First Mass Mortality of Marine Mammals Caused by Highly Pathogenic Influenza Virus (H5N1) in South America

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ABSTRACT

We report the first worldwide infection and massive mortality associated with a Highly Pathogenic Influenza Virus (H5N1) in sea lions of Peru. The transmission pathway of H5N1 may have been through the close contact of sea lions with infected wild birds. We cannot rule out direct transmission among sea lions.

The recent global epidemic event (2020-2022) caused by the highly pathogenic avian influenza (HPAI) A (H5N1) is the largest observed so far, several global outbreaks having been caused (1,2). This is the first time the presence of this pathogen has been registered in some regions, where it has produced massive mortality in wild birds (2–4). This is of great concern and suggests a change has taken place in the dynamics of this pathogen (2).

At the end of 2022, the H5N1 virus reached South America (Peru, Ecuador, Colombia, Venezuela and Chile), with alarming outcomes in Peru (4). This pathogen was detected for the first time in wild birds in Peru on 13 November, as it caused very huge mortality levels (>22,000 birds over 4 weeks within protected areas) (4). Reports on both protected and non-protected areas suggest the virus generated a disease that killed at least >50,000 wild birds by the end of 2022, particularly Peruvian pelicans (*Pelecanus thagus*) and Peruvian boobies (*Sula variegata*) (4, Gamarra-Toledo et al. unpublished data). Most birds showed signs of neurological problems (e.g., incoordination, tremors and partial convulsions) before dying; impressive images of sandy beaches with dozens of dying birds highlighted the magnitude of this catastrophe (Suppl Mat, https://www.youtube.com/watch?v=45UZVY4IxTY). The large biomass of infected wild birds may have led to a spillover event affecting predators and scavengers, including marine mammals cohabiting with them, as reported in other parts of the world (5). In this article, we report the death of 634 sea lions (mainly *Otaria flavescens* and a few *Arctocephalus australis*) on Peruvian coasts over 5 weeks, and give details of the analyses that prove their infection with the Highly Pathogenic Influenza Virus (H5N1).

The Study

During January and the first days of February 2023, more than six hundred sea lions were found dead or dying on Peruvian beaches (e.g., Asia, Chorrillos, Cerro Azul and

Chilca beaches) and in protected areas (e.g., Reserva Nacional Paracas, Reserva Nacional San Fernando, Reserva Nacional Sistema de Islas, Islotes y Puntas Guaneras) (Fig. 1, Table 1). The high mortality observed was worrisome; for instance, up to 100 dead individuals floating together in the sea – an unprecedented observation for this geographical region (Fig. 1, Table 1). The clinical symptoms of dying individuals were mainly neurological, such as tremors, convulsions and paralysis. They also showed respiratory signs such as dyspnea, tachypnea, nasal and buccal secretions and pulmonary edema. Most dead animals were female, and several abortions were observed. There are no other records of such high mortality of aggregated sea lions.

Individuals were examined by veterinarians and some dead animals were necropsied (see Fig. 2 A, B). The body condition of the sea lions necropsied ranged from good to very good, suggesting they died due to an acute health problem. Substantial quantities of whitish secretions filling the upper respiratory tracts (trachea and pharynx) were observed in the necropsies and in dying animals (Fig. 2 C), which explains the severe dyspnea and tachypnea clinically observed by veterinarians. Lungs were congestive, with hemorrhagic focus compatible with interstitial pneumonia. Brains were also congestive with hemorrhagic focus compatible with encephalitis, which explains the neurological signs observed in dying individuals (Fig. 2 D). The small intestine showed necrotic focus compatible with duodenitis. Of epidemiological and conservation concern, a total of 634 dead individuals belonged mainly to *Otaria flavescens* (n=630) and some to *Arctocephalus australis* (n=4). However, the latter species was not tested and there are no other records of high mortality in this species, so we cannot confirm the actual cause of their death. At the time of writing this dispatch (February 2023) sea lions mortalities continue.

76 Given the epidemiologic situation produced by H5N1 in wild birds that cohabit with
77 these sea lions (4), samples were taken in a subset of individuals to diagnose this virus.
78 To diagnose HPIV, samples of *Otaria flavescens* (n=6; including 3 samples from
79 different parts of each animal) were taken inside a protected area (Reserva Nacional
80 Paracas) and sent to the Avian Pathology Laboratory of the Universidad Nacional
81 Mayor de San Marcos. Samples consisted of organs such as kidneys, spleen and lungs,
82 stomach contents, and oral, nasal and rectal swabs (Fig 2 E). Real time RT-PCR
83 targeting the Influenza A matrix segment were performed: all the tested individuals
84 were positive for influenza A (Table 1). Through posterior RT-PCR analysis using
85 specific primers to identify the hemagglutinin and neuraminidase, this virus was
86 characterized as Influenza A of the H5N1 subtype, the virus that had produced massive
87 mortality of wild birds in previous months (4). Almost at the same time (between
88 January 18 and 26) SERFOR (Servicio Nacional Forestal y de Fauna Silvestre) took
89 another 6 samples from dead animals outside protected areas (beaches of the provinces
90 of Lima and Cañete), following the same procedure. These samples were analyzed at
91 the Genomics Laboratory of the Pontificia Universidad Católica del Perú. Three sea lion
92 samples also tested positive for HPAI H5N1 (OFICIO-0017-2023-MIDAGRI-
93 SENASA-DSA), and a dolphin (*Tursiops truncatus*) was also reported as testing
94 positive in Piura, in the northern region of Peru.

95 **Conclusions**

96 We show that sea lions (*Otaria flavescens*) of Peru, an endangered species according to
97 the national legislation (6), were infected by HPAI (H5N1) and developed a deadly
98 associated disease producing massive mortality in several regions of the Peruvian
99 coastline (Fig. 1). The sea lion mass mortality described is compatible with systemic
100 HPIV that resulted in acute encephalitis and pneumonia. Some specific cases of

101 infection and mortality of marine mammals due to HPIV with similar clinical and
102 anatomopathological characteristics have been reported in previous studies around the
103 world (5,7,8). For instance, 1,400 dead harbor seals (*Phoca vitulina*) were found dead in
104 Germany alone; necropsied individuals (n=17) showed congestive lungs and interstitial
105 pneumonia due to HPIV (7). Similarly, in USA, seal mortalities were reported with
106 pneumonia and neurological symptoms due to H5N1 (5). However, to our knowledge,
107 this is the first confident report of massive mortality by disease associated with HPIV
108 (H5N1) in sea lions, and the first report of wild mammal mortality in South America.
109 The source of the HPAI affecting these sea lions was very probably the large number of
110 infected birds/carcasses on the Peruvian coastline (4). Sea lions may be infected by
111 close contact with these carcasses and even through their consumption (see Fig. 2 F).
112 However, the transmission pathway remains unknown until now.

113 Such high levels of mortality in a social animal are worrisome. We cannot exclude
114 direct transmission among sea lions due to their colonial breeding, and because many
115 animals died simultaneously in groups. In fact, based on recent research suggesting the
116 first mammal-to-mammal infection in minks (*Neovison vison*) (9) and the large number
117 of sea lions currently affected, we cannot rule out that the virus has adapted to mammals
118 and that sea lion-sea lion transmission has begun in Peru; this should be urgently
119 investigated. Moreover, the mass mortality of animals that can weigh around 350 kg
120 (10) produces an enormous biomass of infected tissue, which could perpetuate the
121 transmission of H5N1 and other pathogens. This could have serious consequences for
122 the ecosystem and human health.

123 Further research is required to address the transmission pathway in social mammals.
124 This is particularly important in our case, given the complexities of the H5N1 Peruvian
125 outbreak; i.e., severe mortality of wild birds, potential wild bird spillover to mammals,

dead animals on beaches with human presence and human interaction with infected animals (see Supplementary Material Video 1). Since the emergence of the A/goose/Guangdong/1/1996 (Gs/Gd) H5N1 influenza A virus in China in 1996, different Highly Pathogenic Influenza Viruses (HPIV) have produced thousands of human infections with a lethality of 50% (3,11). We would like to call attention to the fact that in this geographical region of the world, human–infected animal interaction is common, so infections might begin to rise (there is already one case of a 9-year-old girl in Ecuador; https://www.eldiario.com.ar/242825-se-confirmo-el-primer-caso-de-gripe-aviar-en-nina-de-9-anos-en-ecuador/) and this must be addressed if we are to avoid the risk of a pandemic through evolutionary emergence (12).

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Figure 1. Map of geographical distribution of high mortalities in sea lions in January and February 2023 and sampling localities (green triangle and yellow star) for sea lions testing positive of Highly Pathogenic Influenza Virus (H5N1) on the Peruvian coastline (SERFOR = Servicio Nacional Forestal y de Fauna Silvestre; SERNANP = Servicio Nacional de Áreas Naturales Protegidas por el Estado).

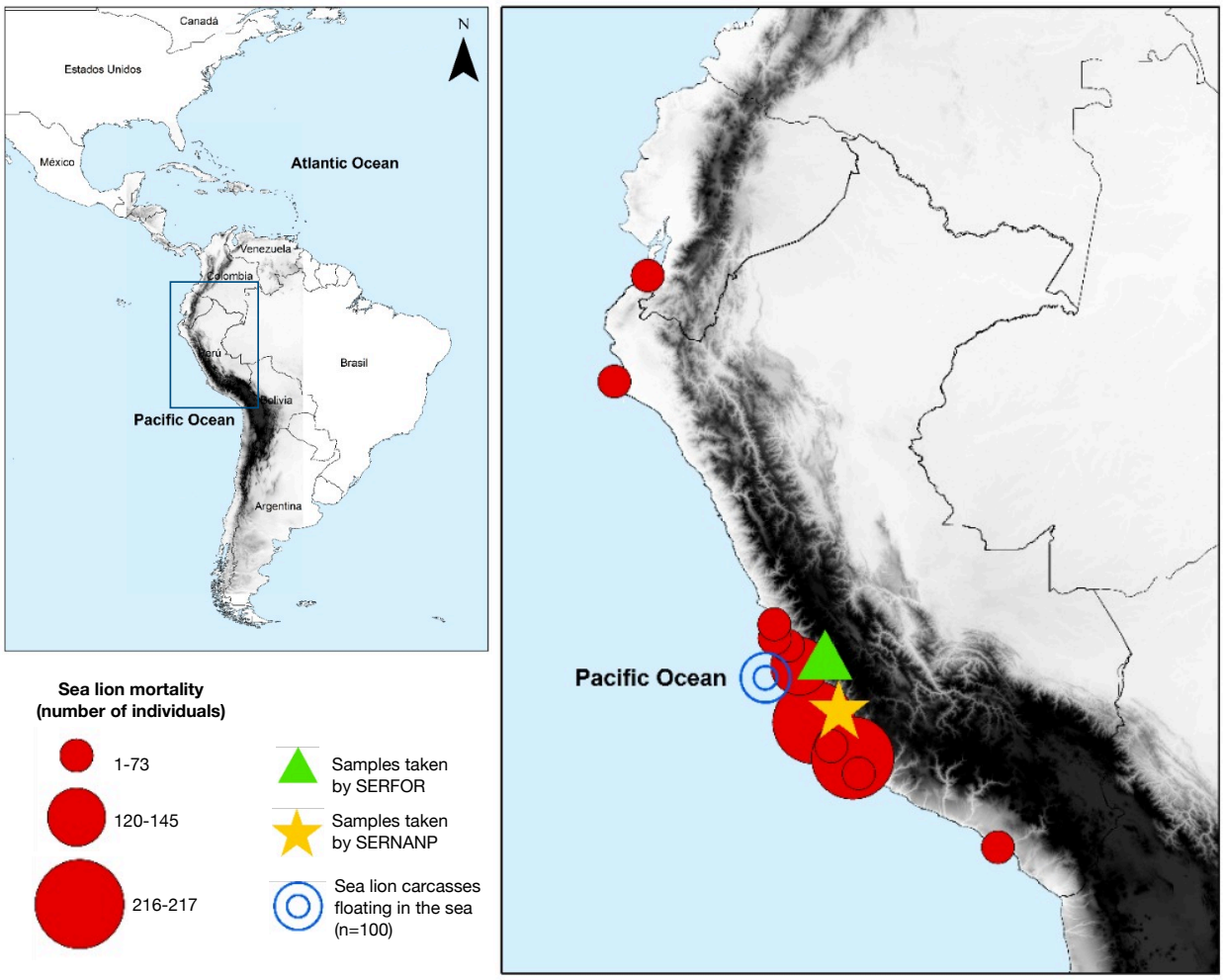

Figure 2. Images showing the field work and sample collection for testing of Highly Pathogenic Influenza Virus (H5N1) in sea lions in the Paracas National Reserve on the Peruvian coastline, February 2023. A) Sea lion carcasses on the beach; B) Dying sea lion with ataxia; C) Dead sea lion with avian flu symptoms; D) Sample collection for RT-PCR; E) Sea lion necropsy; F) Sea lion trapping and eating an infected Guanay cormorant on January 23th of 2023 (Photo: Sandra Lizarme) in the Reserva Nacional Paracas.

188 **Table 1.** Sea lion mortality and positive tests of Highly Pathogenic Influenza Virus

189 (H5N1) in protected areas of Peru between January and February 2023. Abbreviations

190 for the Natural Protected Areas are as follows: RNSF = Reserva Nacional San

191 Fernando, RNILLESCAS = Reserva Nacional Illescas, RNPARACAS = Reserva

192 Nacional Paracas, ZRANCÓN = Zona Reservada Ancón, RNSIIPG = Reserva Nacional

193 Sistema de Islas, Islotes y Puntas Guaneras.

Species	Date	Natural Protected Area	Island & Guano island	Symptoms	Total deaths
South American Fur Seal (*Arctocephalus australis*)					
	26-Jan	RNSF			3
	5-Feb	RNPARACAS	Yuyos, Caracolita, Lagunilla, La Roja		1
South American Sea Lion (*Otaria flavescens*)					
	6-Jan	RNILLESCAS			38
	14-Jan	RNPARACAS	Lagunillas, Supay, Atenas	Eyes closed and watering, nosebleeds and difficulty breathing	3
	16-Jan	ZRANCÓN			4
	18-Jan	RNPARACAS	La Mina		1
	19-Jan	RNPARACAS	Aguada		1
		RNSIIPG	Isla Cavinzas		1
	20-Jan	RNPARACAS	Lagunillas, La Roja, Yumaque, Atenas		32
	21-Jan	RNPARACAS	Santo Domingo		1
		RNSIIPG	Punta Lomitas	Foaming at the mouth, convulsions, paralysis of the forelimbs	1
	23-Jan	RNPARACAS	Barlovento		6
			Isla San Gallan		33
		RNSIIPG	Isla Cavinzas		3
			Isla Pachacamac		7
	24-Jan	RNSIIPG	Isla Cavinzas		4
	25-Jan	RNPARACAS	Lagunillas		36
		RNSIIPG	Isla Cavinzas		6
			Isla Asia		20
	26-Jan	RNPARACAS	Los viejos		24

	RNSF		Carcasses floating in the sea	5
	RNSIIPG	Punta San Juan	Convulsions (dying individuals)	2
27-Jan	RNSF			20
	RNSIIPG	Isla Pescadores		2
		Isla Asia	Carcasses floating in the sea	100
		Punta San Juan		8
	ZRANCÓN			3
28-Jan	RNPARACAS	Arquillo		1
29-Jan	RNSIIPG	Punta San Juan		2
30-Jan	RNPARACAS	Ancla	Dyspnea, tremors (dying individuals)	17
		Caclio		4
		Chucho		4
		La Raya		12
		Punta Prieto		3
		Rancherio		5
31-Jan	RNPARACAS	Santo Domingo		12
		Sequion	Ataxia, tremors (dying individuals)	2
1-Feb	RNPARACAS	Yumaque		5
2-Feb	RNPARACAS	Karwas, La Raya	Dyspnea, tremors (dying individuals)	9*
3-Feb	RNSF			188
4-Feb	RNPARACAS	Yumaque Punta Ballena		3
5-Feb	RNPARACAS	Yuyos, Caracolita, Lagunilla, La Roja		2
			Total	**634**

*6 individuals tested, and all results were positive for the Highly Pathogenic Influenza Virus (H5N1)