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Absence of Coronaviruses, Paramyxoviruses, and Influenza A Viruses in Seabirds in the Southwestern Indian Ocean

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ABSTRACT: We investigated circulation of coronaviruses, paramyxoviruses, and influenza A viruses in eight seabird species of the southwestern Indian Ocean. Viruses were not detected by real-time polymerase chain reactions in the 338 tested cloacal swab samples, supporting that they did not circulate in the studied colonies at the time of sampling.

Coronaviruses (COV), paramyxoviruses (PMV), and influenza A viruses (IAV) have been detected in a large diversity of wild bird species (e.g., Stallknecht and Shane, 1988; Coffee et al., 2010; Muradrasoli et al., 2010). In seabirds, COV, PMV, and IAV infections are commonly reported in species in the order Charadriiformes (gulls, terns, shorebirds). Surveillance programs have provided key information on IAV ecology and epidemiology (Olsen et al., 2006); however, only limited data related to the host reservoirs and spatial and temporal variation in the prevalence of infection with COV and PMV are available.

With 31 species and about 7.4 million pairs, seabirds represent the most abundant avifauna in the southwestern Indian Ocean (Le Corre, unpubl. data). Many seabird species aggregate at very high densities in colonies for breeding. Such aggregations can involve hundreds of thousands of birds and may favor virus transmission. Viruses and ectoparasites have been documented in seabirds in the southwestern Indian Ocean, sometimes associated with mortality and nest desertion (Converse et al., 1975, 1976; Feare, 1976). We investigated COV, PMV, and IAV in colonies of eight seabird species from five islands of the southwestern Indian Ocean: Réunion, Mayotte, Europa, Tromelin, and Madagascar.

Cloacal swabs were collected from 338 birds (adults and chicks) between April 2011 and February 2012 (Table 1). Swabs were stored in 1 ml of RNA NOW™ (BIOGENTEX, Seabrook, Texas, USA), frozen at −20 C in the field, shipped to the laboratory in a cooler with ice packs within 48 hr, and held at −80 C until tested. RNA extraction was performed following RNA NOW isolation and purification protocol; samples were eluted in a final volume of 60 µL. Reverse transcription was performed on 20 µL of RNA product, using 0.1 µg of random hexamers (Promega, Madison, Wisconsin, USA) and the GoScript™ Reverse Transcriptase (Promega, Madison, Wisconsin, USA), under the following thermal conditions: 80 C for 5 min, 25 C for 15 min, 42 C for 60 min, and 70 C for 5 min. The cDNA was diluted 1:2 and stored at −20 C until tested. Before RNA extraction, 10 µl of RNA of the MS2 phage was added to each cloacal sample; after the reverse-transcription step, all samples were tested for cDNA of the MS2 phage (Ninove et al., 2011).

Real-time PCR was performed on MS2-positive samples only, following published protocols optimized for the detection of avian IAV, PMV, and COV (respectively: Spackman et al., 2002; Kim et al., 2008; Muradrasoli et al., 2009). The ABsolute Blue qPCR Low ROX Mix (Thermo

TABLE 1. Location, collection date, status, and number of birds sampled and tested for the detection of coronaviruses, paramyxoviruses, and influenza A viruses southwestern Indian Ocean, April 2011–February 2012.

Order	Common name	Species	Location	Date	Status	*n*
Charadriiformes	Lesser Noddy	*Anous tenuirostris*	Reunion (21°22′S, 55°34′E)	December 2011	Nonbreeding adults	26
Phaethontiformes	White-tailed Tropicbird	*Phaethon lepturus*	Europa (22°21′S, 40°21′E)	December. 2011	Breeding adults	29
			Mayotte (12°50′S, 45°08′E)	February 2012	Breeding adults	20
	Red-tailed Tropicbird	*Phaethon rubricoda*	Europa (22°21′S, 40°21′E)	December 2011	Breeding adults	49
			Europa (22°21′S, 40°21′E)	April 2011	Breeding adults and chicks	9
			NosyVe (23°39′S, 43°37′E)	July 2011	Breeding adults	39
Procellariiformes	Barau's Petrel	*Pterodroma baraui*	Reunion (21°07′S, 55°25′E)	December 2011–January 2012	Breeding adults	35
	Wedge-tailed Shearwater	*Puffinus pacificus*	Reunion (21°22′S, 55°34′E)	December 2011–January 2012	Breeding adults	44
Suliformes	Lesser Frigatebird	*Fregata ariel*	Europa (22°21′S, 40°21′E)	December 2011	Chicks	1
	Great Frigatebird	*Fregata minor*	Europa (22°21′S, 40°21′E)	December 2011	Chicks	18
	Red-footed Booby	*Sula sula*	Europa (22°21′S, 40°21′E)	December 2011	Nonbreeding adults	30
			Europa (22°21′S, 40°21′E)	April 2011	Nonbreeding adults and chicks	7
			Tromelin (15°53′S, 54°31′E)	April 2011	Nonbreeding adults and chicks	31

Fisher Scientific, Surrey, UK) was used in a final volume of 25 μL containing 5 μL of cDNA; PCRs were carried out in a Bio-Rad CFX96 Touch™ (Bio-Rad, Hercules, California, USA) real-time PCR detection system. All PCRs were run with a negative and a positive control.

We did not detect COV, PMV, and IAV in the 338 cloacal swab samples, suggesting that these viruses did not circulate in the populations at the time of sampling. Nevertheless, the low sample size might have limited the probability of detection if prevalence was low. Factors related to the epidemiology of these viruses may also have affected this result. Toennenssen et al. (2011) reported interannual variations in the prevalence of IAV in a breeding colony of Black-legged Kittiwake (*Rissa tridactyla*), ranging from 5% to 15%. The age of sampled birds can also affect virus detection in a colony (Velarde et al., 2010), and spatial variation in prevalence has been shown between nearby colonies of Ring-billed gulls (*Larus delawarensis*) in North America (Velarde et al., 2010).

Few studies have focused on COV, PMV, and IAV detection in seabirds in the Phaethontiformes, Procellariiformes, and Suliformes orders. In Procellariiformes, IAV have been documented but at low prevalence (e.g., 0.3%) as compared with waterbirds such as gulls and ducks (Olsen et al., 2006). In the Indian Ocean, Mackenzie et al. (1984) detected IAV in only three of 531 sampled Wedge-tailed Shearwaters (*Puffinus pacificus*) on the western coast of Australia, although 4% of sampled ducks

were positive. In the latter study, PMV were also isolated from several duck and tern species but not from shearwaters. Although additional studies would be required, our results suggest that seabird species in the Procellariiformes, and likely those in the Phaethontiformes and Suliformes, may not act as important host reservoirs for COV, PMV and IAV in the southwestern Indian Ocean.

Future surveillance should focus on other species, in particular those from the Charadriiformes. Sooty Terns (*Onychoprion fuscatus*), for example, are the most abundant seabird species in the southwestern Indian Ocean and breed at very high densities in colonies (>6 nests/m^2; Feare et al., 1997). In Australia, IAV and PMV have been documented in this species and in Lesser Noddy (*Anous tenuirostris*; Mackenzie et al., 1984), highlighting the potential importance of terns in virus epidemiology. In these hosts, genetic variants may also exist, such as the H15 subtype of IAV isolated in Australia (Röhm et al., 1996), suggesting that their circulation could be limited to seabird populations in the Indian Ocean.

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