1984, 1986; Mandler et al. 1990; Callan et al. 1995). On the other hand, a direct transmission of influenza A virus of the H7N7 subtype from seals to man, which caused conjunctivitis among the people handling diseased and dead seals, was recorded (Webster et al. 1981b). In addition, systemic infection and death of a monkey during an experimental infection with this seal virus has also been shown (Murphy et al. 1983). On the other hand, the infection of Northern elephant seals with the pandemic H1N1 (pdm H1N1) influenza virus has recently shown the potential for transmission of mammalian influenza viruses to marine mammals (Goldstein et al. 2013).

Interspecies transmission is an important factor in the evolution and ecology of influenza viruses; transmission from avian to mammalian species, such as marine mammals or pigs, may play an important role in the evolution of new mammalian viral strains (Reperant et al. 2012). After interspecies transmission of influenza viruses and successful

spread of the virus in the new host, virus adaptation or other unknown processes (such as co-infection with other pathogens or predisposing environmental conditions), may increase the pathogenicity of the virus both for marine and terrestrial mammals. For example, the pathogenicity of seal H7N7 for mammalian species such as mice, ferrets and rats increased after several passages of the virus in MDCK and chicken embryo cells (Li et al. 1990; Scheiblauer et al. 1995). Serological and virological data have shown that influenza B viruses could have persisted in the seal population for a long time without significant changes, a fact which may be considered as a risk for the re-introduction of the viruses to the human populations. However, at present no evidence is convincing enough to make us conclude that seals play a role as reservoir species for human influenza B viruses. More detailed and validated monitoring as well as experimental studies using negative and positive seal serums and also naïve seals are necessary to clarify the ecological and zoonotic importance of influenza viruses circulating in marine mammals.

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