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## Serologic Evidence of Influenza A and B Viruses in South American Fur Seals (*Arctocephalus australis*)

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**ABSTRACT:** The aim of this work was to detect serologic evidence of influenza virus infections in South American fur seals (*Arctocephalus australis*) that inhabit the Uruguayan coast. In 29 of 37 serum samples that were analyzed, we identified antibodies to at least one of the following antigens: H1N1 (A/NewCaledonia/20/99), B/Beijing/184/93-like viruses, B/Hong Kong/330/01, and B/Sichuan/379/99 by means of the hemagglutination inhibition assay (HAI). Results confirmed that influenza A viruses circulate in marine mammals and also showed, for the first time, indirect evidence of influenza B infections in *Arctocephalus australis*.

**Key words:** *Arctocephalus australis*, hemagglutination inhibition assay, influenza virus, South American fur seals, Uruguay.

Influenza viruses belong to the family Orthomyxoviridae, which have enveloped and segmented, single-stranded negative-sense RNAs. There are three types of influenza viruses: A, B, and C, of which only types A and B cause widespread outbreaks in humans.

Influenza A virus infects a variety of avian and mammalian species including humans, horses, swine, and marine mammals such as seals and cetaceans (Webster et al., 1992; Murphy and Webster, 1996). It was believed that influenza B virus only infects humans, but in 2000 an influenza B virus isolate from an infected harbor seal (*Phoca vitulina*) was reported (Osterhaus et al., 2000).

Since the end of the 1970s, evidence of influenza A infections in marine mammals has been reported based on virus isolation (Lvov et al., 1978; Lang et al., 1981; Webster et al., 1981; Geraci et al., 1982; Hinshaw et al., 1982, 1984, 1986; Callan et

al., 1995) or on antibody detection (de Boer et al., 1990; Steuen et al., 1994; Danner and McGregor, 1998; Nielsen et al., 2001; Ohishi et al., 2002, 2004, 2006; Fujii et al., 2007). However, very few have been reported for influenza B (Osterhaus et al., 2000; Ohishi et al., 2002).

Uruguay has one of the largest colonies of both *Arctocephalus australis* (South American fur seal) and *Otaria byronia* (South American sea lion) in the world. These species inhabit the Lobos Island, located in the Atlantic Ocean 8.5 km from the coast (35°1'24"S, 54°52'54"W). The islands have a rocky surface that covers 41 ha.

Serum samples were collected in September 2004 from 37 South American fur seals (12 female and 25 male) estimated to be 10 mo old. The presence of antibodies against influenza A and B virus was tested using the hemagglutination inhibition assay (HAI) according to the WHO Manual on Animal Influenza Diagnosis and Surveillance (2002a). Briefly, all serum samples were incubated overnight in a 37 C waterbath with receptor-destroying enzyme (RDE) in a relationship 1:3, respectively, to remove nonspecific inhibitors and then heated at 56 C during 30 min to inactivate the RDE. After that, the serum samples were absorbed with turkey red blood cells to inactivate nonspecific agglutinins. The HAI was performed in microtiter plates using turkey erythrocytes at a concentration of 0.5% in phosphate-buffered saline. A sample was considered antibody positive when the HA titer was  $\geq 80$ , as reported by Ohishi et al., 2004.

TABLE 1. Hemagglutination-inhibition assay (HAI) of fur seal serum samples with influenza A and B virus antigens. The highest titers for each antigen tested appear in boldface.

Influenza virus antigens	Positive rate <sup>a</sup> with titer<80	Positive rate with titer=80	Positive rate with titer=160	Positive rate with titer=320	Positive rate with titer=640
IA/H1N1/NewCaledonia/20/99	16/37(43.3%)	10/37(27%)	10/37(27%)	<b>1/37(2.7%)</b>	0/37(0%)
IA/H3N2/ Panamá/2007/99	37/37(100%)	0/37(0%)	0/37(0%)	0/37(0%)	0/37(0%)
IB/Beijing/184/93	12/37(32.4%)	13/37(35.2%)	10/37(27%)	<b>2/37(5.4%)</b>	0/37(0%)
IB/Hong Kong/330/01	17/37(46%)	14/37(37.8%)	<b>6/37(16.2%)</b>	0/37(0%)	0/37(0%)
IB/Sichuan/379/99	13/37(35%)	4/37(11%)	8/37(22%)	10/37(27%)	<b>2/37(5%)</b>

<sup>a</sup> Number positive/number tested (positive percent).

We used the following influenza virus antigens: 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 (WHO Influenza Reagent Kit, Center for Disease Control and Prevention, Atlanta, Georgia, USA, given by Departamento de Laboratorios de Salud Pública of Ministerio de Salud Pública).

Antibodies to influenza were detected in most seal-serum samples tested (Table 1). Thus, among 37 serum samples, only eight did not inhibit hemagglutination of any antigen, while five inhibited hemagglutination of one antigen assayed; and three, five, and 16 sera inhibited two, three, and four antigens, respectively. None of them reacted with all the antigens considered, but 17 reacted with all B antigens tested and 16 of them with H1N1 antigen.

Regarding influenza A virus, we detected antibodies reaching HAI titers of 320 only in one (3%) serum sample against H1 subtype. On the other hand, we found antibodies against three influenza B virus strains that have been previously detected in humans in Uruguay. The Beijing strain circulated in 1999 (WHO, 1999), the Sichuan strain in 2001 (WHO, 2001), and the Hong Kong strain in 2002 (WHO, 2002b). The highest HAI titers (640) were found against the Sichuan strain in 5% of the seals studied (Table 1), but the HAI titers of some sera against antigens of the other strains were also high (320 against Beijing in 5% of the samples

and 160 against Hong Kong in 16% of the samples studied). The results were consistent with those proposed by Osterhaus et al. (2000) and Ohishi et al. (2002), which suggested that influenza B virus was not restricted to humans.

To our knowledge, this is the first report about influenza virus A and B circulation in this marine mammal species (*Arctocephalus australis*).

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