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SEROLOGICAL SURVEY FOR SELECT INFECTIOUS AGENTS IN WILD MAGELLANIC PENGUINS (*SPHENISCUS MAGELLANICUS*) IN ARGENTINA, 1994–2008

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ABSTRACT: Despite being the most numerous penguin species in South America, exposure of the Magellanic Penguin (*Spheniscus magellanicus*) to pathogens has not yet been thoroughly assessed. We collected serum from 1,058 Magellanic Penguins at 10 breeding colonies along the entire latitudinal range of this species in Argentina. The work spanned 10 breeding seasons over 15 yr (1994–2008). Sera were tested for antibodies to select infectious agents. Antibodies reacting against 16 pathogens were detected (seroprevalence): *Aspergillus* sp. (15.1%), *Chlamydia psittaci* (6.5%), *Salmonella* Pullorum (3.1%), *Salmonella* Typhimurium (81.3%), *Aviadenovirus* sp. (18.1%), *Duck atadenovirus A* (23.6%), *Anatid herpesvirus 1* (0.7%), *Avian orthoreovirus* (3.3%), *Avian coronavirus* M41 (43.5%), *Avian coronavirus* C46 (59.8%), *Avian coronavirus* A99 (37.4%), *Avian coronavirus* JMK (40.2%), *Tremovirus A* (0.3%), *Avian avulavirus 1* (44.0%), *Avian avulavirus 2* (43.8%), and *Avian avulavirus 3* (46.6%). No antibodies were detected against nine infectious agents: *Gallid alphaherpesvirus 1*, *Gallid alphaherpesvirus 2*, *Infectious bursal disease virus*, *Avastrovirus 2*, *West Nile virus*, *Eastern equine encephalitis virus*, *Venezuelan equine encephalitis virus*, *Western equine encephalitis virus*, and *Influenza A virus*. While restricted by limitations inherent to serological methods, our results provide baseline knowledge for a key species in the South Atlantic Ocean. This information is valuable for adaptive conservation management in a time of increasing environmental stressors affecting the Patagonian Sea, one of the world's richest pelagic seabird communities.

Key words: Adaptive management, environmental stressors, health, penguin, seabird, serology, South America, Sphenisciformes.

INTRODUCTION

Penguins are currently the second most threatened group of seabirds in the world (after albatrosses) with 10 of 18 species under threat of extinction (International Union for Conservation of Nature 2018). While penguin declines are multifactorial, recent mortality events from infectious diseases (Cooper et al. 2009; Alley et al. 2017; Khomenko et al. 2018) highlight the relevance of disease surveillance for conservation management of species at risk.

The Patagonian Sea has one of the world's richest pelagic seabird communities, and Magellanic Penguins (*Spheniscus magellanicus*) are the most abundant penguin species in this region (Falabella et al. 2009). This species breeds along the coast of Argentina (66 sites), Chile (31 sites), and the Malvinas/Falkland Islands (at least 100 sites; BirdLife International 2018). The species' population is estimated at 1.3 million breeding pairs, 65% of which are concentrated in large breeding colonies along the Patagonian coast of Argentina (Falabella et al. 2009; Boersma et al. 2013). Magellanic Penguins are currently classified as Near Threatened, and their main conservation threats are fisheries bycatch and competition, marine pollution, disease, cli-

mate variability and change, and habitat degradation (Trathan et al. 2015; BirdLife International 2018).

Penguins kept in zoos and rehabilitation centers are known to be susceptible to a number of viral, bacterial, and fungal pathogens (Cranfield 2003; Wallace 2014), but there is limited information on the occurrence of infectious agents in free-ranging penguin populations. In recent years and with advances in diagnostic capability several new viruses and strains have been reported in penguins in Antarctica (e.g., Lee et al. 2014; Varsani et al. 2014, 2015). However, much less information is available for penguins breeding in South America. A few serological surveys of penguins breeding in South America have revealed exposure to potentially infectious agents of conservation significance (Karesh et al. 1999; Travis et al. 2006; Smith et al. 2008), and a novel avian paramyxovirus was identified in Southern Rockhopper Penguins (*Eudyptes chrysocome*; Miller et al. 2010). Yet, despite being the most numerous penguin species on this continent, serological surveys of Magellanic Penguins have been conducted only in Brazil, outside of their breeding distribution (Nunes et al. 2012).

We employed serological methods to assess exposure to infectious agents of Magellanic Penguins at breeding colonies along the Argentinean Patagonia coast to inform adaptive conservation management in the face of increasing global and local environmental stressors.

MATERIALS AND METHODS

Magellanic Penguins were sampled at 10 colonies along the coast of Argentina (Fig. 1), with sampling undertaken during 10 breeding seasons from 1994 to 2008 (Supplementary Material Tables S1, S2). Penguins were caught and manually restrained, and blood (<1% of body mass) was drawn by venipuncture of the jugular vein, using 10 or 20 mL heparinized syringes and 0.7×22 mm needles. Blood samples were kept cool on ice for 4–6 hr and then were centrifuged at 1000 × G for 20 min using a portable centrifuge (Mobilespin, Vulcan Technologies, Grandview, Missouri, USA). Plasma was harvested and frozen in liquid nitrogen. Samples were treated in a water bath at 56 C for 2 hr in accordance with US Department of Agriculture regulations before importation to the US and then were transferred to –80 C freezer until serological analysis.

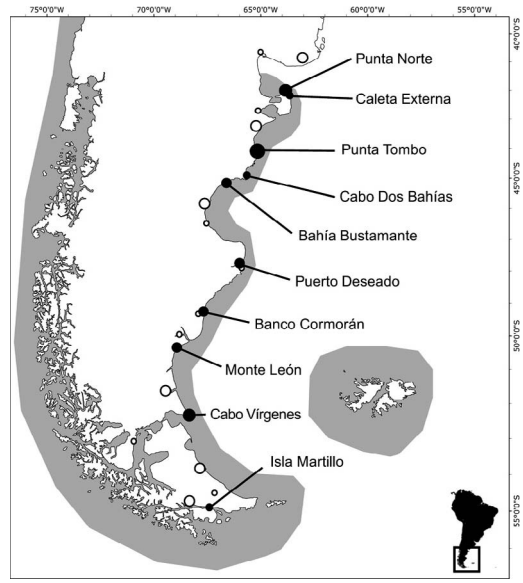

FIGURE 1. Geographic distribution of Magellanic Penguin (*Spheniscus magellanicus*) colonies on the Argentinean coast. Study colonies (black circles) and urban settlements (white circles) are drawn proportionally to their population size. The gray area corresponds to the breeding distribution of Magellanic Penguins (BirdLife International 2018).

Table 1 summarizes the serological tests performed, methods used, and, where appropriate, the cutoff value used to define a sample as positive in this study. Serological testing was performed at the National Veterinary Diagnostic Laboratory (Ames, Iowa, USA), except for *Aspergillus* sp. and *West Nile virus*, which were, respectively, performed at the National Center for Avian Clinical Services, University of Minnesota (St. Paul, Minnesota, USA) and at the Animal Health Diagnostic Center, Cornell University College of Veterinary Medicine (Ithaca, New York, USA). Due to protocol changes in the laboratories to which the samples were submitted during the study period, different serological tests or cutoff values were used to test different batches of samples for *Salmonella*, *Aviadenovirus*, and *Avian orthoreovirus* (Table 1). Antibody titers were expressed as the inverse of the highest dilution with a positive signal and were quantified for samples testing positive for *Chlamydia psittaci*, *Coronaviridae*, and *Paramyxoviridae*. For *C. psittaci*, two cutoff values (1:10 and 1:40) were

TABLE 1. Summary of the serological tests employed for testing of sera from Magellanic Penguins (*Spheniscus magellanicus*) from the Argentinean coast, 1994–2008.

Disease	Pathogen (Virus family)	Test[a,b]	Cutoff value[c]	Acronym
Aspergillosis	*Aspergillus* spp.	ELISA[1]	0.1	ASPER
Avian chlamydiosis	*Chlamydia psittaci*	CFT[2]	1:10	CHLAM-10
			1:40	CHLAM-40
Pullorum disease	*Salmonella enterica enterica* Pullorum	MA[2]	1:20	PULLO-20
			1:40	PULLO-40
Paratyphoid	*Salmonella enterica enterica* Typhimurium	MA[2]	1:25	TYPHI-25
			1:40	TYPHI-40
Avian adenovirus infection	*Aviadenovirus* spp. (*Adenoviridae*)	AGID[3]	—	ADV-AG
		HI[3]	1:10	ADV-HI
Egg drop syndrome	*Duck atadenovirus A* (*Adenoviridae*)	AGID[3]	—	EDS
Infectious laryngotracheitis	*Gallid alphaherpesvirus 1* (*Herpesviridae*)	IFA[2]	1:10	ILTV
Marek's disease	*Gallid alphaherpesvirus 2* (*Herpesviridae*)	AGID[2]	—	MDV
Duck virus enteritis	*Anatid herpesvirus 1* (*Herpesviridae*)	SN[2]	1:4	DVE
Infectious bursal disease	*Infectious bursal disease virus* (*Birnaviridae*)	AGID[2]	—	IBDV
Avian reovirus infection	*Avian orthoreovirus* (*Reoviridae*)	IFA[3]	1:10	ARV-10
			1:20	ARV-20
Avian nephritis	*Avastrovirus 2* (*Astroviridae*)	IFA[3]	1:10	ANV
Infectious bronchitis	*Avian coronavirus* Massachusetts 41 (*Coronaviridae*)	HI[2]	1:10	ACV-M41
	Avian coronavirus Connecticut 46 (*Coronaviridae*)	HI[2]	1:10	ACV-C46
	Avian coronavirus Arkansas 99 (*Coronaviridae*)	HI[2]	1:10	ACV-A99
	Avian coronavirus JMK (*Coronaviridae*)	HI[2]	1:10	ACV-JMK
West Nile fever	*West Nile virus* (*Flaviviridae*)	PRNT[4]	1:10	WNV
Avian encephalomyelitis	*Tremovirus A* (*Picornaviridae*)	AGID[3]	—	AEV
Eastern equine encephalitis	*Eastern equine encephalitis virus* (*Togaviridae*)	HI[2]	1:10	EEE
Venezuelan equine encephalitis	*Venezuelan equine encephalitis virus* (*Togaviridae*)	HI[2]	1:10	VEE
Western equine encephalitis	*Western equine encephalitis virus* (*Togaviridae*)	HI[2]	1:10	WEE
Avian influenza	*Influenza A virus* (*Orthomyxoviridae*)	AGID[2]	—	IAV
Avian paramyxovirus infection	*Avian avulavirus 1* (*Paramyxoviridae*)	HI[2]	1:8	APMV-1
	Avian avulavirus 2 (*Paramyxoviridae*)	HI[2]	1:8	APMV-2
	Avian avulavirus 3 (*Paramyxoviridae*)	HI[2]	1:8	APMV-3

[a] ELISA = enzyme-linked immunosorbent assay; CFT = complement fixation test; MA = microscopic agglutination; AGID = agar gel immunodiffusion; HI = hemagglutination inhibition; IFA = indirect immunofluorescence assay; SN = serum neutralization; PNRT = plaque reduction neutralization.

[b] References: [1]Rettig et al. (1986); [2]Office International des Epizooties (1996); [3]Purchase et al. (1989); [4]Okeson et al. (2007).

[c] — = not applicable.

used to evaluate the effect on the estimated seroprevalence based on the same samples.

Seroprevalence was defined as the percentage of animals that were seropositive, and the Wilson score method without continuity correction was used to calculate the upper and lower limits of the 95% confidence interval (Newcombe 1998). Chi-square tests followed by the calculation of Goodman and Kruskal's gamma (G) were used to quantify the association among serological tests; only serological tests with at least five positive results were included in this analysis.

RESULTS

All sampled penguins were adult-plumaged, were not molting, appeared to be healthy, and were either resting in the colony or sitting on nests with chicks (Gallo et al. 2019). Penguins were selected randomly for sampling in 100 m radius areas across different habitat types given known variations in nest density and reproductive success (Rebstock et al. 2016). Edge effect is negligible in this species (Rebstock et al. 2016), but, as visitation is a known disturbance factor (Villanueva et al. 2012), sampling was conducted at least 200 m from tourist boardwalks or trails. To avoid repeated sampling of nonbanded individuals returning to the same nest in different years, different sections of colonies were selected for recurring sampling. For logistical reasons, the sampling effort was unevenly distributed among colonies and years (Supplementary Material Table S3).

Serum from 1,058 free-ranging Magellanic Penguins were tested, yet due to logistical constraints, the sample size for specific serological tests varied between 42 and 648 individuals depending on the test (Tables 2, 3 and Supplementary Material Table S3). Antibodies reacting against 16 pathogens were detected (seroprevalence): *Aspergillus* sp. (15.1%), *C. psittaci* (6.5%), *Salmonella* Pullorum (3.1%), *Salmonella* Typhimurium (81.3%), *Aviadenovirus* sp. (18.1%), *Duck atadenovirus A* (23.6%), *Anatid herpesvirus 1* (0.7%), *Avian orthoreovirus* (3.3%), *Avian coronavirus* M41 (43.5%), *Avian coronavirus* C46 (59.8%), *Avian coronavirus* A99 (37.4%), *Avian coronavirus* JMK (40.2%), *Tremovirus A* (0.3%), *Avian avulavirus 1* (44.0%), *Avian avulavirus 2* (43.8%), and *Avian avulavirus 3* (46.6%). No antibodies were detected against nine infectious agents: *Gallid alphaherpesvirus 1*, *Gallid alphaherpesvirus 2*, *Infectious bursal disease virus*, *Avastrovirus* 2, *West Nile virus*, *Eastern equine encephalitis virus*, *Venezuelan equine encephalitis virus*, *Western equine encephalitis virus*, and *Influenza A virus*.

A strong positive association ($G \geq 0.7$) of serological results was identified among the M41, C46, and A99 strains of *Avian coronavirus*, as well as among APMV-1, APMV-2, and APMV-3 (Supplementary Material Table S4). The highest antibody titers recorded were 80 for CHLAM, 128 for APMV-2, 160 for ACV-A99 and ACV-JMK, $\geq$256 for APMV-1 and APMV-3, and $\geq$320 for ACV-M41 and ACV-C46 (Fig. 2 and Supplementary Material Table S5).

DISCUSSION

We evaluated a sizable sample of serum collected over two decades from a large number of Magellanic Penguins, spanning the entire latitudinal distribution of the species in Argentina, providing a basis for future disease risk assessments and adaptive conservation management to protect seabird communities in the Patagonian Sea. However, caution is warranted interpreting our results, considering that our sampling effort was unevenly distributed across colonies and years, the serological tests were not specifically validated for Magellanic Penguins and therefore their sensitivity and specificity when applied to this species is not known, and the viral and microbial communities of penguins are still poorly characterized, and it is possible that yet-undiscovered organisms could cross-react with the serological tests employed in this study.

Aspergillus

Aspergillosis is a common respiratory disease of aquatic birds in captivity (Converse 2007; Burco et al. 2014), including Magellanic Penguins (Xavier et al. 2011; Silva-Filho et al. 2015). Due to the ubiquitous nature of *Aspergillus* spp., however, the interpretation of serological results is challenging because there is a high background seroprevalence in healthy individuals (Savelieff et al. 2018). Furthermore, not all infected birds show a reliable antibody response, potentially resulting in a high frequency of false negative results (França et al. 2012).

A serological survey of penguins in the Pacific Ocean revealed that burrow-nesting

TABLE 2. Seroprevalence for select infectious agents among 10 breeding colonies of Magellanic Penguins (*Spheniscus magellanicus*) on the Argentinean coast, 1994–2008.

Serological test[a]	Prevalence (%) and sample size (*n*) at each breeding colon[b]									
	Punta Norte	Caleta Externa	Punta Tombo	Cabo Dos Bahías	Bahía Bustamante	Puerto Deseado	Banco Cormorán	Monte León	Cabo Vírgenes	Isla Martillo
ASPER	20 (81)	19 (27)	13 (88)	19 (43)	—	17 (46)	7 (14)	10 (40)	4 (45)	29 (21)
CHLAM-10	43 (138)	13 (30)	36 (144)	55 (92)	77 (26)	29 (56)	24 (25)	26 (58)	30 (54)	0 (21)
CHLAM-40	7 (138)	3 (30)	4 (144)	8 (92)	12 (26)	9 (56)	0 (25)	9 (58)	9 (54)	0 (21)
PULLO-20	9 (89)	0 (30)	2 (93)	0 (44)	—	0 (46)	13 (15)	3 (39)	0 (45)	0 (21)
PULLO-40	0 (9)	—	0 (10)	0 (8)	0 (15)	—	—	—	—	—
TYPHI-25	77 (30)	—	81 (32)	86 (29)	—	—	—	—	—	—
TYPHI-40	0 (9)	—	0 (21)	0 (10)	0 (12)	0 (10)	0 (10)	0 (18)	0 (9)	—
ADV-AG	7 (44)	—	9 (80)	32 (19)	15 (26)	30 (10)	60 (10)	53 (17)	11 (9)	—
ADV-HI	0 (30)	—	0 (32)	0 (29)	—	—	—	—	—	—
EDS	—	12 (25)	9 (33)	15 (20)	—	55 (20)	—	—	36 (25)	—
ILTV	0 (92)	0 (25)	0 (135)	0 (68)	0 (26)	0 (30)	0 (10)	0 (18)	0 (34)	—
MDV	0 (18)	0 (10)	0 (19)	—	—	—	—	—	—	—
DVE	0 (50)	0 (24)	0 (40)	3 (38)	—	0 (40)	0 (15)	0 (40)	0 (23)	5 (19)
IBDV	0 (62)	0 (25)	0 (82)	0 (39)	0 (26)	0 (28)	0 (14)	0 (23)	0 (38)	—
ARV-10	0 (45)	—	0 (47)	0 (29)	—	—	—	—	—	—
ARV-20	0 (43)	12 (25)	2 (84)	3 (36)	0 (26)	4 (28)	—	0 (6)	9 (23)	—
ANV	0 (18)	0 (10)	0 (19)	—	—	—	—	—	—	—
ACV-M41	22 (64)	0 (15)	17 (70)	40 (48)	40 (15)	56 (41)	60 (25)	78 (60)	47 (34)	90 (21)
ACV-C46	59 (64)	0 (15)	46 (70)	67 (48)	67 (15)	68 (41)	56 (25)	65 (60)	62 (34)	100 (21)
ACV-A99	30 (64)	0 (15)	19 (70)	42 (48)	33 (15)	41 (41)	52 (25)	67 (60)	35 (34)	38 (21)
ACV-JMK	25 (64)	0 (15)	23 (70)	33 (48)	0 (15)	51 (41)	48 (25)	65 (60)	59 (34)	86 (21)
WNV	0 (9)	—	0 (10)	0 (9)	0 (15)	—	0 (10)	0 (20)	0 (9)	—
AEV	0 (77)	0 (25)	0 (120)	1 (68)	0 (11)	0 (28)	0 (10)	0 (18)	0 (34)	—
EEE	0 (18)	0 (10)	0 (19)	—	—	—	—	—	—	—
VEE	0 (18)	0 (10)	0 (19)	—	—	—	—	—	—	—
WEE	0 (18)	0 (10)	0 (19)	—	—	—	—	—	—	—
IAV	0 (92)	0 (25)	0 (140)	0 (68)	0 (26)	0 (30)	0 (14)	0 (21)	0 (38)	—
APMV-1	37 (138)	10 (30)	50 (137)	51 (92)	19 (26)	59 (56)	62 (29)	57 (61)	26 (58)	43 (21)
APMV-2	35 (138)	20 (30)	46 (137)	50 (92)	15 (26)	64 (56)	68 (25)	53 (58)	39 (54)	33 (21)
APMV-3	38 (128)	10 (30)	51 (158)	57 (82)	47 (15)	52 (46)	87 (15)	73 (40)	11 (45)	67 (21)

[a] See Table 1 for definitions of the tests used.

[b] — = not applicable.

penguins in temperate areas have higher *Aspergillus* spp. seroprevalence than did surface-nesting penguins in Subantarctic and Antarctic colonies (Graczyk and Cockrem 1995), suggesting that nesting behavior and environmental conditions might play an important role in determining exposure to this ubiquitous agent. This is consistent with other studies showing that environmental conditions, especially humidity and rainfall, are determinant of exposure to *Aspergillus* spp. in seabirds (Burco et al. 2014) and humans (Panackal et al. 2010). The higher prevalence of antibodies against *Aspergillus* sp. at Isla Martillo (Table 2) is consistent with reports of high incidence of aspergillosis in Magellanic Penguins at Isla Magdalena (Godoy et al. 2013), suggesting that the higher rainfall in the southern tip of South America (Vanstreels et al. 2017) causes penguins to experience a greater exposure to *Aspergillus* spp., poten-

TABLE 3. Seroprevalence for select infectious agents in free-ranging Magellanic Penguins (*Spheniscus magellanicus*) on the Argentinean coast, 1994–2008, compared to prevalences in Southern Rockhopper (*Eudyptes chrysocome*) from Argentina, Galapagos Penguins (*Spheniscus mendiculus*) from the Galapagos Islands, and Humboldt Penguins (*Spheniscus humboldti*) from Peru.

		Penguins					
		Magellanic			Southern Rockhopper[b]	Galapagos[c]	Humboldt[d]
Pathogen	Test and cutoff[a]	Prevalence	*n* positive/ *n* tested	95% CI	Prevalence[e]	Prevalence[e]	Prevalence[e]
Aspergillus sp.	ELISA	15.1	61/405	11.9–18.9	0	—	0
Chlamydia psittaci	CFT 1:10	37.1	239/644	33.5–40.9	2.5	89.0	62.0
Chlamydia psittaci	CFT 1:40	6.5	42/644	4.9–8.7	—	—	—
Salmonella Pullorum	MA 1:20	3.1	13/422	1.8–5.2	0	—	6.6
Salmonella Typhimurium	MA 1:25	81.3	74/91	72.1–88.0	—	—	—
Aviadenovirus sp.	AGID	18.1	39/215	13.6–23.8	—	0	—
Duck atadenovirus A	AGID	23.6	29/123	17.0–31.8	23.0	—	6.6
Gallid alphaherpesvirus 1	IFA 1:10	0	0/438	0–0.9	0	—	0
Gallid alphaherpesvirus 2	AGID	0	0/47	0–7.6	—	0	—
Anatid herpesvirus 1	SN 1:4	0.7	2/289	0.2–2.5	0	—	0
Infectious bursal disease virus	AGID	0	0/337	0–1.1	0	0	0
Avian orthoreovirus	IFA 1:20	3.3	9/271	1.8–6.2	23.0	0	1.6
Avastrovirus 2	IFA 1:10	0	0/47	0–7.6	—	—	—
Avian coronavirus M41	HI 1:10	43.5	171/393	38.7–48.5	23.0	—	0
Avian coronavirus C46	HI 1:10	59.8	235/393	54.9–64.5	47.0	—	5.0
Avian coronavirus A99	HI 1:10	37.4	147/393	32.8–42.3	23.0	—	5.0
Avian coronavirus JMK	HI 1:10	40.2	158/393	35.5–45.1	30.0	—	5.0
West Nile virus	PRNT	0	0/82	0–4.5	—	0	—
Tremovirus A	AGID	0.3	1/391	0.1–1.4	3.3	0	0
Eastern equine encephalitis virus	HI 1:10	0	0/47	0–7.6	—	0	0
Venezuelan equine encephalitis virus	HI 1:10	0	0/47	0–7.6	—	0	0
Western equine encephalitis virus	HI 1:10	0	0/47	0–7.6	—	0	0
Influenza A virus	AGID	0	0/454	0–0.8	0	0	0
Avian avulavirus 1	HI 1:8	44.0	285/648	40.2–47.8	20.0	0	1.6
Avian avulavirus 2	HI 1:8	43.8	279/637	40.0–47.7	37.0	0	6.6
Avian avulavirus 3	HI 1:8	46.6	270/580	42.5–50.6	3.3	0	1.6

[a] ELISA = enzyme-linked immunosorbent assay; CFT = complement fixation test; MA = microscopic agglutination; AGID = agar gel immunodiffusion; HI = hemagglutination inhibition; IFA = indirect immunofluorescence assay; SN = serum neutralization; PNRT = plaque reduction neutralization.

[b] Karesh et al. (1999).

[c] Travis et al. (2006).

[d] Smith et al. (2008).

[e] — = not tested.

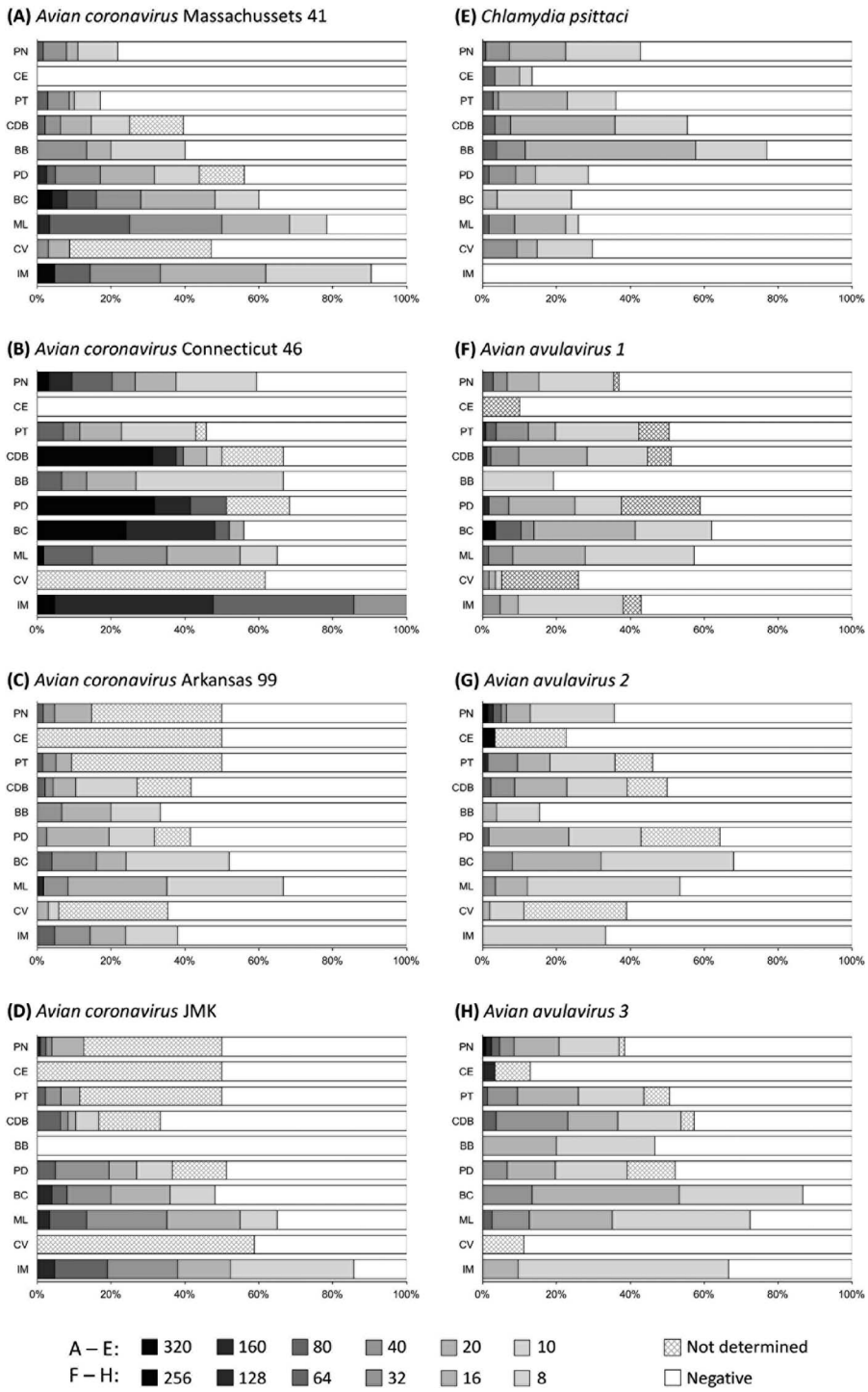

FIGURE 2. Comparison of the distribution of antibody titers for select infectious agents among breeding colonies of Magellanic Penguins (*Spheniscus magellanicus*) on the Argentinean coast, 1994–2008. Colonies: PN=Punta Norte; CE=Caleta Externa; PT=Punta Tombo; CDB=Cabo Dos Bahías; BB=Bahía Bustamante; PD=Puerto Deseado; BC=Banco Cormorán; ML=Monte León; CV=Cabo Vírgenes; IM=Isla Martillo.

tially leading to a greater risk of developing aspergillosis.

Chlamydia

Chlamydia psittaci can cause significant mortality in wild birds such as pigeons, ducks, geese, and gulls (Andersen and Vanrompay 2003; Andersen and Franson 2007). Penguins are also susceptible to this pathogen, as demonstrated by an outbreak in Magellanic Penguins at a zoo (Jencek et al. 2012).

Because cross-reaction with other Gram-negative bacteria can occur with the complement fixation test (CFT), only titers higher than 32 should be interpreted as positive (Office International des Epizooties 1996). When this threshold is applied, CFT's sensitivity and specificity are 72.2% and 71.9%, respectively (Salinas et al. 1993). High seroprevalence (>60%) for *C. psittaci* was reported in Galapagos (*Spheniscus mendiculus*) and Humboldt penguins (*Spheniscus humboldti;* Travis et al. 2006; Smith et al. 2008). However, the 1:10 cutoff used in those studies could have led to an overestimation of positives. For instance, if we had used a similar cutoff value, our estimated seroprevalence would have been almost six times greater (37.1%).

The failure to isolate *C. psittaci* despite relatively high CFT seroprevalences (Travis et al. 2006; González-Acuña et al. 2013) suggests cross-reactivity with antigenically-similar organisms. *Chlamydia psittaci* was demonstrated to infect captive Little Penguins (*Eudyptula minor*; Gedye et al. 2018), but other yet unidentified Chlamydiales are also known to infect wild penguins (Isaksson et al. 2015). A high prevalence (32.3%) of Chlamydiaceae species other than *C. psittaci* was recently detected in cloacal swabs of Magellanic Penguins in Argentina, and some of these were related to a strain previously found in feces of seabirds from the northern hemisphere (Gallo-Vaulet et al. 2017). Considering the low titers that we detected, it seems probable that our results reflected exposure to Chlamydiaceae species but not necessarily to *C. psittaci*.

Salmonella

Chickens are the primary hosts of *Salmonella* serotype Pullorum, but naturally occurring outbreaks have also been reported in ducks, passerines, pheasants, and parrots (Friend and Franson 1999; Shivaprasad and Barrow 2013). *Salmonella* serotype Typhimurium has been extensively documented to infect wild birds worldwide, including aquatic species such as gulls, terns, ducks, and herons (Friend and Franson 1999; Gast 2013). Although aquatic birds are often asymptomatic (Daoust and Prescott 2007), an outbreak of *S.* Typhimurium in African Penguins (*Spheniscus demersus*) at a European zoo was associated with lethargy, ruffled feathers, diarrhea, and death (Cockburn 1947).

The sera in this study were reactive against *S.* Pullorum and *S.* Typhimurium only at low dilutions, and it is, therefore, possible that cross-reactivity of antibodies against other salmonella serotypes occurred (Williams and Whittemore 1976; Lee et al. 2002). Numerous studies have isolated *S.* Typhimurium and other salmonella serotypes (but not *S.* Pullorum) from penguins elsewhere (Olsen et al. 1996; Palmgren et al. 2000; Iveson et al. 2009). Dougnac et al. (2015) isolated *S.* Enteriditis and *S.* Agona from fecal and cloacal samples of Magellanic Penguins in southern Chile, and some strains showed genotypic similarity with salmonella strains recovered from humans and gulls, suggesting interspecies transmission. Magellanic Penguin colonies are commonly surrounded by other seabird species such as Kelp Gulls (*Larus dominicanus*), which are often associated with human dwellings and waste (Yorio et al. 1998; Giaccardi and Yorio 2004). *Salmonella* Typhimurium has been isolated from free-ranging Kelp Gulls in Argentina (Frere et al. 2000), lending further credence to the hypothesis of interspecies transmission.

Adenoviridae

The genus *Aviadenovirus* comprises a number of species isolated from birds (McFerran and Smyth 2000; Fitzgerald

2007). Although most infections are subclinical, some strains have been implicated in respiratory disease and inclusion body hepatitis in domestic and wild birds (Fitzgerald 2007; Hess 2013). Infections with *Duck atadenovirus A* are mostly asymptomatic in aquatic birds (Gulka et al. 1984; McFerran and Smyth 2000). Yet severe acute respiratory disease has been reported in experimentally infected goslings (Ivanics et al. 2001).

The only *Adenoviridae* demonstrated to infect penguins is the *Chinstrap penguin adenovirus 1*, a putative species of the genus *Siadenovirus* that was detected in carcasses of Chinstrap Penguins (*Pygoscelis antarcticus*) in Antarctica (Lee et al. 2014, 2016). The agar gel immunodiffusion (AGID) tests targeting *Aviadenovirus* or *Atadenovirus* can cross-react (Adair et al. 1986), and it is unknown whether or not antibodies against *Siadenovirus* interfere with serological tests targeting these genera. Of note, we found a high seroprevalence (≥30%) for *Aviadenovirus* and *Duck atadenovirus A* at Magellanic Penguin colonies that are relatively close to breeding colonies of cormorants (*Phalacrocorax* spp.) (Gandini and Frere 1998; Frere et al. 2005). Cormorants are known to have high seroprevalence (40–67%) for *Aviadenovirus* in Argentina (Gallo et al. 2013), suggesting a potential interface for interspecies transmission.

Herpesviridae

Duck virus enteritis causes episodic mortality and decreased egg production and viability in ducks and teals. Attempts to experimentally infect other avian species have failed (Hansen and Gough 2007; Metwally 2013). There are no records of *Anatid herpesvirus 1* infection or seropositivity in penguins (Karesh et al. 1999; Smith et al. 2008), and the only reports of this virus in South America are from suspected cases at zoos in Brazil (Odend'hal 1983; Hansen and Gough 2007). Although we found antibodies against *Anatid herpesvirus 1* in two Magellanic Penguins, the positive predictive value of these two samples will be low due to the low predicted population seroprevalence (i.e., they are most likely false positive results).

The lack of detection of antibodies against *Gallid alphaherpesvirus 1* and *Gallid alphaherpesvirus 2* was not unexpected, considering that Galliformes and Anseriformes are the only known hosts to these viruses (García et al. 2013; Schat and Nair 2013), and that previous serological and PCR surveys in penguins also failed to detect their circulation (Miller et al. 2001; Travis et al. 2006; Smith et al. 2008). Previous studies show that some respiratory herpesviruses infecting penguins may elude detection by serological and molecular tests targeting gallid alphaherpesviruses (Parsons et al. 2015). Therefore, the negative results in this study should not be interpreted as an absence of exposure to other putative herpesvirus species such as *Magellanic penguin herpesvirus 1* and *Magellanic penguin herpesvirus 2*, the latter recently reported in several colonies included in this study (Niemeyer et al. 2017).

Reoviridae

Reoviral infections can cause a variety of disease syndromes in domestic and wild birds, including arthritis/tenosynovitis, decreased reproductive success, and gastrointestinal and respiratory disease, among others (Hollmén and Docherty 2007; Jones 2013). There are no studies conclusively demonstrating *Avian orthoreovirus* infection in penguins, but Gough et al. (2002) isolated reovirus-like particles from the tissues of African Penguins that died with gastrointestinal and respiratory lesions at a zoo in the UK. Previous surveys of *Avian orthoreovirus* in penguins found seroprevalences ranging from 0% to 23% (Karesh et al. 1999; Travis et al. 2006; Smith et al. 2008; Parsons et al. 2016). In addition, antibodies to this pathogen were also reported in other seabirds that share breeding habitat with Magellanic Penguins in Patagonia (Gallo et al. 2013), and it therefore seems likely that wild penguins are sporadically exposed to *Avian orthoreovirus*.

Coronaviridae

Avian coronavirus causes respiratory disease in chickens, and asymptomatic infections have been detected in numerous wild bird species (Cavanagh 2007; Hughes et al. 2009; Jackwood and de Wit 2013). The strong positive association among the serological results for M41, C46, and A99 strains in our results suggests antibody cross-reactivity occurred, which is not unexpected considering their phylogenetic relatedness (Callison et al. 2001).

The highest seroprevalence and antibody titers were found for the C46 and M41 serotypes, which have been extensively used for vaccination by the poultry industry (De Wit et al. 2011; Jackwood and de Wit 2013). This is consistent with previous studies on Southern Rockhopper Penguins in Argentina (Karesh et al. 1999) and can be interpreted as indicative of exposure to vaccinia variants (Hughes et al. 2009). Likewise, the only records of coronavirus infection in Magellanic Penguins are from individuals undergoing rehabilitation in Brazil, for which genetic analysis revealed close proximity to the H120 and M41 vaccinia variants (Niemeyer 2015). Moreover, cormorants in the Argentinean coast are also known to have high seroprevalence for *Avian coronavirus* (Gallo et al. 2013), suggesting a complex interface for the transmission of infectious agents from domestic or bridge species to wild seabirds.

Picornaviridae

Avian encephalomyelitis causes ataxia, tremors, decreased egg production, and mortality to Galliformes and Columbiformes (Suarez 2013). An antibody response to *Tremovirus A* has been occasionally reported in wild birds, albeit without evidence of disease (Padilla et al. 2003; Suarez 2013).

Only one penguin was seropositive for *Tremovirus A* in our study. Using the same AGID test as this study, Karesh et al. (1999) found one seropositive Southern Rockhopper Penguin in Argentina, whereas other researchers failed to detect seropositive penguins in South America (Travis et al. 2006; Smith et al. 2008). Given the low positive predictive value, we would interpret our findings as potentially false positive results. In contrast, enzyme-linked immunosorbent assay (ELISA) testing of African Penguins for antibodies against this virus found seroprevalence ranging from 1% to 5% in wild populations (Parsons et al. 2016). It is unclear, however, whether these serological results can be attributed to exposure to *Tremovirus A* or to cross-reactivity with other antigenically similar agents. The detection of a yet unidentified astrovirus in cloacal swabs from Adélie Penguins (*Pygoscelis adeliae*) in Antarctica (Grimaldi et al. 2015) suggests that penguins may be hosts to *Picornaviridae* other than *Tremovirus A*.

Paramyxoviridae

Avian paramyxovirus 1 (*Avian avulavirus 1*) affects a broad variety of domestic and wild birds worldwide, causing clinical signs ranging from decreased egg production to tremors, paralysis, and death (Leighton and Heckert 2007; Miller and Koch 2013). Low and high virulence strains of APMV-1 have been isolated from Antarctic penguins (Morgan and Westbury 1981; Alexander et al. 1989; Austin and Webster 1993; Thomazelli et al. 2010). Other avian paramyxoviruses are usually less pathogenic, but there are reports of APMV-2 and APMV-3 causing respiratory disease and decreased egg production in turkeys (Leighton and Heckert 2007; Miller and Koch 2013). A new *Avulavirus*, APMV-10, was identified in Southern Rockhopper Penguins from the Malvinas/Falkland Islands (Miller et al. 2010). Recently APMV-2 and APMV-10 have been reported in asymptomatic Magellanic Penguins sampled at a rehabilitation center in Brazil (Fornells et al. 2012).

The strong positive association in the serological results among APMV-1, APMV-2, and APMV-3 in this study suggests that antibody cross-reactivity occurred (Nayak et al. 2012; Tsunekuni et al. 2014). Considering recent studies showing the circulation of low pathogenicity APMV-1, APMV-2, and APMV-

10 in Magellanic Penguins and other seabirds in Brazil and Argentina (Zanetti et al. 2005; Miller et al. 2010; Fornells et al. 2012), it is difficult to determine to which paramyxoviruses the penguins in this study were exposed.

The high seroprevalence observed in this study contrasted with the lack of detection of antibodies against APMV-1 in Magellanic Penguins in Brazil (Nunes et al. 2012). Nunes et al. (2012) employed an ELISA test, which is less likely to produce false positive results due to cross-reactivity with other avian paramyxoviruses than the hemagglutination inhibition (HI) test we employed (Nayak et al. 2012). Other HI-based studies have also found high prevalence of antibodies against APMV-1 (20–33%) in penguins in Argentina and in the Antarctic Peninsula (Karesh et al. 1999; Thomazelli et al. 2010). Furthermore, a high APMV-1 seroprevalence (56%) was detected in apparently healthy cormorants in Argentina (Gallo et al. 2013), and virulent APMV-1 strains were detected in dead cormorants in southern Chile (Moreno et al. 2009), corroborating that there is *Paramyxoviridae* circulation among seabirds in South America.

Other viruses

Previous studies detected *West Nile virus* (Ludwig et al. 2002) and *Eastern equine encephalitis virus* (Tuttle et al. 2005) in captive Magellanic and African Penguins and *Influenza A virus* in Adélie Penguins in Antarctica (Hurt et al. 2014; Barriga et al. 2016). We found no evidence of exposure to these viruses in free-ranging Magellanic Penguins. Our failure to detect antibodies against *Venezuelan equine encephalitis virus* or *Western equine encephalitis virus* is consistent with the lack of detection in previous studies on penguins (Travis et al. 2006; Smith et al. 2008). We also failed to find antibodies against *Avastrovirus* 2, a pathogen that had not been evaluated in previous serological surveys of penguins.

There is conflicting evidence regarding the occurrence of *Birnaviridae* infections in penguins. Gough et al. (2002) isolated birnavirus-like particles from tissues of African and Macaroni Penguins (*Eudyptes chrysolophus*) that died at a zoo in the UK, yet they were unable to further characterize the strains. Serological surveys in other species of penguins in South America have not found sera reacting against *Infectious bursal disease virus* (Karesh et al. 1999; Travis et al. 2006; Smith et al. 2008). In contrast with these negative results and with our negative results, Nunes et al. (2012) found that 47% of Magellanic Penguins at rehabilitation centers in Brazil were seropositive. Although seropositivity to *Infectious bursal disease virus* has been consistently observed in penguins in Antarctica (Gardner et al. 1997; Gauthier-Clerc et al. 2002; Grimaldi et al. 2018), it has been proposed that these could be false positives due to exposure to crustacean birnaviruses in their diet (Grimaldi et al. 2018). These conflicting reports show that the question of whether or not *Infectious bursal disease virus* can infect penguins remains unresolved.

Although disease outbreaks in free-ranging Magellanic Penguins have been relatively uncommon so far, disease is considered a significant potential threat for this species (Kane et al. 2010, 2012; Trathan et al. 2015). The recent outbreak of high pathogenicity avian influenza in southern Africa, which caused mass mortality of endangered African Penguins (Khomenko et al. 2018), highlights the relevance of pathogens as significant threats to the conservation of penguins. While restricted by limitations inherent to serological methods, our results provide baseline knowledge for this key species in the South Atlantic Ocean. This information is valuable to guide adaptive management to protect seabird communities in coastal Patagonia, such as the establishment of biosecurity measures with regard to tourism activities and the importation and maintenance of exotic birds and poultry in the region.

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