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# Uruguayan Pinnipeds (*Arctocephalus australis* and *Otaria flavescens*): Evidence of Influenza Virus and *Mycobacterium pinnipedii* Infections

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Additional information is available at the end of the chapter

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## 1. Introduction

### 1.1. Location and general characteristics of the pinniped population

#### 1.1.1. Otariid and phocid species of Uruguay

Uruguay has 450 km of shorelines along the La Plata River and 220 km along the Atlantic Ocean (MTOP-PNUD-UNESCO, 1980). Two species of Otariids breed and reproduce on Uruguayan Atlantic islands: the South American fur seal, *Arctocephalus australis* (Zimmermann, 1783) (Fig. 1), and the South American sea lion, *Otaria flavescens* (Shaw, 1800), (Fig. 2), (Ponce de León, 2000; Ponce de León & Pin 2006; Vaz-Ferreira, 1976, 1982). Both are polygynous, gregarious and show strong sexual dimorphism (Bartholomew, 1970). South American fur seal adult males reach lengths of 1.9 m and weigh from 120 kg to 200 kg, while females can reach 1.4 m long and weigh from 40 kg to 55 kg, and newborns can be from 0.4 m to 0.5 m long and weigh from 3.5 kg to 5.5 kg (Vaz-Ferreira, 1982). Sea lion males may reach 2.8 m and weigh up to 354 kg while adult females are much smaller, reaching up to 1.9 m long and weighing as much as 150.0 kg (Ponce de León, 2000). Newborns in this species are between 0.7 m and 0.9 m long and weigh from 10.0 kg to 17.0 kg (Cappozzo et al., 1994). A third pinniped species, the southern elephant seal *Mirounga leonina* (Fig. 3), is a frequent visitor of Uruguayan islands and shorelines, although its reproductive areas are located in Argentina. Elephant seals can reach up to 5 m, 3 m or 1.3 m in length for males, females and pups respectively, and they can weigh as much as 5,000 kg, 800 kg from 40 kg to 50 kg (Reeves et al., 1992).



**Figure 1.** Group of South American fur seal *Arctocephalus australis* males, females and pups on Lobos Island. Photograph: A. Ponce de León.



**Figure 2.** South American sea lion *Otaria flavescens* reproductive groups with pups on Marco Island with pups. Photograph: A. Ponce de León.





**Figure 3.** Young male southern elephant seal *Mirounga leonina* on Coronilla's Islet. Photograph: A. Ponce de León.

## 1.2. Brief exploitation history

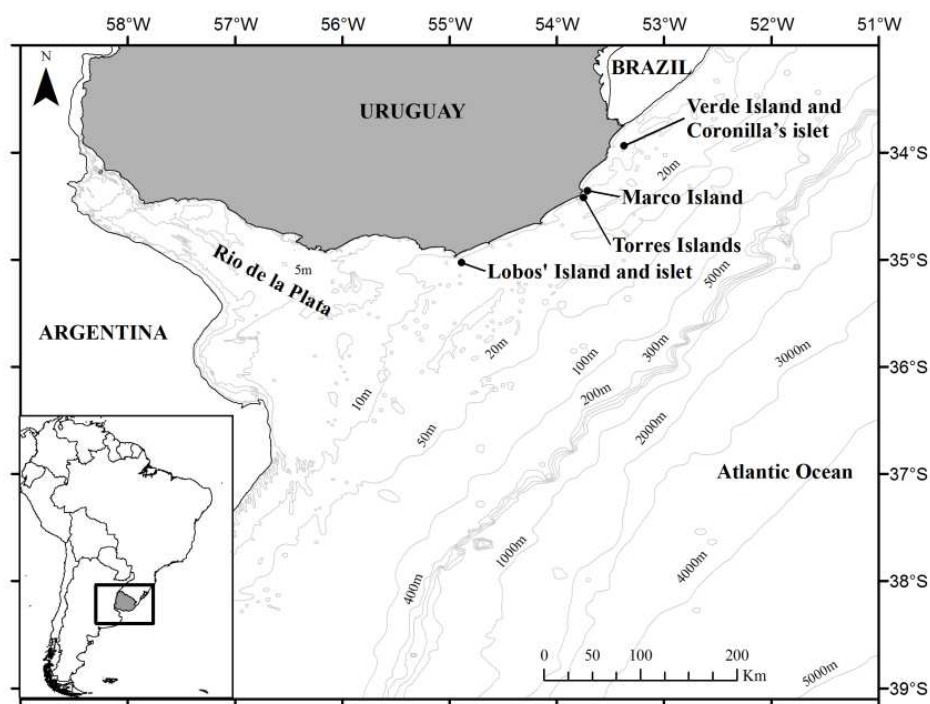
The exploitation of fur seals by Europeans in Uruguay is known to have begun in 1516, soon after the Spaniards explored the South Western Atlantic Ocean. During this exploration, Juan Díaz de Solís discovered the La Plata River and his crew landed on Isla de Lobos, where they killed 66 seals for their meat to be salted and consumed on their way back to Europe. The first semi-organized commercial exploitation took place in 1724, and the seal oil obtained was used for illuminating the city of Maldonado. From 1792 the Real Compañía Marítima, under direct instructions of the King of Spain, was responsible for sealing, until England invaded the territory in 1808. Shortly after, seal harvesting was carried out by private concessionaries and controlled by the local Government. From 1873 to 1900 a total of 440,000 seals were slaughtered (annual average of 16,000 pelts), whereas no records are available from 1901 to 1909. Further on, from 1910 to 1942, 72,000 South American fur seals were killed, as well as 17,000 more between 1943 and 1947. Due to the uncontrolled exploitation, populations of both seal species began to decrease. After 1950 a new management scheme started on Isla de Lobos, based on the system used for Northern fur seals (*Callorhinus ursinus*) in Pribilof Islands (Alaska), and the harvest was restricted to males. Also, private sector concessions were suspended, and the Government directly organized the harvesting program and related activities. Between 1959 and 1991 a total of 276,000 South American fur seals were removed (about 8,400 animals per year) and from 1967 to 1978, 36,400 sea lions were also slaughtered (3,000 animals per year). Products taken were crude skins, oil, meat and male genitals. Pelts were tanned and prepared in specific areas in Uruguay. Carcasses and fat were processed to obtain special oil for making soap, cosmetics and paints. In the XIX century, seal oil was used for illuminating the main streets of some cities. The meat was sometimes dried and given to the Montevideo Zoo for feeding big cats, eagles and condors. Since 1980, genitals were processed and sold for preparing

medicines and aphrodisiacs (Acosta y Lara, 1884; DINARA, 2006; Pérez Fontana, 1943; Ponce de León, 2000; Vaz-Ferreira 1982; Vaz-Ferreira & Ponce de León, 1984, 1987).

Harvesting and slaughtering of Uruguayan seals stopped in 1978 for South American sea lions and in 1991 for South American fur seals. From 1992 to the present day, the conservation and preservation of pinnipeds and cetacean species are under control of the National Direction of Aquatic Resources (DINARA: Dirección Nacional de Recursos Acuáticos).

### 1.3. Local geographical distribution

Uruguayan South American fur seal and sea lion colonies are located on three main islands in the Atlantic Ocean: 1) Isla de Lobos and Lobos Islet, 9,260 m off Punta del Este (Department of Maldonado); 2) Torres Group Islands (Rasa Island, Encantada Island and Islet) close to Polonio's Cape (Department of Rocha) and 3) Marco Island close to Valizas (Department of Rocha). There are two more small islets close to La Coronilla (Department of Rocha), where small groups of sea lions aggregate in reproductive areas (Fig. 4). Sometimes, a few South American fur seals also appear on these islands (Ponce de León, 2000; Ponce de León & Pin, 2006; Smith, 1934; Vaz Ferreira, 1950, 1952, 1956; Vaz Ferreira & Ponce de León, 1984, 1987).



**Figure 4.** Location of Uruguayan South American fur seal and sea lion calving, breeding and mating islands close to the shorelines of the Departments of Maldonado and Rocha : Isla de Lobos ( $35^{\circ} 01' 38''$  S –  $54^{\circ} 52' 55''$  W) and Lobos Islet; Rasa Island ( $34^{\circ} 24' 12''$  S –  $53^{\circ} 46' 10''$  W), Encantada Island ( $34^{\circ} 24' 26''$  S –  $53^{\circ} 45' 56''$  W), Torres Islet ( $34^{\circ} 24' 09''$  S –  $53^{\circ} 44' 59''$  W); Marco Island ( $34^{\circ} 20' 59''$  S –  $53^{\circ} 44' 26''$  W); 3) Verde Island and Coronilla's Islet ( $33^{\circ} 56' 21''$  S –  $53^{\circ} 29' 15''$  W). Isobath data layer obtained from FREPLATA-Proyecto de Protección Ambiental del Río de la Plata y su Frente Marítimo ([www.freplata.org](http://www.freplata.org)).

#### 1.4. Biology, population state and trends

Parturition and mating occur between November and January for South American fur seals and during January and February for South American sea lions (Franco-Trecu, 2005; Ponce de León, 2000, 2001; Ponce de León & Pin, 2006; Trimble, 2008). Gestation lasts around one year. In South American fur seals, lactation extends for several months and weaning begins between the 8<sup>th</sup> and the 12<sup>th</sup> month of age (Ponce de León, 1983, 1984, 2000; Ponce de León & Pin, 2006). From the 6<sup>th</sup> month of age, pups start eating fish and small mollusks as can be seen when analyzing stomach contents and gastrointestinal parasites of indirect cycle (Katz et al., 2012; Morgades et al., 2006). In some cases, South American fur seal lactation can be extended further, and the mother has to feed two pups from two consecutive breeding seasons: the yearling pup and the new one (Vaz-Ferreira & Ponce de León, 1987). In sea lions, there is a mother-pup relation for one year and in some cases for two (Vaz-Ferreira & Achaval, 1979) or possibly, up to three years (Soto, 1999). Little is known about the exact time of weaning, and whether pups are mixing milk with solid prey. It was suggested that weaning occurs when the mother actively rejects the older pup because a new one is born (Vaz-Ferreira, 1981; Vaz-Ferreira & Achaval, 1979).

South American fur seals have a lek reproductive system (Franco-Trecu, 2005). During the reproductive season, males fight each other to defend territories in very violent battles that can result in serious wounds and scars (Ponce de León, 2000; Ponce de León & Pin, 2006; Vaz-Ferreira, 1976, 1982; Vaz-Ferreira & Ponce de León, 1984, 1985, 1987). Females have no strong bonds with the areas defended by males. Fur seal colonies on islands are occupied by individuals from different age classes during the entire year. During the reproductive season there is a high density of animals in rocky areas as compared to sandy surfaces. As a consequence of the high environmental temperature, territorial males may abandon the reproductive areas in order to refresh themselves in the water (Vaz-Ferreira & Palerm, 1962; Vaz-Ferreira & Sierra de Soriano, 1962). After giving birth, South American fur seal females may remain with their pups for 6 to 11 days (Franco-Trecu, 2010) before starting short foraging trips that gradually become longer as the pups grow bigger and more independent (Ponce de León & Pin, 2006; Franco-Trecu, 2010).

The Uruguayan South American fur seal population is the biggest in South America (Vaz-Ferreira, 1982), with an annual growth rate of 3.3% (Páez, 2006) and an actual size estimated at 400,000 individuals. However, this species is included by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) in the list of globally protected species because of population decline of other South American colonies (de Oliveira et al., 2006). For the International Union for Conservation of Nature (IUCN) the same species is listed as "Of low concern".

South American sea lions have a polygynous breeding system (Ponce de Leon & Pin, 2006; Trimble, 2008; Vaz-Ferreira, 1981; Vaz-Ferreira & Sierra de Soriano, 1962). The reproductive season extends from mid December to mid February. During this time males fight each other to establish territories and to defend females from other males (Campagna & Le Boeuf, 1988; Ponce de Leon & Pin, 2006). Pregnancy has an estimated duration of 363 days (Franco-



Trecu & Trimble, unpublished data). Territorial males display violent fights that may last at least one hour, and end with serious wounds. After parturition, sea lion females remain with their pups in order to suckle them for approximately one week and then start short foraging trips of three days, alternated with two-day suckling periods on land (Campagna & Le Boeuf, 1988). There is a decrease in the number of adult males and females at the end of the reproductive season because, after fasting during the breeding season, males begin their foraging period at sea. In addition, adult females alternate foraging trips at sea and suckling periods ashore, and move to other areas of the island (Franco-Trecu & Trimble, unpublished data; Ponce de León & Pin, 2006). Despite the fact that sea lions abandon the islands in order to move to feeding areas, there are generally some animals in the rookeries, even outside the reproductive season. In many other South American colonies this species maintains an increasing population growth (Grandi et al., 2008; Sepúlveda et al., 2006) and has been classified as low risk by the IUCN. However, sea lions in Uruguay are considered a highly endangered species due to their population decrease of 1.7 to 2% annually, with a total population estimated at only 12,000 individuals (Páez, 2006; Pedraza et al., 2009).

On Lobos Island and Lobos Islet, South American sea lion groups are found in small patches, surrounded by large groups of South American fur seals. However, on the Torres Islands (Rasa Island, Encantada Island and Islet), Marco Island and other islets, groups of sea lions are more numerous than on the bigger Lobos Island. According to Pedraza et al., (2009) and Ponce de León (unpublished data), sea lion populations are stable or increasing (2.4% annually) in Polonio's Cape islands, while in Lobos Island their growth have a negative tendency. This is related to a positive trend in the *A. australis* population and may be an indicator of competition for territory (breeding areas), a process that occurs only on Lobos Island and Lobos Islet.

The breeding and reproductive areas of the elephant seal *Mirounga leonina* are located in sub Antarctic regions along the coast of South America (Campagna & Lewis, 1992; Lewis et al., 1998) and smaller colonies are formed in the Antarctic (Le Boeuf & Laws, 1994). The only large breeding colony of southern elephant seals on the South American continent is found in Península Valdés (42°04'S, 63°45'W) (Campagna & Lewis, 1992; Le Boeuf & Laws 1994; Lewis et al., 2004). Young, juvenile and adult animals migrate to northern regions (Lewis et al., 2006) and occur at different points all along the Uruguayan shoreline of the La Plata River and Atlantic Ocean beaches. During practically the whole year, elephant seals of both sexes are frequently seen in the flat areas of access to Lobos Island, Lobos Islet, Rasa Island and Coronilla Islet (Fig. 3 and 4). Mother and pup couples have been seen on Lobos Island in October, and in some odd cases, individuals have swum up the waters of the Uruguay River to the Departments of Rio Negro and Paysandú (Ponce de León & Pin, 2000).

### 1.5. Feeding, diet and diving behaviour

South American fur seals have mainly pelagic feeding habits (Naya et al., 2002; Ponce de León & Pin, 2000, 2006; Vaz-Ferreira, 1976) but also feed in shallower waters (Franco-Trecu, 2010). Their diet is basically composed of anchovies (*Engraulis anchoita*, *Anchoa mitchilli*),

squid (*Illex argentinus*, *Loligo sanpaulensis*), hake (*Merluccius hubbsi*), striped weakfish (*Cynoscion guatucupa*), oceanic shrimp (*Pleoticus muelleri*) and cutlassfish (*Trichiurus lepturus*) (Frau & Franco-Trecu, 2010; Naya et al., 2002; Pin et al., 1996; Ponce de León et al., 1988; Ponce de León et al., 2000; Ponce de León & Pin, 2006; Vaz Ferreira 1976; Vaz-Ferreira & Ponce de León, 1984, 1987). Uruguayan fur seals usually do not interfere directly with artisanal and industrial fisheries, as they do not eat from nets nor destroy fishing gears (Ponce de León & Pin, 2006), though there are a few records of fur seal by-catch in artisanal (Franco-Trecu et al., 2009) and industrial fisheries (Szephegyi et al., 2010).

Diving records obtained by different researchers showed that during lactation, female fur seals perform dives of up to 186 m (media: 23.5 m  $\pm$  19.5 m) in depth with an average duration of 1.2 min  $\pm$  0.8 min (max. 5.3 min.) (Riet et al., 2010; York et al., 1998). These data suggest that females use both benthic and pelagic foraging strategies, and demonstrate their huge endurance for deep dives, apnea resistance and swimming ability. Diurnal dives were shallower and shorter than nocturnal ones (Riet et al., 2010). It was determined that lactating females consume different prey species, adapting their diving strategies to variations in food resources (Ponce de León & Páez, 1996; Ponce de León & Pin, 2006; Riet et al., 2010; York et al., 1998).

During early lactation, female sea lions perform dives of 21 m  $\pm$  8 m in depth with an average time of 1.9 min  $\pm$  0.7 min. Mean distance traveled per trip was 62.2 km  $\pm$  63.0 km. Foraging trips lasted 1.3  $\pm$  0.8 days and did not exceed the continental shelf (>50 m of depth). Maximum distance from the colony was 98.60 km  $\pm$  31.3 km. These results indicate that during the breeding season females forage in coastal and shallow continental shelf areas (Riet et al., 2009, 2012). In autumn, foraging trips last 5 days (range: 1-14 days). Most animals seemed to complete round trips along the same tracks, meaning that each animal uses the same path on successive trips, with low overlap between individuals. Site fidelity to Lobos Island was highly remarkable for all animals, independently of their reproductive condition (Rodríguez et al., 2012).

Sea lions compete directly with small-scale coastal fishing and artisanal fisheries, feeding on species that are part of the fishermen's daily catch by stealing prey trapped in nets and longlines, and sometimes causing important damage or cracks in the gear (Franco-Trecu et al., 2012; Lezama & Szteren, 2003; Ponce de León & Pin, 2006; Szteren & Páez, 2002). According to different authors, the sea lions' diet is mainly made up of coastal prey and some pelagic fishes: whitemouth croaker (*Micropogonias furnieri*), striped weakfish (*Cynoscion guatucupa*), Brazilian codling (*Urophycis brasiliensis*), cutlassfish (*Trichiurus lepturus*), mackerel (*Trachurus lathami*), Argentinean conger (*Conger orbignyanus*), carangid (*Parona signata*), two species of anchovies (*Engraulis anchoita* and *Anchoa mitchilli*), and Argentinean croaker (*Umbrina canosai*) (Franco-Trecu, 2010; Naya et al., 2000; Pinedo & Barros, 1983; Ponce de León & Pin, 2006; Riet et al., 2011, 2012; Vaz Ferreira, 1981). As a consequence of interactions with sea lions, fishermen lost prey with high local commercial market value. Sometimes, fishermen find small shark specimens in their nets (*Mustelus schmitti*, *Galeorhinus galeus*, *Myliobatis* spp.) which have bite marks in their abdominal area



(Fig. 5) from sea lions that learned to exploit this energy reservoir (Ponce de León & Pin, 2006). Recent reports show that during the early lactation period, foraging home ranges of sea lion females overlapped with fishing effort areas of coastal bottom trawl fisheries (15%) and artisanal fisheries (>1%). For both fisheries the resource overlap per fisheries impact index identified the “hotspots” which are distributed along the coast, west of the breeding colony (56°W - 55W°) (Riet et al., 2011).



**Figure 5.** Artisan fishery capture of *Mylobatis* spp. The opened abdominal areas of the sharks are seen, from where sea lions have taken highly nutritive and energy rich livers and pancreas. Photograph: A. Ponce de León.

### 1.6. Future exploitation for tourism

South American fur seals and South American sea lions could represent an important tourism attraction. Since the seals are only a few meters away from visitors on Lobos Island and Polonio's Cape, seal watching in both of these popular natural areas could be exploited for tourism activities (Ponce de León & Pin, 2000, 2006; Ponce de León & Barreiro 2010). This type of exploitation should be regulated by serious and responsible Government rules in order to assure sustainable coastal management of environmental resources. New employment opportunities for local people in the Departments of Maldonado and Rocha would be created. This kind of offer would also contribute to public awareness-raising programs for conservation of these charismatic species and for conservation of aquatic ecosystems.

### 1.7. Management considerations

Nowadays, fur seals are an important nontraditional exportation item: between 60 and 80 living young fur seals are captured annually on Lobos Island and exported to aquaria and

theme parks all over the world. The principal objective of these parks is to educate people about environmental issues and about the conservation of aquatic and marine resources and ecosystems. Although live sea lions had been sold by the Uruguayan Government since 1980, the exportation of living specimens of this Otariid was suspended in 2006 by DINARA-MGAP due to decreasing population numbers. Captures of animals are held in Lobos Island from mid March to mid November. This special period was defined in order to avoid disturbances and to be respectful of parturition, breeding and mating periods of the two Otariid species present in the island (Ponce de León, 2000; Ponce de León & Pin, 2006).

As far as research is concerned, DINARA maintains the old seal factory plant on Lobos Island, using parts of the buildings for providing accommodation to researchers who are developing studies in both species. Through the development of various research projects, the Government institution DINARA, students and graduates of the University of Uruguay (Universidad de la República) as well as from foreign countries, are gathering and collecting data to increase knowledge about the population dynamics of both species, which will also help in developing appropriate rules and guidelines for their management, and ensuring the conservation of Uruguayan natural resources.

## 2. Evidence of influenza virus infections in *Arctocephalus australis* individuals

Marine mammals are susceptible to a variety of pathogens including influenza viruses. In humans, influenza causes annual epidemics and occasional pandemic diseases, with a significant threat to human health. In wild animals, several outbreaks have been reported and especially marine mammals experienced several devastating episodes that highlight the importance of monitoring wild populations to perform conservation programs and to evaluate possible risks to human health.

Influenza viruses belong to the *Orthomyxoviridae* family and are enveloped viruses with a segmented, negative-sense RNA genome (Webster et al., 1992). Embedded in the lipid envelope, the hemagglutinin (HA) and neuraminidase (NA) proteins are responsible for virus attachment and release from host cells, respectively (Webster et al., 1992). This family of viruses is composed of four genera: influenza A, B and C viruses and Thogoviruses (Wright & Webster, 2001). While influenza B and C viruses are primarily "human" viruses, influenza A viruses infect a variety of avian and mammalian species including humans, horses, swine and marine mammals such as seals and cetaceans (Wright & Webster, 2001; Webster et al., 1992). Influenza B virus was isolated from a harbor seal (*Phoca vitulina*) for the first time in the year 2000 (Osterhaus et al., 2000) becoming a possible second reservoir of this virus.

Influenza viruses are unique among respiratory tract viruses as they undergo considerable antigenic variation. Both surface antigens of the influenza A viruses are subject to two types of variation: drift and shift. Antigenic drift involves minor changes in the hemagglutinin (HA) and neuraminidase (NA) and plays a role in influenza epidemics, which occur sporadically. Antigenic shifts involve major changes in these molecules resulting from

replacement of the gene segment, producing new pandemic strains (Wright & Webster, 2001).

Phylogenetic evidence suggests that influenza epidemics in humans and other mammals, including seals, come from mutation and antigenic drift of viruses originating from aquatic birds (Webster et al., 1992). Several influenza events which have affected marine mammals have been described since the late seventies. The New England coast was the scene of an episode of influenza virus between December 1979 and November 1980. More than 400 harbor seals (*Phoca vitulina*) died of acute pneumonia associated with the influenza virus A/Seal/Massachusetts/1/80 (H7N7). This was the first evidence of an influenza virus antigenically and genetically related to avian viruses that could be associated with severe disease in wild animals (Geraci et al., 1982; Lang et al., 1981; Webster et al., 1981). This H7N7 strain was associated with an approximate 20% mortality of the seal population and also showed potential for causing conjunctivitis in humans. However, it was not transmitted among humans.

A new event was described along the New England coast from June 1982 through March 1983. This time the influenza virus isolation was an H4N5 subtype, which had previously been detected only in birds. It was recovered from harbor seals dying of viral pneumonia (Hinshaw et al., 1984). This strain, which caused an estimated mortality of 2 % to 4 %, was found to be genetically and serologically related to avian strains.

In January 1991 and January to February 1992, influenza A viruses were isolated from seals that died of pneumonia along the Cape Cod Peninsula in Massachusetts. Antigenic characterization identified two H4N6 and three H3N3 viruses. This was the first isolation of an H3 influenza virus from seals, although this subtype is frequently detected in birds, pigs, horses and humans (Callan et al., 1995). Genetic analysis indicated that the viruses were both of avian origin and that transmission from birds to seals was the most likely possibility.

Also, indirect evidence of influenza infection was reported from a variety of marine mammal species. In pinnipeds, antibodies against influenza A virus were detected in sera from harp seals (*Phoca groenlandica*) and hooded seals (*Cystophora cristata*) collected between 1991 and 1992 in the Barents Sea (Steuin et al., 1994), as well as from sea lions (Otariidae) and seals in the North and Bering seas (De Boer et al., 1990), and a ringed seal (*Pusa hispida*) in Alaska (Danner et al., 1998). A serological survey of influenza A antibodies from five species of marine mammals collected from Arctic Canada between 1984 and 1998, revealed that 2.5% of ringed seals (*Phoca hispida*) were serologically positive (Nielsen et al., 2001). A serological study of influenza virus infection in Caspian seals (*Phoca caspica*) detected antibodies to human-related (H3N2) virus in 36% of the seals (Ohishi et al., 2002). Two years later, another study suggested that human-related H3 viruses were prevalent in Baikal seals (*Phoca sibirica*) and ringed seals (*Pusa hispida*) inhabiting the central Russian Arctic (Ohishi et al., 2004). Serological evidence of influenza A virus infection was reported in Kuril harbor seals (*Phoca vitulina stejnegeri*) of Hokkaido, Japan, from samples collected between 1998 and 2005 (Fujii et al., 2007). In this study, antibodies to H3 and H6 subtypes of influenza A virus were detected. This was the first time that H6 antibodies were identified in seals (Fujii et al., 2007).



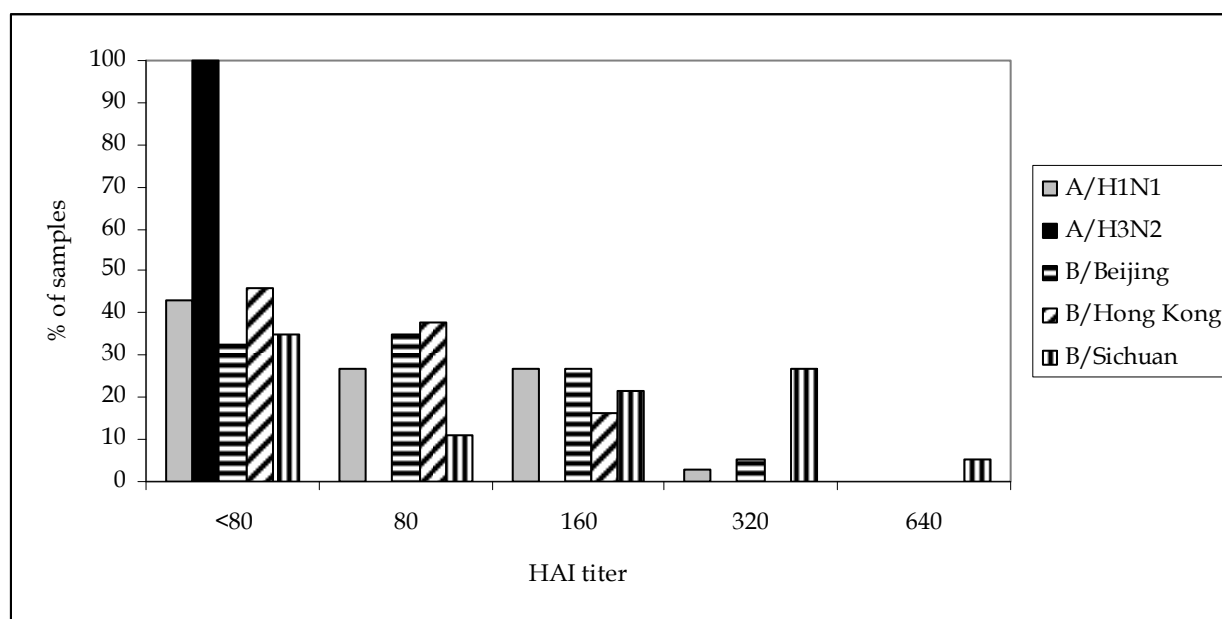
Indirect evidence of influenza A viruses has been reported in 27% of the South American fur seals sampled in Uruguay (Blanc et al., 2009). By Hemagglutination Inhibition Assay (HAI) it was found that all the positive samples reacted with A/New Caledonia/20/99(H1N1) antigen reaching HAI titer of 320 but none of the sampled serum reacted with A/Panamá/2007/99(H3N2) antigen. For the first time, the presence of influenza A in *A. australis* was confirmed (Blanc et al., 2009) (Fig. 6).

Influenza viruses have also been detected in whales. An H1N3 virus was isolated from a striped whale in the South Pacific (Lvov et al., 1978). In 1984 influenza A viruses of the H13N2 and H13N9 subtypes were isolated from a pilot whale (*Globicephala melas*) (Hinshaw et al., 1986). Serological, molecular, and biological analyses indicate that the whale isolates are closely related to the H13 influenza viruses from gulls (Hinshaw et al., 1986). In cetaceans, specific antibodies were observed in a low portion of sera from belugas (*Delphinapterus leucas*) in Arctic Canada (Nielsen et al., 2001).

Few studies have been reported regarding the detection of influenza B viruses in marine mammals. The first one reported the isolation of influenza B virus (B/seal/Netherlands/1/99) from a naturally infected harbor seal in the year 2000. Sequence analyses as well as serology indicated that this influenza B virus is closely related to strains that circulated in humans 4 to 5 years earlier. Retrospective analyses of sera collected from 971 seals showed a prevalence of antibodies of the influenza B virus in 2% of the animals after 1995, and in none before that year, suggesting that the virus was introduced in the seal population from a human source around 1995 (Osterhaus et al., 2000). Antibodies to influenza B viruses were detected by ELISA in 14% and 10% of serum samples collected from Caspian seals in 1997 and 2000, respectively (Ohishi et al., 2002).

Serologic evidence of influenza B virus has been reported from South American Uruguayan fur seals *A. australis* (Blanc et al., 2009). Thirty of the 37 serum samples assayed by HAI reacted against one of the three antigens used: 25/37 (68%) reacted against B/Beijing/184/93-like viruses, 20/37 (54%) reacted against B/Hong Kong/330/01, and 24/37 (65%) reacted against B/Sichuan/379/99. The results show that 17 sera reacted against all B antigens, only six reacted against two antigens and eight sera did not react against any of them. The highest titer reached was (640) against B/sichuan antigen. The results demonstrated influenza B virus circulation in South American fur seals for the first time in our country and in this species. The antigens assayed correspond to strains that circulated in humans between the years 1999 and 2001, 3 to 5 years after the study was carried out, confirming the hypothesis of other authors that marine mammals could be a reservoir of influenza strains that circulated in the past (Fig. 6).

It is important to consider that marine mammals share their habitat with several different wild shorebirds as well as with aquatic birds, the main influenza A virus hosts. The presence of bird feces in water, which can shed high concentrations of Avian Influenza viruses, and the close contact during feeding activities between birds and seals, increase the probability of fecal-oral transmission.



**Figure 6.** Antibodies to Influenza A and B virus by HAI in fur seal sera. Percent (number positive/number tested) of samples bearing antibodies vs. HAI titer for each Influenza antigen assayed. Titers  $\geq 80$  were considered positive. Antigens used: A/New Caledonia/20/99(H1N1), A/Panamá/2007/99(H3N2), B/Beijing/184/93-like viruses, B/Yamanashi/166/98, B/Hong Kong/330/01, and B/Sichuan/379/99.

Monitoring the distribution of the influenza virus in wild animal species including marine mammals is important for understanding the ecology and evolution of the virus, and also to understand how the virus can mutate and re-emerge more virulent, producing devastating epidemic diseases.

### 3. *Mycobacterium pinnipedii* infections in *Arctocephalus australis*, *Otaria flavescens* and *Mirounga leonina* individuals

Bacteria belonging to the Genera *Mycobacterium* are acid-fast bacilli (AFB) classified in different complexes and species according to biochemical, cultural and genetic features. The first communication of tuberculosis (TB) in captive seals dates from the early twentieth century (Blair, 1913). However, the diagnosis and study of tuberculosis and mycobacteriosis in different species of marine mammals is fairly recent. Ehlers (1965) reported a tuberculosis case in a Northern seal (*Cystophora cristata*). Subsequently, Kinne (1985) described tuberculosis cases in several marine mammal species. In 1986, the first isolates of *Mycobacterium* spp. were obtained in Australian fur seals and sea lions (*Arctocephalus pusillus doriferus* and *Neophoca cinerea* respectively) and New Zealand fur seals (*Arctocephalus forsteri*) in captive and wild conditions (Forshaw & Phelps, 1991; Woods et al., 1995). Successive isolations were made from wild pinniped species in the Southern Hemisphere (Bastida et al., 1999; Bernardelli et al., 1996; Cousins et al., 1993; Hunter et al., 1998; Romano et al., 1995; Woods et al., 1995; Zumárraga et al., 1999).

In Uruguay, the first isolation of *Mycobacterium* spp. in pinnipeds was conducted in 1987 from samples of South American sea lion *O. flavescens* specimens kept in "Villa Dolores" municipal zoo. Of the ten animals studied, one died and nine others were tuberculin-positive and were later euthanized. Seven animals showed typical histo-pathological lesions of tuberculosis, and a total of 6 strains were isolated. Initially, it was considered that the bacillus was *M. bovis* according to results from a smear, biochemical tests and culture features. The strains were inoculated to guinea pigs (0,1 mg) that developed characteristic lesions and subsequently *Mycobacterium* spp. were isolated, fulfilling Koch's postulates (Castro-Ramos et al., 1998). In 1997 *Mycobacterium* spp. was isolated from lung samples of an adult South American fur seal stranded on the coast of Montevideo. The animal was collected from the beach by a NGO and sent to quarantine in a zoo for recovery, but died four days after admission. The observed granulomatous lung lesions were typical of TB, and a *Mycobacterium* spp. strain was isolated (Castro-Ramos et al., 2001).

Between 2001 and 2006, pathological, microbiological and genetic studies were conducted on dead stranded animals of different species of pinnipeds found along ocean shores of Uruguay: South American fur seal (n = 129), South American sea lion (n = 24) and Southern elephant seal (n = 1). Necropsies were performed using standard methods (Dierauf, 1990). Samples from several organs with or without lesions (lung, mediastinal lymph nodes, spleen, liver) (n = 36) were stored at 4° C, frozen at -20° C or fixed in 10% formalin.

Formalin fixed samples were processed by standard histological methods: 4-5 cuts were made at 5-6 µm and stained with Hematoxylin-Eosin and Ziehl-Neelsen (ZN) (Luna, 1968). Mycobacteriological studies were performed according to the methodology described by the Pan American Zoonoses Center (Centro Panamericano de Zoonosis (OPS/OMS) (1979), Office International des Epizooties (OIE) (2000), Runyon et al., (1980) and Tacquet et al., (1967). Smears from single or pooled samples of each animal were performed and then cultured in Stonebrink and Lowenstein Jensen media. Cultures were kept for eight weeks at 37° C and periodically reviewed. Culture tests were based on microscopic features, morphology of the colony, growth temperature, time of development and chromogenicity of isolates. The identification was completed with the following biochemical tests: niacin, nitrate reduction, catalase at 22° C and 68° C, hydrolysis of Tween 80 at 5 and 10 days, reduction of potassium tellurite 0.2% at 3 days, urease and pyrazinamidase. A total of 14 strains were isolated (Table 1).

Strains isolated in 1987 (N° 01073, adult male *O. flavescens*), 1997 (N ° 01337, juvenile male *A. australis*) and 2002 (N ° 2493, juvenile female *O. flavescens*) were analyzed through amplification of 200 bp of the Internal Transcribed Spacer (ITS) region through Polymerase Chain Reaction (PCR) as in Roth et al., (2000). Sequences obtained were compared to those available at GenBank database through a maximum parsimony phylogenetic tree, and strains were grouped with sequences of *M. tuberculosis* / *M. pinnipedii*.

During the necropsies, granulomatous lesions were observed in only five animals: two South American sea lions (juvenile male and female) and three South American fur seals (two adult and one juvenile male) (Fig. 7), from which *M. tuberculosis* / *M. pinnipedii* complex



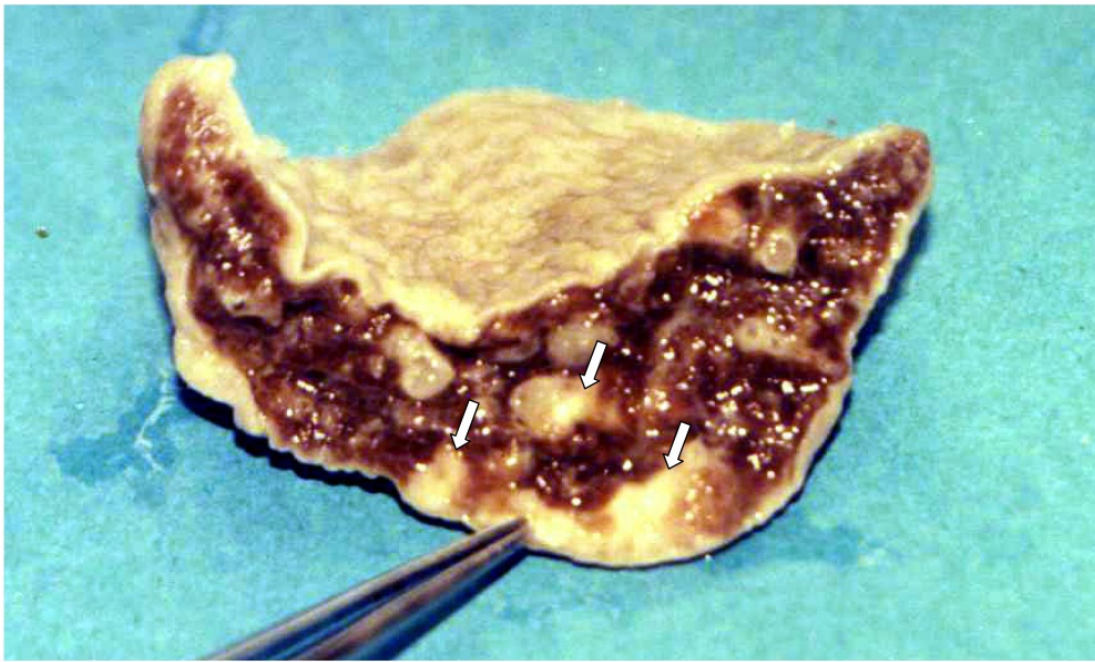
strains were isolated. Isolates were also obtained from organs without gross lesions belonging to pups and juvenile fur seals ( $n = 9$ ) and from a sub-adult male elephant seal (Castro-Ramos et al., 2005, 2006) (Fig. 9).

Code	Year of sampling	Species	Sex	Category	Origin	Bacilloscopy	Culture
9/2001	2001	Aa	♂	Adult	w	---	---
2493	2002	Of	♀	Juvenile	w	+	+
0874	2002	Aa	♂	Adult	w	+	+
0873	2003	Aa	♀	Pup	w	+	+
0875	2003	Aa	♀	Pup	w	+	+
1405	2003	Of	♂	Juvenile	w	+	+
1332/3	2004	Aa	♀	Pup	w	---	+
2172/1	2005	Aa	♀	Pup	w	-	+
2172/2	2005	Aa	♀	Pup	w	-	+
2172/3	2005	Aa	♀	Pup	w	-	+
2172/4	2005	Aa	♂	Pup	w	-	+
2172/6	2005	Aa	♂	Pup	w	-	+
2172/7	2005	Aa	♂	Pup	w	-	+
2173	2005	Of	♀	Pup	w	-	+
2174	2005	Ml	♂	Juvenile	w	-	+

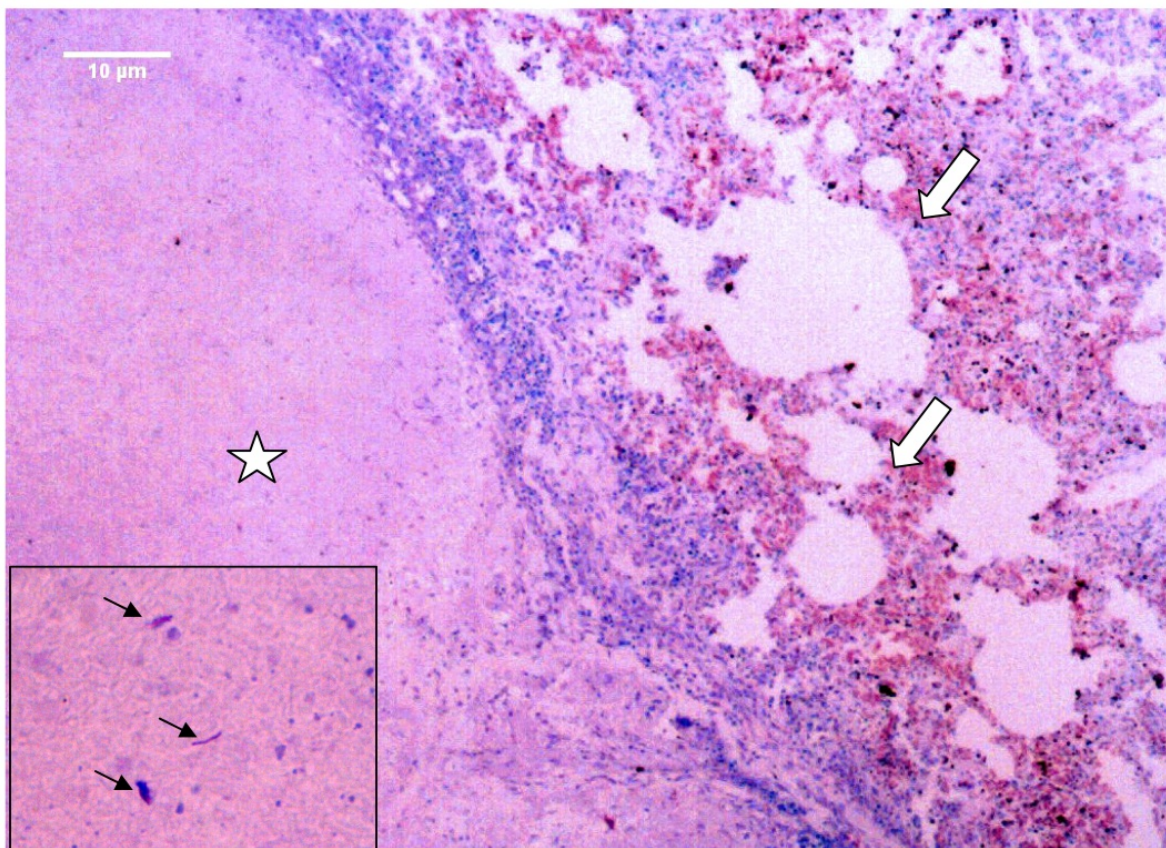
Aa= *Arctocephalus australis*; Of= *Otaria flavescens*; Ml= *Mirounga leonina*; c= captive, w= wild.

**Table 1.** Isolation of *Mycobacterium* spp / *M. pinnipedii* in *Arctocephalus australis*, *Otaria flavescens* and *Mirounga leonina* in Uruguay.

Macro and microscopic lesions were recorded. Hydrothorax and hemothorax were found in two adult animals. Papillary and proliferative lesions in parietal and visceral pleura (Fig. 7) were associated with a chronic inflammatory process, mononuclear and lymphocytic infiltration, and in some cases congestion and hemorrhage. Lungs presented yellowish-white nodules on surface and deep pulmonary parenchyma, which corresponded to granulomas with AFB. Histology showed mononuclear infiltration throughout the parenchyma as well as congestion, emphysema and atelectasia near nodules. Granulomas showed the typical structure with a necrotic center surrounded by a mild fibroblastic reaction and mononuclear infiltration with AFB in single arrangements or small groups (Fig. 8). In bronchi and bronchioles mononuclear exudates at the lumen and lymphocyte/macrophage aggregates below the cartilage were present. Necrotic foci were also recorded in mediastinal lymph nodes with AFB. In one juvenile sea lion a mediastinal abscess and hematoma were found between the great vessels near the heart, which was associated with a chronic inflammatory process.



**Figure 7.** Macroscopic view of an *Arctocephalus australis* lung. Numerous granulomas were present on the surface and deep parenchyma (white arrows) and a significant thickening of the visceral pleura.



**Figure 8.** Histological section from an *Arctocephalus australis* lung. A granuloma in the lung parenchyma (star) surrounded by areas of congestion and atelectasis (arrows) can be observed. Inset: AFB groups within the granuloma (immersion) (black arrows). (Ziehl-Neelsen. 400x)





**Figure 9.** *Mycobacterium* spp. / *M. pinnipedii* colonies in Stonebrink medium culture. Sample obtained from a *Mirounga leonina* lung.

These results indicate a wide distribution of the disease in Uruguayan fur seal and sea lion colonies associated with a high prevalence of the disease in zoos and aquaria from South American native pinnipeds populations (Jurzinsky et al., 2011; Lacave, 2009; Lacave et al., 2009; Moser et al., 2008). The region used for genetic analysis can only discriminate between two large complexes within the Genera *Mycobacterium*, *Mycobacterium tuberculosis* complex (MTC) and *M. avium* (MAC). Based on these results we can conclude that the sequences studied are part of the MTC and correspond to *M. pinnipedii* as determined by more precise genetic studies (Cousins et al., 2003, Jurczynski et al., 2012; Kriz et al., 2011; Moser et al., 2008) which include a strain isolated in Uruguay (Cousins et al., 2003). Discrimination between different members of the MTC is essential for epidemiological investigations of wild populations, as well as the diagnosis of human cases associated with an adequate chemotherapy (de la Rua-Domenech, 2006).

The isolation of bacilli in pups and juvenile animals without apparent lesions indicates early transmission of the organism even though the animals do not show symptoms of respiratory disease. Similar findings were reported for wild carnivores for which positive cultures for *M. bovis* were not accompanied by gross or histological lung lesions (Bruning-Fann et al., 2001; Little et al., 1982). Furthermore, cattle with *M. bovis* developed a non-progressive disease, with small lesions in lymph nodes (retropharyngeal and mediastinal), which in turn are positive reactors to diagnostic tests for delayed cutaneous hypersensitivity (Tuberculin



skin test). Most individuals of the population carrying the dormant bacilli may become ill with TB at some point in their life, if an immunodeficient situation develops. It is therefore necessary to consider all infected individuals as potentially diseased animals (Rider, 1999).

The fact that most gross and microscopic lesions are located in the respiratory system of pinnipeds (Bastida et al., 1999; Bernardelli et al., 1996; Castro-Ramos et al., 1998, 2001; Forshaw & Phelps, 1991; Katz et al., 2002; Moser et al., 2008; Woods et al., 1995) indicates that the main transmission is by aerosols. Less frequently, lesions were located in liver, spleen, kidney and abdominal lymph nodes (Bernardelli et al., 1996; Forshaw & Phelps 1991; Kiers et al., 2008; Kriz et al., 2011), cases in which the bacilli probably spread through blood flow or swallowing of sputum containing mycobacteria (Forshaw & Phelps, 1991; Kriz et al., 2011). In wildlife, it is expected that transmission takes place mainly through spray during coughing and sneezing which is frequently observed in pups and adults (H. Katz, pers. obs.) or the typical naso-nasal contact between pinnipeds.

*M. pinnipedii* have been isolated from fur seals, sea lions and elephant seals from the Southern Hemisphere (*Arctocephalus australis*, *A. forsteri*, *A. pussilus doriferus*, *A. tropicalis*, *Otaria flavescens*, *Neophoca cinerea*, *Mirounga leonina*, *Phocarcos hookeri*). They have been found both in wild animals and specimens kept in European aquaria or theme parks taken from South American colonies (Bernardelli et al., 1996; Castro-Ramos et al., 2005; Cousins et al., 2003; Duignan et al., 2003; Kiers et al., 2008; Kriz et al., 2011; Lacave, 2009). Micobacteriosis was diagnosed in only one Otariid species of the Northern Hemisphere (California sea lion, *Zalophus californianus*, Ehlers, 1965; Gutter et al., 1987). Most diagnoses were made in captive *Otaria flavescens*, probably because it is the most common species in aquaria as it is easily trained. Uruguay exported this species destined for aquaria from 1980 to 2006, when captures were restricted due to population decline (Páez, 2006). Nowadays, only live juvenile specimens of *A. australis* are caught for exportation to different destinations (Asia, Latin America and Europe). As this species is also carrier of *M. pinnipedii* (Cousins et al., 2003; Castro-Ramos et al., 2006; Katz et al., 2002) it is important to establish accurate diagnostic methods. The isolation of *M. pinnipedii* from different mammalian species (cattle, Bactrian camels, Malayan tapirs, Guinea pigs and humans), indistinguishable from strains isolated from pinnipeds, suggests that the bacillus has significant potential to infect a wide range of hosts, particularly when animals are in captivity (Cousins et al., 2003; Kiers et al., 2008; Moser et al., 2008).

Presently, the diagnostic methods in living specimens have certain inaccuracies or deficiencies that make it difficult to establish a universal technique or golden standard. This is particularly important given that individuals carrying the microorganism can take years to show signs of the disease; only in terminal cases have nonspecific symptoms including anorexia, dysphagia, lethargy and weight loss been described. Coughing has not been described as a sign accompanying respiratory infection although significant lung lesions were present (Bernardelli, 1996; Castro-Ramos et al., 1998, 2006; Cousins et al., 1993; Kiers et al., 2008; Kriz et al., 2011; Lacave, 2009). In necropsied seals, tuberculosis diagnosis had been made based on mycobacterial isolation, histopathology and genetic characterization of strains. Imaging methods (radiography, computer tomography) have been used in captive

animals, but in wild conditions these procedures are impractical. Chest radiographs were performed on pinnipeds of different sizes, but in cases of very small lesions in large animals with thick blubber, radiological images do not give appropriate information (Forshaw & Phelps, 1991; Jurczynski et al., 2012). In some zoos computer tomography has been used for detection of small calcified granulomas (Jurczynski et al., 2011, 2012), but it is very difficult to be used routinely. Different serological tests (rapid test, Elephant TB STAT-PAK, Chembio; multiantigen print immunoassay (MAPIA) Chembio; dual path platform assay (DPP Vet; Chembio) have been used in *O. flavescens* individuals in captivity, with DPP technique demonstrating greater sensitivity (87.5%) (Jurczynski et al., 2012). The tuberculin skin test (TST) with purified protein derivative (PPD bovine and avian) for screening has been done in *O. flavescens* and *A. australis* individuals (Bernardelli et al., 1990; Castro-Ramos et al., 1998; Kiers et al., 2008; Lacave, 2009) and reported by Forshaw & Phelps (1991) in *A. forsteri*, *A. pusillus doriferus* and *N. cinerea*. This technique is very sensitive, economical and easy to perform. The possible occurrence of false negatives must be taken into consideration in cases of advanced infection with anergy or very recent infections that have not yet generated an appropriate immune response (Jurczynski et al., 2011). False positives may also occur in nonspecific cases of exposure to non-tuberculous mycobacteria (*Mycobacterium avium*, *M. chelonae*, *M. fortuitum* and *M. smegmatis*) (Bernardelli et al., 1990; Forshaw & Phelps, 1991). Tissue and bronchial secretion smears have been used for diagnosis (Jurczynski et al., 2012), but the AFB may correspond to mycobacteria other than *M. pinnipedii* and, therefore, other confirmation methods must be used. Molecular techniques (PCR, spoligotyping and MIRU / VNTR) applied to samples from purified cultures, tissues and sputum, have produced quick results, allowing the identification of strains involved and their origin (Cousins et al., 2003; Jurczynski et al., 2011; Kiers et al., 2008; Kriz et al., 2011; Moser et al., 2008). In zoo collections, it is suggested that the final diagnosis should be based on the simultaneous use of three different methods, needing a minimum of two positive tests to increase the overall sensitivity when making the final decision for euthanasia. In case of wild animals, the possible diagnostic methods could include the TST, culture and molecular analysis of sputum (PCR) and serology (DPP). This would be extremely important in order to establish or confirm the endemic conditions of the disease in wild colonies and prevent the exportation or handling of carrier animals.

#### **4. Importance of infectious diseases in Uruguayan pinniped colonies with zoonotic potential**

It has been documented that the introduction of novel pathogens into a native animal population without previous exposure could result in epizootics. Human and wildlife populations share a wide range of diseases. While the most common disease transmissions reported are between wild and domestic animals (though many zoonoses do have wildlife origin), emerging diseases of animal origin represent one of the greatest potential threats to public health.

The emergence and re-emergence of over 30 agents have been reported in marine mammals (Miller et al., 2001). There are several reports about their susceptibility to virus, bacterial,

fungus and parasitic agents, provoking diseases that result in mass mortality events (Gulland et al., 1996; Miller et al., 2004; Ostehaus, 2000). Once new or previous pathogens are established in the host, they can represent a health risk to other marine vertebrates, humans, or both. It is, however, very difficult to know which routes these zoonotic marine mammal infections take in the marine environment.

The dynamic governing the relationship between infectious diseases affecting humans and marine vertebrates, including sea mammals is very complex and generally poorly understood. It is probable that human activity is a greater threat to marine vertebrate health than vice versa (Mos et al., 2006). However, it is very difficult to establish the role of marine animals as vectors or carriers of zoonotic diseases. Although the role of sea mammals in transmission of potential zoonotic pathogens is not well established, several risk factors, including frequent and prolonged direct contact with live specimens, were clearly identified in workers exposed to these animals (Hunt et al. 2008). Thus, these animals may present a zoonotic potential and also the potential for epizootic events which could cause health problems in marine animals. Since the influenza virus is transmitted by direct contact with infected individuals, by aerosol, or contact with infected objects, several incidents of influenza A virus transmission have occurred from infected seals and whales maintained in captivity to humans (Webster et al., 1981).

Due to their life span, high trophic feeding and the continuous exposition to emerging pathogens, sea mammals might be considered as sentinel species of emerging/re-emerging diseases. Pinnipeds share these characteristics and may serve as effective sentinels providing information about public and aquatic ecosystem health, and indicating the current or potential negative impacts on animal health at the individual or population level.

Implementing a proper management and the accurate execution of conservation policies of wildlife requires the analysis of the vulnerability of the animals to infectious diseases. The majority of animals included in our studies belonged to colonies near cities or towns in proximity to shore (Punta del Este, Polonio's Cape), regions that are commonly used by humans, marine and terrestrial mammals and different avian species, establishing an optimal opportunity for zoonotic disease transmission and long-term disease maintenance. The dense aggregations of pinniped colonies make fur seals and sea lions vulnerable to epizootics. Also, movements between adjacent latitudinal domains are common in both pinniped species, which could transmit or acquire pathogens during feeding trips to other parts of the Atlantic Ocean and the La Plata River.

The diagnosis of influenza virus in high-density seal populations in Uruguay generated considerable concern about the potential impacts on South American fur seal and sea lion colonies, as well as potential health risks to humans and domestic animals. In the evolution and ecology of influenza viruses, interspecies transmission is an important factor; seabirds and marine mammals are conspicuous animals that integrate changes in the ecosystem and reflect the existing state of the environment (Aguirre & Tabor, 2004; Boersma, 2008; Moore, 2008; Thiele et al., 2004). Transmission of the influenza virus occurs between avian and several marine mammal species (Mandler et al., 1990) at least for influenza A virus,



representing an important step in the evolution and emergence of new mammalian viral strains. Fur seals have the potential to serve as an influenza reservoir for other mammal species. However, more detailed studies are needed to elucidate the role of seals in the epidemiology of influenza along the Uruguayan coasts and in other South American countries.

Our findings confirm that fur seals can act as reservoirs of human influenza strains that circulated in the past, and also suggest that influenza A and B viruses may be transmitted from humans to seals as has been mentioned by other authors (Ohishi et al., 2002, 2004; Osterhaus, 2000). This transmission is due to the highly social lifestyle of pinnipeds, which congregate at sea and on land, and frequently associate with seals from other colonies. It is important to note that there is a strong interaction between seals and humans on Lobos Island and Polonio's Cape during live animal captures; nowadays, most interactions occur during capture and research activities, as well as in rehabilitation centers and sometimes with divers that swim near the seal islands. These events constitute opportunities for new influenza strains to jump between humans and seals, providing the potential for an epidemic event. Gaining information on the full spectrum of influenza viruses circulating in our seal colonies and detecting these viruses will remain an important task for its surveillance, outbreak control, and animal and public health.

As described previously, tuberculosis in pinnipeds had been recorded from the beginning of the twentieth century but it was not until mid 80's and later that several publications appeared with data from stranded animals and zoological collections. It is important to note that most records are from Otariids from the Southern Hemisphere, a single diagnosis is from a southern elephant seal and one from one native Northern Otariid species kept in captivity in the Northern Hemisphere.

Despite the fact that the seal harvest in Uruguay extended for 200 years, there are no records of macroscopic lesions observed in animals, nor of tuberculosis diagnosis from the staff working in the capture, slaughter or processing of the by-products, that could contribute retrospective data to this disease in Uruguayan pinniped colonies. The disease is considered endemic because of the numerous cases diagnosed in wild animals and the high prevalence of South American seals kept in aquaria and zoos. Unfortunately, to date non epidemiological studies have been conducted in any of the seal colonies from the Southern Hemisphere which could indicate the prevalence of the disease in its natural environment.

Information presented in this work, including the isolation of *M. pinnipedii* in pups and juvenile seals, indicates the early transmission of the organism. However, it is difficult to establish the course of the disease or immune mechanisms that may develop in each individual to control the infection or, in other cases, allow individuals to act as healthy carriers for several years until some trigger factor determines the development of the disease.

The most important mechanism of transmission of *M. tuberculosis*, and probably *M. pinnipedii*, is through the air by droplets produced when an individual with respiratory tract TB eliminates aerosolized microorganisms by coughing or sneezing. Large droplets fall

quickly due to their weight and reach the ground without evaporation. Smaller drops evaporate and decrease in size, becoming infectious droplet nuclei, which remain suspended in ambient air for a long time and can be airborne for days. Successful transmission requires that these micro droplets charged with bacilli are of sufficiently small size (1 to 5  $\mu\text{m}$ ) to enable them to reach very deep into the lungs and alveoli (Rider, 1999). This phenomenon is more frequent in captive conditions in zoos and aquaria where spray released during enclosure hygiene, poor ventilation and exposure among animals in confined environments, constitute increased risk factors for infection as has been shown in different zoos (Cousins et al., 1993 and 2003; Kiers et al., 2008; Thompson et al., 1993). In wild pinniped colonies, natural ventilation and increased exposure to UV is a natural way of microorganism control that could be the reason for the less common occurrence of TB in these colonies.

In the early course of host-pathogen interaction, mycobacteria are phagocytized by alveolar macrophages. In case the macrophage cannot destroy the pathogen, it resides in a quiescent state with a relatively low multiplication until cell mediated immunity is compromised transiently or permanently. Among the risk factors for immune-compromise, are age, history of a spontaneous TB cure with residual fibrotic lesions, and the time elapsed since infection. Other medical conditions such as endocrine disorders, tumors, malnutrition and stress may influence the progression of infection to disease (Musser et al., 2000; Rider, 1999). This evolution of the disease is consistent with the findings by different authors who have isolated AFB in organs without lesions from pups and juveniles (Bruning-Fann et al., 2001), identified the presence of calcified granulomas in mediastinal lymph nodes in adult and some juvenile animals in captivity and documented tuberculosis lesions in older animals (Jurczynski et al., 2011, 2012).

Keepers and veterinarians at zoos, aquaria and rehabilitation centers are at increased risk of infection because of their extensive contact with the animals. The degree of risk depends on the type of accommodation and sanitizing procedures of the enclosures (Kiers et al., 2008; Thompson et al., 1993) in association with the presence of open tuberculosis cases. Given the zoonotic condition of the disease, it is important to take preventive measures in all personnel working near or with access to wild and captive pinnipeds (researchers, fishermen, seal hunters, rehabilitation centers, aquaria and zoos staff) (Kiers et al., 2008; Thompson et al., 1993) and to establish hygiene measures for reducing the chances of spreading the infection.

Since development of the disease is triggered under immunosuppressive conditions, it is important to assess which factors directly or indirectly affect the immune system and allow development of the disease in wild populations of the Southern Hemisphere. This also applies to other diseases that may be affecting the health status of wild colonies, particularly in *O. flavescens* from Uruguay, whose population is declining.

Further investigation is needed in order to establish the sources of zoonotic potential in the marine environment and to better understand the nature of health risks for sea mammals and human. Additional epidemiologic studies are also required, to investigate epizootic

episodes assessing its impact and to elucidate how these diseases spread among and within marine mammal's populations.

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