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SEROSURVEY FOR SELECTED INFECTIOUS AGENTS IN TWO SYMPATRIC SPECIES OF CORMORANTS (*PHALACROCORAX ATRICEPS* AND *PHALACROCORAX MAGELLANICUS*) FROM COASTAL PATAGONIA, ARGENTINA

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ABSTRACT: We conducted a serologic survey for selected infectious agents on two sympatric cormorants, the Imperial Cormorant (*Phalacrocorax atriceps*) and the Rock Shag (*Phalacrocorax magellanicus*). Blood was collected from 267 Imperial Cormorants and 106 Rock Shags at 17 colonies along the Patagonia Atlantic shore during nine breeding seasons (1994, 1999–2001, 2005–2008, 2010). Antibodies to four pathogens were common to both species and frequently observed: avian paramyxovirus type 1 (56% of Imperial Cormorants and 56% of Rock Shags); avian adenovirus (67% of Imperial Cormorants and 40% of Rock Shags); infectious bronchitis virus serotypes IBV-41, IBV-46, IBV-99, and IBV-JMK (53% of Imperial Cormorants and 64% of Rock Shags); and *Salmonella pullorum* (18% of Imperial Cormorants and 7% of Rock Shags). Antibody prevalence for these pathogens varied significantly between species, except for avian paramyxovirus type 1. Exposure to avian paramyxovirus type 1 and all serotypes of infectious bronchitis virus varied significantly among seasons in both species. In contrast, the sporadic occurrence of positive titers suggest that cormorants had occasional exposure to *Aspergillus* spp. (3% of Rock Shags, only in 2000), avian paramyxovirus type 3 (5% of Rock Shags, only in 2008), *Chlamydophila* spp. (1% of Imperial Cormorants, only in 2010), and avian reovirus (1% of Rock Shags, only in 1999; 29% of Imperial Cormorants, in 2008 and 2010). Both species were antibody negative for avian encephalomyelitis virus, avian influenza virus, avian laryngotracheitis virus, avian paramyxovirus type 2, and infectious bursal disease virus. We provide the first information on pathogen exposure, indicated by detection of antibody in blood samples, for two sympatric species of South Atlantic cormorants. To determine major causes of morbidity and mortality in these birds future efforts should focus on necropsy surveys in cormorant colonies.

Key words: Antibodies, cormorants, infectious diseases, Patagonia, *Phalacrocorax atriceps*, *Phalacrocorax magellanicus*, seabirds, serology.

INTRODUCTION

Infectious diseases can regulate wild populations by reducing reproductive success and increasing mortality rates (Delahay et al., 2009). More importantly, pathogens can interact with other factors, such as habitat loss, climate change, overexploitation, invasive species, and environmental pollution, to drive populations temporarily or permanently to low numbers or population densities, predisposing them to local and global extinctions (de Castro and Bolker, 2005; Gerber et al., 2005; Smith et al., 2009). Lack of knowledge about the diversity and abundance of pathogens in natural systems has made it difficult to establish the relative importance of disease as a determinant of population trends, and the context in which this is most likely to occur.

Globally, many seabirds are threatened by human activities such as bycatch, overfishing or inappropriate spatial management of fisheries, human disturbance at breeding sites, and infrastructure/commercial/residential development near colonies (Croxall et al., 2012). Also, because of their gregarious breeding habits (Harrison, 1990), seabirds are particularly susceptible to catastrophic events and disease epidemics (Work, 1996). However, information on exposure to pathogens in seabirds is not readily accessible for most

species or geographic areas. Particularly for the Southern Hemisphere, the majority of literature on marine bird diseases covers sub-Antarctic and Antarctic species (Barbosa and Palacios, 2009), and only a few authors discuss the health status of continental seabirds in the southwest Atlantic (Karesh et al., 1999; Moreno et al., 2002; Uhart et al., 2003, 2008). Antibodies in blood samples, as indirect evidence of pathogen exposure, have been reported for seabirds breeding in Argentina including antibodies to avian adenovirus; avian encephalomyelitis virus; infectious bronchitis virus; avian reovirus; avian paramyxovirus types 1, 2 and 3; *Salmonella pullorum*; and *Chlamydophila* sp. (Karesh et al., 1999; Uhart et al., 2003, 2008).

The Imperial Cormorant (IC, *Phalacrocorax atriceps*) and Rock Shag (RS, *Phalacrocorax magellanicus*) are colonial seabird species widely distributed along the Patagonian coast in Argentina, with some of their colonies located near growing urban centers (Yorio et al., 1998). At the majority of locations, both species reproduce syntopically, nesting in adjacent or mixed colonies (Frere et al., 2005). Imperial Cormorants are the fourth most abundant species of seabird in Patagonia, with colonies of several thousand individuals, although colony size is highly variable (Frere et al., 2005). The population size of the RS is much smaller, and their breeding colonies are relatively small, ranging between two and 400 nests (Frere et al., 2005). Both species reproduce in dense aggregations, which could favor pathogen transfer (McCallum et al., 2001). Furthermore, cormorant colonies are commonly surrounded by other seabird species such as kelp gulls (*Larus dominicanus*; Yorio et al., 1998) which are often associated with human dwellings and waste (Giaccardi and Yorio, 2004). This has resulted in the infection of gulls with enterobacteria, some of which are potentially pathogenic to other seabird species and humans (Frere et al., 2000).

There are no studies on the health status of cormorants in Patagonia, and most disease reports in cormorants worldwide are limited to outbreaks of Newcastle disease (Blaxland, 1951; Wobeser et al., 1993; Glaser et al., 1999; Artois et al., 2002, Diel et al., 2012). Notwithstanding, Travis et al. (2006) found antibodies for avian adenovirus and *Chlamydophila psittaci* in the Flightless Cormorant (*Phalacrocorax harrisi*) in the Galápagos Islands, Ecuador.

As part of an ongoing long-term study on the reproductive and foraging ecology of IC and RS along the coasts of Patagonia, we determined exposure to selected avian infectious agents as evidenced by antibodies in blood samples. We also explored interspecific differences and interannual variability in antibody prevalences. Monitoring diseases in cormorants is important for the interpretation of future ecologic or disease disturbances, to predict population trends, and to assess the overall status of the marine ecosystem in which they live (Spalding and Forrester, 1993; Deem et al., 2001; Mörner et al., 2002).

MATERIALS AND METHODS

We sampled during the incubation and chick rearing periods of nine breeding seasons (1994, 1999–2001, 2005–2008, 2010). We sampled 267 IC and 106 RS in apparent good health (e.g., normal clinical behavior, no evidence of morphologic abnormalities or disease, and average body weights [Svagelj and Quintana, 2007]) at 17 colonies along coastal Patagonia, Argentina (between 42°30′S, 68°38′W and 50°15′S, 64°29′W; Fig. 1). Based on plumage coloration and development of caruncles all cormorants sampled were adults (Siegel-Causey, 1986).

Most colonies ($n=12$) were located to the north of Golfo San Jorge in Chubut, Argentina, where approximately 35% and 45% of the entire Patagonian population of IC and RS reproduce (Frere et al., 2005). Details on each breeding colony sampled have been described (Yorio et al., 1998). Birds were manually restrained and blood collection was added to routine clinical examination. Observational data on banded IC and RS suggests that individuals typically nest in the same area of the colony every year (Sapoznikow and Quintana, 2008; Svagelj and Quintana, 2011). Therefore, to avoid repeated sampling of

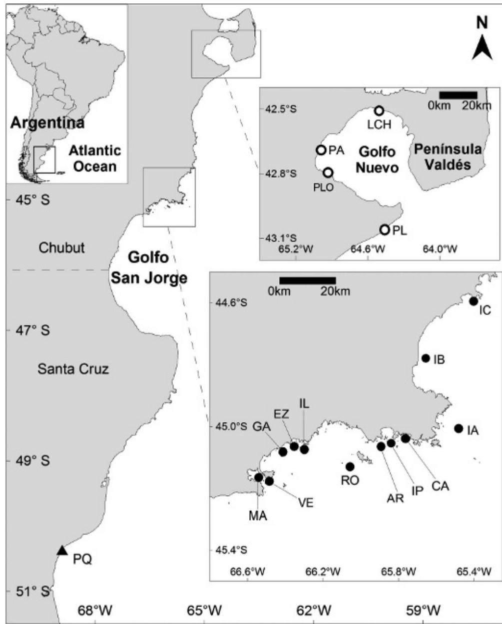

FIGURE 1. Location of sampling sites for cormorants tested for antibodies to selected infectious agents in coastal Patagonia, Argentina. Open circles represent colonies located near Golfo Nuevo, Chubut Province: LCH = Las Charas, PA = Punta Arco, PLO = Punta Loma, and PL = Punta León. Solid circles represent colonies located North of Golfo San Jorge, Chubut Province: IC = Isla Cumbre, IB = Islas Blancas, IA = Isla Arce, CA = Isla Cayetano, IP = Islote Puente, AR = Islotes Arellano, EZ = Isla Ezquerra, GA = Isla Galiano Sur, IL = Isla Lobos, RO = Isla Gran Robredo, MA = Roca Malaspina, and VE = Isla Vernacci Este. Solid triangle marks a single colony located in Santa Cruz Province: PQ = Pico Quebrado.

nonbanded individuals, different sections of colonies were selected in cases where recurring sampling occurred. Blood was drawn by venipuncture of the jugular vein, with the use of 10-cc heparinized syringes and 22 or 23 G × 2.5-cm needles. All samples were kept cool on ice until centrifuged at 1,000 × G for 20 min within 4–6 hr postcollection in a portable 12-volt centrifuge (Mobilespin, Vulcan Technologies, Grandview, Missouri, USA). Plasma was removed and frozen in liquid nitrogen. Samples were heat treated in a water bath (56 C for 3 hr) prior to being imported to the United States for testing and in accordance with US Department of Agriculture (USDA) regulations.

Serologic test selection was directed by positive results from other seabird species breeding in Patagonia, and by pathogen exposure reported in cormorants in other parts of the world. These included *Aspergillus* spp.; avian adenovirus; avian encephalomyelitis virus; avian influenza virus; avian paramyxovirus types 1, 2, and 3; avian reovirus; *Chlamydophila* spp.; infectious bronchitis virus serotypes Massachusetts 41 (IBV-41), Connecticut 46 (IBV-46), Arkansas 99 (IBV-99), and JMK (IBV-JMK); infectious bursal disease virus; infectious laryngotracheitis virus; and *Salmonella pullorum*. Tests were run at the Oklahoma Animal Disease Diagnostic Laboratory (Oklahoma State University, Stillwater, Oklahoma, USA) for 1999 samples and at the National Veterinary Service Laboratory (USDA, Ames, Iowa, USA) for samples collected in all remaining seasons. Table 1 summarizes the pathogens screened and serologic tests employed, except for *Aspergillus* spp. (detailed below). Where appropriate, antibody titers defined as positive in this study are reported. Samples from 1994/2000 and 1999/2006 were tested for *Aspergillus* spp. by agar gel immunodiffusion at the Raptor Center (University of Minnesota, St. Paul, Minnesota, USA) and at the Oklahoma Animal Disease Diagnostic Laboratory (Oklahoma State University), respectively.

Prevalence was defined as the percentage of animals that were antibody positive. Ninety-five percent confidence intervals are provided (Thrusfield, 2007). Generalized linear models with binomial family distribution and logit-link function (Crawley, 2007) were used to explore differences in antibody prevalence between species. The categorical explanatory variable "species" included in the models was evaluated using the likelihood ratio test.

Antibody titers were expressed as the inverse of the highest dilution with positive signal, and only were available for infectious bronchitis virus and avian paramyxovirus type 1. Differences in median antibody titers between species were evaluated using the nonparametric Mann-Whitney *U*-test due to the nonnormality and heteroscedasticity of the data (Sokal and Rohlf, 1995).

To explore changes in pathogen exposure over time, defined as changes in antibody prevalence across sampling periods, we only used data from colonies located at the North of Golfo San Jorge (Fig. 1), where both species were sampled in several seasons (IC=1999–2001, 2006, and 2007; RS=1999, 2000, and 2008). This analysis was performed only for pathogen-specific antibodies that were frequently observed in both species and for which prevalences were high (>50%) in at least one species (avian paramyxovirus type 1, all serotypes of infectious bronchitis virus and

TABLE 1. Serology of selected pathogens in Imperial Cormorants (*Phalacrocorax atriceps*) and Rock Shags (*Phalacrocorax magellanicus*) from coastal Patagonia, Argentina.

Pathogen	Test (NVSL)[a]	Positive titer	Test (OADDL)[a]	Positive titer
Avian adenovirus	AGID	NA	AGID	NA
Avian encephalomyelitis virus	AGID	NA	AGID	NA
Avian influenza virus	AGID	NA	AGID	NA
Avian paramyxovirus (types 1, 2 and 3)	HI	≥1:8	HI	≥1:40
Avian reovirus	IFA	≥1:20	AGID	NA
Chlamydophila spp.	CF	≥1:10	EBA	NA
Infectious bronchitis virus (serotypes IBV-41/46/99 and JMK)	HI	≥1:10/≥1:8[b]		
Infectious bursal disease virus	AGID	NA	ELISA	NA
Infectious laryngotracheitis virus	IFA	≥1:10	ELISA	NA
Salmonella pullorum	MA	≥1:40/≥1:20[c]	MA	≥1:40

[a] NVSL = National Veterinary Services Laboratory, US Department of Agriculture, 13th Street and Dayton Avenue, Ames, Iowa 50010, USA; OADDL = Oklahoma Animal Disease Diagnostic Laboratory, Oklahoma State University, Stillwater, Oklahoma, USA; ELISA = enzyme-linked immunosorbent assay; AGID = agar gel immunodiffusion; HI = hemagglutination inhibition; IFA = indirect immunofluorescence; CF = complement fixation; MA = microscopic agglutination; NA = not applicable; EBA = element body agglutination.

[b] Cutoff value provided by the laboratory for samples processed in 2006 for serotype IBV-99.

[c] Cutoff value provided by the laboratory for samples processed in 2000 and 2001.

avian adenovirus). Generalized linear models (GLM) with binomial family distribution and logit-link function (Crawley, 2007) were employed, and the categorical explanatory variable "season" included in the models was evaluated with the use of the likelihood ratio test. Statistical analyses were carried out using R software, Version 2.10.1 (R Development Core Team, 2010) and results were considered significant at $P<0.05$.

RESULTS

All cormorants examined ($n=372$) appeared healthy at the time of sample collection, indicated by normal clinical behavior, no evidence of morphologic abnormalities or disease, and average body weights (Svagelj and Quintana, 2007). Both species were negative for antibodies to avian encephalomyelitis virus, avian influenza virus, avian laryngotracheitis virus, avian paramyxovirus type 2, and infectious bursal disease virus. We saw evidence of exposure in both IC and RS for the rest of the infectious agents (avian paramyxovirus type 1, avian adenovirus, *Salmonella pullorum*, infectious bronchitis virus, and avian reovirus) except for *Aspergillus* spp. (IC) and *Chlamydophila* spp. (RS). For IC, highest (>50%) antibody prevalence was for avian adenovirus and avian paramyxovirus type 1, whereas for RS it was for infectious bronchitis virus (serotypes IBV-41 and IBV-99) and avian paramyxovirus type 1 (Table 2). The sporadic occurrence of positive titers suggests that cormorants had only occasional exposure to *Aspergillus* spp., avian paramyxovirus type 3, *Chlamydophila* spp. and avian reovirus.

Although some individuals had high antibody titers, titers were low in the majority of paramyxovirus type 1 antibody-positive animals of both species ($P>0.05$; Fig. 2). Conversely, antibody titers for infectious bronchitis virus were significantly greater in RS than in IC ($P<0.05$; Fig. 2). Exposure to selected pathogens varied significantly among seasons in both species (see methods), except for avian adenovirus (Table 3). Significant interannual variation in prevalence was observed for avian paramyxovirus type 1, and avian bronchitis virus (all serotypes; Fig. 3).

DISCUSSION

We provide the first information on pathogen exposure, indicated by the

TABLE 2. Number of animals tested (n), antibody prevalence (% positive), and 95% confidence intervals (CI) for selected pathogens in Imperial Cormorants (*Phalacrocorax atriceps*) and Rock Shags (*Phalacrocorax magellanicus*) from coastal Patagonia, Argentina. Only pathogens for which one or both species were positive are included. Asterisks show pathogens for which prevalences differed significantly between species ($P<0.05$). Both species were antibody negative for avian encephalomyelitis virus, avian influenza virus, avian paramyxovirus type 2, infectious laryngotracheitis virus, and infectious bursal disease virus.

	Imperial Cormorant			Rock Shag		
Pathogens	n	%	95% CI	n	%	95% CI
Aspergillus spp.	60	0.0	0.0–7.2	30	3.3[a]	0.0–18.1
Avian adenovirus*	165	67.3	59.8–74.0	92	40.2	30.8–50.4
Avian paramyxovirus						
Type 1	162	56.2	48.5–63.6	86	55.8	45.3–65.8
Type 3	120	0.0	0.0–3.73	84	4.8[b]	0.1–12.0
Avian reovirus*	96	29.4[c]	32.3–51.7	88	1.1[c]	0.01–6.8
Chlamydophila spp.	128	0.8[d]	0.01–4.73	86	0.0	0.0–5.1
Infectious bronchitis virus						
Serotype IBV-41*	117	21.4	14.9–29.7	72	51.4	40.1–62.6
Serotype IBV-46*	139	7.2	3.8–12.9	72	26.4	17.5–37.6
Serotype IBV-99*	139	47.5	39.4–55.7	72	62.5	50.9–72.8
Serotype IBV-JMK*	123	4.9	2.0–10.5	72	36.1	26.0–47.7
*Salmonella pullorum**	147	17.7	12.3–24.7	91	6.6	2.8–14.0

[a] One positive RS in Isla Galiano Sur in 2000.

[b] One RS positive in Punta Loma and four positive RS in Isla Vernacci Este, in 2008.

[c] One positive IC in 2008 in Isla Galiano Sur, 39 positives IC in 2010 in Punta León, and one positive RS in Punta Loma in 1999.

[d] One positive IC in Punta León in 2010.

presence of detectable antibody in blood of IC and RS. Repeated occurrence, high antibody prevalence, or antibody-positive animals found in most colonies sampled suggest that avian paramyxovirus type 1, avian adenovirus, avian infectious bronchitis virus, and *Salmonella pullorum* might be enzootic in cormorant populations in Patagonia. Variation in antibody exposure between species over time could be attributed to individual susceptibility (Cross et al., 2009) or frequency of exposure (Rogers et al., 2002; Koelle et al., 2005). Future efforts should focus on necropsy surveys in cormorant colonies to determine major causes of morbidity and mortality.

Exposure to avian paramyxovirus type 1 was seen over years in both species suggesting either enzootic presence of the virus or repeated exposure. Avian paramyxovirus type 1 has caused significant mortality in cormorants elsewhere (Gerlach, 1994; Kuiken, 1999), and exposure has been documented in other seabirds in Patagonia (Karesh et al., 1999; Uhart et al., 2003). Given its importance for poultry (Leighton and Heckert, 2007) and its pathogenicity for cormorants (Nisbet, 1995; Kuiken et al., 1999), future investigations should focus on isolating and characterizing this virus from Argentine cormorants.

Both species of cormorants were antibody positive for avian adenovirus at all sites with highest prevalences in IC. The recurring finding of antibodies to this virus could suggest continual reinfections given antibody short duration (McFerran and Adair, 2003). Avian adenoviruses are widely distributed and infected animals are often asymptomatic (Gerlach, 1994). Antibodies to avian adenovirus in seabirds sharing IC and RS habitats were reported in Patagonia (Karesh et al., 1999; Uhart et al., 2003, 2008). Exposure to this virus

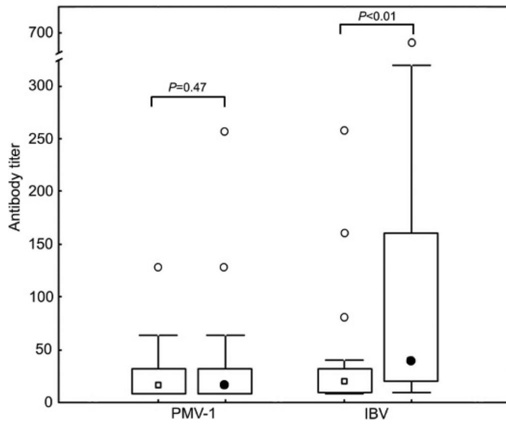

FIGURE 2. Antibody titers to avian paramyxovirus type 1 (PMV-1) and all serotypes of infectious bronchitis virus (IBV) in Imperial Cormorants (*Phalacrocorax atriceps*, open squares) and Rock Shags (*Phalacrocorax magellanicus*, filled circle) from coastal Patagonia, Argentina. Overall *P* values represent comparisons by the Mann-Whitney *U*-test. The central symbol marks the median titer, each box shows the interquartile range (25 and 75 percentile), the vertical lines (whisker) indicate nonoutlier ranges and open circles represent outliers.

should continue to be monitored because latent infections can become activated in immunocompromised animals (Ritchie, 1995) or by stress associated with environmental degradation and contamination (Leighton and Heckert, 2007).

Antibodies to infectious bronchitis virus were found in cormorants from most of the colonies sampled, though variability among seasons and between species was noted. Coronaviruses causing infectious bronchitis are widely distributed and generally considered pathogens of poultry (Gerlach, 1994; Cavanagh and Naqi, 2003). Karesh et al. (1999) reported coronavirus antibodies in rockhopper penguins at a breeding colony on the Patagonia coast. To our knowledge, infectious bronchitis exposure has not been described before in cormorants, limiting interpretation of its significance.

Salmonella pullorum antibody was found in both species of cormorants, but was higher in IC. An outbreak of *Salmonella enteritidis* killed several seabird species along the coast of Brazil (Cubas, 1993) and more recently, *Salmonella typhimurium* was isolated from dead Double-crested Cormorants (*Phalacrocorax auritus*) in Canada (Clavijo et al., 2001). This bacterium has been isolated from free-ranging Kelp Gulls in Patagonia (Frere et al., 2000) and penguins (Spheniscidae) in Antarctica and Patagonia (Cubas, 1993). Necropsies and isolation of the bacteria are needed to interpret the meaning of our findings.

Rock Shags and Imperial Cormorants were antibody positive, albeit at very low prevalences, to avian reovirus, *Aspergillus* spp. (RS), avian paramyxovirus type 3 (RS), and *Chlamydophila* spp. (IC). Previous investigators reported antibodies to these pathogens in other seabirds that share breeding and feeding habitat with cormorants (Karesh et al., 1999; Uhart et al., 2008). Although these agents can cause disease in birds (Ritchie and Carter, 1995), given the uncertain performance of the serologic tests used in cormorants, the significance of these results remains

TABLE 3. Results from generalized linear models assessing the effect of sampling season on pathogen-specific antibody prevalence in cormorants from northern Golfo San Jorge, Argentina.

	Likelihood ratio test					
	Imperial Cormorant			Rock Shag		
Pathogen	χ^2	df[a]	*P* value	χ^2	df[a]	*P* value
Avian paramyxovirus type 1	88.15	4	<0.001	8.18	2	0.017
Avian adenovirus	4.60	3	0.203	1.78	2	0.410
Infectious bronchitis virus (all serotypes)	28.99	3	<0.001	5.81	1	0.016

[a] df = degrees of freedom.

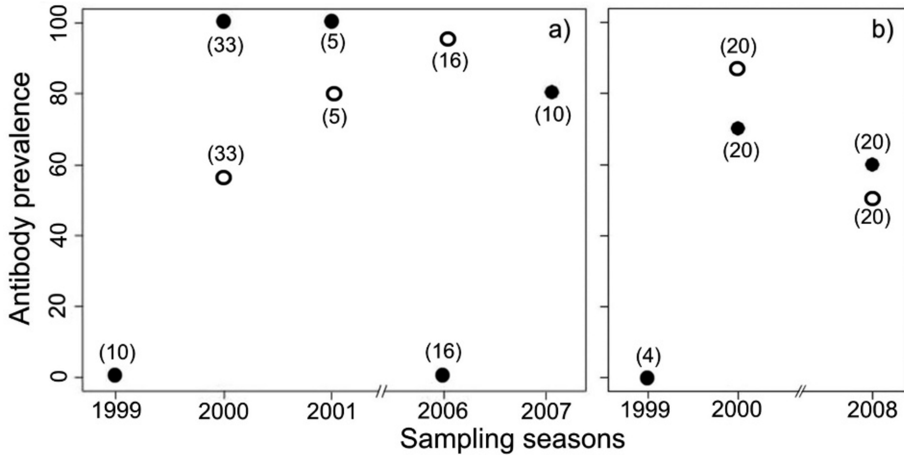

FIGURE 3. Variability in antibody prevalence (%) across sampling seasons was observed for avian paramyxovirus type 1 (filled circles) and all serotypes of avian bronchitis virus (open circles) in Imperial Cormorant (*Phalacrocorax atriceps*) (a) and Rock Shag (*Phalacrocorax magellanicus*) (b) from colonies north of Golfo San Jorge, Argentina.

speculative and requires further data from morbidity and mortality surveys.

Although we found antibodies to numerous common avian pathogens, serologic tests used were not validated for cormorants. Thus, interpretation of findings is limited. Future efforts should focus on the isolation and molecular characterization of cormorant pathogens to assess their significance for cormorant and seabird conservation in Patagonia.

Coastal Patagonia is undergoing significant changes, and current trends in urban and industrial development could increase the vulnerability of seabird colonies to human disturbance (Yorio et al., 1999; Uhart et al., 2008). Long-term monitoring of pathogen exposure and necropsy surveys coupled with information on environmental stressors, reproductive success, and population trends are important to understand drivers of Patagonia seabird population dynamics and implement adaptive conservation management.

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