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Source: Journal of Wildlife Diseases, 39(2) : 359-365

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-39.2.359

Journal of Wildlife Diseases, 39(2), 2003, pp. 359–365

HEMATOLOGY, PLASMA BIOCHEMISTRY, AND SEROSURVEY FOR SELECTED INFECTIOUS AGENTS IN SOUTHERN GIANT PETRELS FROM PATAGONIA, ARGENTINA

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ABSTRACT: In conjunction with reproductive and feeding ecology studies on southern giant petrels (SGP, *Macronectes giganteus*) blood samples were collected for baseline health evaluations. Twenty-five adult SGP from a breeding colony in Chubut, Argentina, were sampled during two consecutive breeding seasons, 1999–2000 ($n=15$) and 2000–01 ($n=10$). Values for hematology, plasma biochemistry, and minerals are described for 20 birds in apparent good physical condition. A serologic survey of exposure to selected infectious agents was also conducted on all 25 birds sampled. Southern giant petrels were serologically negative for evidence of exposure to infectious laryngotracheitis virus, avian encephalomyelitis virus, avian influenza virus, avian reovirus, infectious bursal disease virus, infectious bronchitis virus, paramyxovirus 1, 2, and 3 virus, *Chlamydophila,* and *Aspergillus.* Antibodies to avian adenovirus were found in 14% of SGP during the first sampling season, and 60% in the second year. Additionally, all birds were negative for antibodies to *Salmonella pullorum* at the first sampling date, but 90% had low titers the following breeding season. This study contributes to understanding the health status of South Atlantic seabirds and to establishment of baseline information for SGP. Long-term monitoring of pelagic predator-scavenger seabirds such as SGP should be established for the surveillance of marine ecosystem health.

Key words: Biochemistry, health evaluation, hematology, *Macronectes giganteus,* pelagic seabird, serology, southern giant petrel.

INTRODUCTION

The health status of Patagonian seabirds is poorly known (Karesh et al., 1999) and there is no report on the health status of pelagic feeders such as southern giant petrels (SGP, *Macronectes giganteus*) in the southwestern Atlantic Ocean.The SGP is the largest avian predator-scavenger in the southern ocean ecosystem (Hunter, 1985). Its circumpolar distribution includes colonies on the Antarctic Peninsula, many subantarctic islands, and on the Patagonian coast in Argentina (Hunter, 1984; Patterson et al., 2000). There are four colonies in Patagonia: two in Chubut (45°S, 65°W) and two in Tierra del Fuego (54°S, 64°W; Yorio et al., 1998). Despite its important ecologic role in the South Atlantic ecosystem (Hunter, 1985), basic aspects of their breeding biology remain unknown for the Patagonian colonies. Recent studies showed that breeding birds from Gran Robredo Island (Patagonia, Argentina) forage through ample oceanic areas and exploit diverse marine habitats on the Patagonian shelf (Quintana and Dell'Arciprete, 2002).

Reproductive populations of SGP are decreasing in most of colonies within their worldwide distribution range (Patterson et al., 2000); population trends are unknown for this species in Argentina. The causes of large-scale population decline have not yet been determined, although they could be influenced by environmental disturbance and degradation, contamination, disease, reduction of food sources, use and discard of non-selective fishing gear, and by incidental catch in commercial fisheries (Schia-

vini et al., 1997; Patterson et al., 2000). Southern giant petrels recently were considered threatened by the International Convention on the Conservation of Migratory Species of Wild Animals (United Nations Environment Programme) and the BirdLife International Seabird Conservation Program.

As part of an on-going study of the reproductive and foraging ecology of SGP we conducted health evaluations of adult SGP breeding in Patagonia. Our objectives were to establish baseline hematologic and plasma biochemical indices and evaluate serologic evidence of exposure to selected infectious agents. Monitoring population health is necessary for interpretation of future ecologic or disease disturbances, to predict population trends, and to evaluate the overall status of the marine ecosystem (Spalding and Forrester, 1993; Deem et al., 2001; Mörner et al., 2002).

MATERIALS AND METHODS

Study area

Southern giant petrel blood samples were collected at Isla Gran Robredo (45°08′S, 66°03′W), which holds a reproductive population of 1,600 breeding pairs (Yorio et al., 1998). The island is located at 14 km from the continental shore. The terrain is rocky with no vegetation. Breeding colonies of terns (*Sterna* spp.), imperial cormorants (*Phallacrocorax albiventer*), rock shags (*Phallacrocorax magellanicus*), kelp gulls (*Larus dominicanus*), and dolphin gulls (*Larus scoresbii*), and a non breeding rookery of southern sea lion (*Otaria flavescens*), share the island with the SGP (Yorio et al., 1998).

Sample collection and storage

Field studies were conducted during incubation and chick rearing periods of the breeding seasons (November–January) of 1999–2000 and 2000–01. A total of 25 blood samples, 15 during the first year and 10 in the second, were collected from manually restrained apparently healthy adult SGP. Handling procedures included body measurements, weight, notation of visual or palpable abnormalities, and blood collection. Five body measurements were taken: wing length, head length, bill length and depth, and tarsus length. Both wing and tarsus measurements were taken on the right side of the body. For bill and tarsus size, we used a digital vernier caliper (±0.01 mm) and two stopped rulers were designed for head and wing measurements (±1 mm). Body weights were measured by use of a 5 kg spring scale to the nearest 100 g.

Approximately 20 ml of blood (<1% of body weight) were drawn by jugular venipuncture using a heparinized syringe and 21G×2.5 cm needle. All samples were stored in plain glass vacuum tubes (Benton-Dickinson, Rutherford, New Jersey, USA) and kept cool on ice, until processing, within 6 hr of collection.

Sample processing

Once at the field laboratory, thin blood smears were prepared from heparinized blood and fixed with 99% methanol. Microhematocrit tubes were centrifuged in a portable 12 volt centrifuge (Mobilespin, Vulcan Technologies, Grandview, Missouri, USA) for packed cell volume (PCV) determination. Plasma total solids (TS) were measured using a hand-held refractometer (Schulco, Toledo, Ohio, USA) calibrated at the site. Granulocytes were counted using the Unopette Test 5877 (Benton-Dickinson) following manufacturer instructions (Campbell, 1994). The remaining blood was centrifuged for 20 min and plasma was removed and frozen in liquid nitrogen. Plasma samples were heat treated in a water bath at 56 C for 3 hr in accordance with United States Department of Agriculture regulations before importation to the US.

Sample and data analysis

For white blood cell (WBC) differential counts, blood smears were stained with modified Wright-Giemsa stain (Hematology Threestep Stain, Accra Lab, Bridgeport, New Jersey). Total white cell counts were determined using the methodology described by Dein et al. (1994). Plasma chemistries and enzymes were processed on a wet automated analyzer (Hitachi 747 Wet Chemistry Analyzer, Boehringer Mannheim Corporation, Indianapolis, Indiana, USA) at a commercial veterinary laboratory (Idexx Veterinary Services Laboratory, Totowa, New Jersey). The chemistry and enzyme panels were tested on a sub-sample of 20 individuals, 10 from each sampling year. Tests conducted are shown in Table 1.

Serum mineral panels were tested by inductively coupled argon plasma emission spectroscopy as described by Stowe et al. (1985) at the Animal Health Diagnostic Lab (Michigan State University, East Lansing, Michigan, USA). Tests conducted are shown in Table 2.

Serologic tests for antibodies to selected in-

TABLE 1. Hematology, plasma chemistry, and enzyme and mineral values for southern giant petrels in Argentina.

Test (units)	Mean±SD	Range	*n*
Hematocrit (%)	47.4±4.0	38–53.5	20
White blood cells (cells/mm^3 × 10^3)	4.0±1.2	2.1–6.58	25
Heterophils (cells/mm^3 × 10^3)	2.1±0.7	0.9–3.9	25
Lymphocytes (cells/mm^3 × 10^3)	1.3±0.5	0.4–2.5	25
Monocytes (cells/mm^3 × 10^3)	0.2±0.1	0.02–0.37	25
Eosinophils (cells/mm^3 × 10^3)	0.4±0.2	0.11–1.10	25
Basophils (cells/mm^3 × 10^3)	0.006±0.023	0–0.01	25
Total solids (g/dl)	6.2±1.1	4.2–8.4	25
Glucose (mg/dl)	285±39	198–367	20
Blood urea nitrogen (mg/dl)	3.4±2.5	1–10	20
Uric acid (mg/dl)	8.9±2.3	4.3–12.3	20
Total protein (g/dl)	3.4±1.0	2.2–6.1	20
Albumin (g/dl)	1.5±0.3	0.8–2	20
Globulins (g/dl)	2.3±0.86	1.4–4.6	20
Albumin/Globulin ratio	0.68±0.16	0.3–0.9	20
Alkaline phosphatase (IU/l)	<3[a]		14
	4.3±2.4	3–11	6
Lactate dehydrogenase (IU/l)	18.0±9.9	6–48	20
Amylase (IU/l)	11.8±16.86	0–53	20
Creatine kinase (IU/l)	<4[a]		17
	5.7±1.5	4–7	3
Aspartate aminotransferase (IU/l)	93.1±29.6	37–180	20
Alanine aminotransferase (IU/l)	<4[a]		10
Calcium (mg/dl)	9.1±1.2	5.6–10.6	20
Phosphorus (mg/dl)	2.4±2.0	1.9–7	20
Potassium (mEq/l)	<1.5[a]		15
	2.2±0.29	1.9–2.6	5
Sodium (mEq/l)	154.5±15.5	100–171	20
Chloride (mEq/l)	121.3±5.8	108–128	10
Bile acids (μmol/l)	36.3±13.7	15.7–63.3	10

[a] Animals with values below detectable limits.

TABLE 2. Soluble plasma elements for southern giant petrels in Argentina.

Test (units)	Mean±SD	Range	*n*
Total iron (ppm)	1.36±0.57	0.87–2.51	10
Soluble iron (ppm)	<0.50		10
Total phosphorus (ppm)	228.2±27.52	196–293	10
Inorganic phosphorus (ppm)	41.83±23.76	11.1–72.2	10
Potassium (ppm)	45.01±24.15	16.8–85.3	20
Sodium (ppm)	3870.5±172.4	3470–4330	20
Calcium (ppm)	107.02±8.89	87.5–122	20
Magnesium (ppm)	36.7±12.56	21.3–63.1	20
Zinc (ppm)	2.33±0.49	1.44–3.55	20
Boron (ppm)		<1.00–<1.67	20
Copper (ppm)	0.19±0.04	0.09–0.26	20
Manganese (ppm)		<0.062–<0.083	10
Chromium (ppm)		<0.1–<0.33	20
Cobalt (ppm)		<0.125–<0.167	10
Molybdenum (ppm)		<0.25–<0.33	10
Barium (ppm)	0.097±0.01	0.01–0.07	10

TABLE 3. Serologic test procedures, level of titers defined as positive, and results for each test used for analysis of exposure to infectious agents for southern giant petrels from Patagonia, Argentina.

Disease or pathogen	Test procedure	Positive titer	Number positive/number tested (% positive) 1999–2000	2000–01
Aspergillus	Enzyme linked immunosorbent assay	0.5	0/13	0/10
Avian adenovirus	Agar gel immunodiffusion	NA[a]	2/14 (14%)	6/10 (60%)
Avian encephalomyelitis virus	Agar gel immunodiffusion	NA	0/14	0/10
Avian influenza virus	Agar gel immunodiffusion	NA	0/14	0/10
Avian paramyxovirus-1	Hemagglutination inhibition	1:8	0/14	0/10
Avian paramyxovirus-2	Hemagglutination inhibition	1:8	0/14	0/10
Avian paramyxovirus-3	Hemagglutination inhibition	1:8	0/14	0/10
Avian reovirus	Indirect immunofluorescence	1:20	0/10	0/10
Chlamydophila spp.	Complement fixation	1:10	0/14	0/10
Infectious bronchitis virus	Hemagglutination inhibition	1:10	0/14	0/10
Infectious bursal disease virus	Agar gel immunodiffusion	NA	0/14	0/10
Infectious laryngotracheitis virus	Indirect immunofluorescence	1:10	0/10	0/10
Salmonella pullorum	Microscopic agglutination	1:20	0/14	9/10[b] (90%)

[a] NA=not applicable.
[b] Antibody titers=1:20.

fectious agents (Table 3) were conducted at the Oklahoma Animal Disease Diagnostic Laboratory (Oklahoma State University, Stillwater, Oklahoma, USA) for samples collected in 1999–2000 (n=15) and at the National Veterinary Service Laboratory (US Department of Agriculture, Ames, Iowa, USA) for those collected in 2000–01 (n=10). Serology for antibodies against *Aspergillus* sp. was conducted at the Oklahoma Animal Disease Diagnostic Laboratory for the 1999–2000 samples and at the Raptor Center (University of Minnesota, St. Paul, Minnesota, USA) for the 2000–2001 samples.

Descriptive statistics were conducted for results of hematology, plasma chemistry, and mineral analyses using Statistica for Windows (version 5.1 97, StatSoft, Inc., Tulsa, Oklahoma).

RESULTS

All SGP examined (n=25) were in good physical condition; body weights ranged between 2.5 and 4.1 kg (mean 3.2, SD=0.5, n=24). Hematology and plasma biochemistry results are provided in Table 1. Results of analyses for plasma elements are show in Table 2.

All SGP were negative for antibodies to infectious laryngotracheitis virus, avian encephalomyelitis virus, avian influenza virus, avian reovirus, infectious bursal disease virus, infectious bronchitis virus, paramyxovirus 1, 2, and 3, *Chlamydophila* and *Aspergillus.* Antibodies were detected to avian adenovirus and *Salmonella pullorum* (Table 3).

DISCUSSION

All animals sampled were in apparent good health. Hematologic and plasma biochemical data were clinically unremarkable and similar to reported values (Work, 1996; Newman et al., 1997). Overall, SGP total WBC and differential cell values were lower than those reported by Work (1996) for adult Hawaiian dark-rumped petrels (*Pterodoma phaeopygia*) and Laysan albatross (*Diomedea immutabilis*), but similar or higher than those found by Newman et al. (1997) for northern fulmar (*Fulmarus glacialis*). Plasma biochemistries were similar to values reported for northern fulmar, similar or lower than those reported for dark-rumped petrels, and similar or higher than those reported for Laysan albatross. When compared to biochemistry values reported by Work

(1996) for Laysan albatross, the values for SGP found in our study were similar only to those of the albatross sampled post-incubation, which coincided with the sampling period of our study. To our knowledge, there are no previous reports of hematology and plasma biochemistry values for free-ranging SGP and our study provides a valuable baseline for comparison with other populations and for the study population over time.

Antibodies were found for only two infectious agents (Table 3). Antibodies to avian adenovirus were found in 14% of SGP examined in 99–00 and in 60% SGP tested during the following breeding season. The significance of these findings is unknown because there are no previous reports of this virus in Procelariiformes. Additionally, the agar gel immunodiffusion test used to detect avian adenovirus antibody has only moderate specificity, has not been validated for this species, and could result in false positives (Hietala and Gardner, 1999; Ritchie et al., 2001). Nonetheless, avian adenovirus are distributed worldwide and many avian species are known to be susceptible. This agent causes disease in galliformes and has also been reported as a possible pathogen in pigeons, raptors, psittaciformes, and waterfowl (Gerlach, 1994b). Antibodies have been found by Karesh et al. (1999) in rockhopper penguins (*Eudyptes chrysocomes*) from Patagonia and in other seabirds such as Magellanic penguins (*Spheniscus magellanicus*), imperial cormorants, and rock shags sharing SGP habitat (Uhart et al., unpubl. data).

Ninety percent of SGP sampled in the second year of our study had low antibody titers to *S. pullorum,* while all birds from the previous year tested negative. The finding of seropositive SGP with antibody titers at the cut-off value for the test used and only during one season may indicate that the sensitivity of the test was not appropriate for this species (Hietala and Gardner, 1999). However, these results indicate the need for future studies to evaluate exposure to *Salmonella* by more specific methods such as culture. Even though we cannot interpret the significance of the *Salmonella* antibodies found in this population of SGP, some avian species, such as penguins and gulls, are particularly susceptible to some species of *Salmonella,* suffering acute disease and high flock mortality (Steele and Galton, 1977; Friend, 1987; Gerlach, 1994a). *Salmonella typhimurium* has been isolated from kelp gulls in Patagonia (Frere et al., 2000) and penguins in Antarctica and Patagonia (Cubas, 1993). Reports of wild bird habitat contamination with *Salmonella* sp. from sewage, wastewater, and livestock and poultry operations are abundant (Steele and Galton, 1977; Fenlon, 1981; Fricker, 1984; Stroud and Friend, 1987).

This study provides the first baseline health parameters of this little known species. Long-term monitoring of the health of SGP and other longed-lived seabird populations is necessary for the interpretation of future ecologic or disease disturbances (Holmes, 1982; Mörner et al., 2002). When combined with population dynamics data, such information will contribute to the prediction of population trends and to evaluation of the overall status of the marine ecosystem in which they live. The growing development of coastal Patagonia for tourism, industry, and fisheries could lead to an increase in spread of pathogens by scavenger seabirds such as gulls (Fenlon, 1981; Giaccardi et al.,1997; Frere et al., 2000). Understanding changes in disease exposure in sentinel species will allow us to establish and interpret the present and future impacts of their interaction with other marine species sharing their habitat and with increasing human activities.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the following individuals and institutions for their assistance and support without which this study could not have been completed: V. Greco, L. Starr, K. Poppe, and W. Weisman, Wildlife Conservation Society for sample processing and

general logistic support; C. Marull and P. de Diego for laboratory and data entry; C. B. Marinelli, R.E. Cepeda, and S. Torcida, NUCOMPA, Fac. Cs. Exactas, UNICEN, Tandil, for statistical analysis; M. A. "Hormiga" Díaz and R. "Bebote" Vera for continuously watching for our lives while working at the islands; J. E. Sala for field assistance; and F. Milano and S. L. Deem for their critical review of this manuscript. We thank the Organismo Provincial de Turismo and the Dirección de Fauna y Flora Silvestres of Chubut Province for issuing the work permits, the Wildlife Conservation Society, Agencia Nacional de Promoción Científica y Tecnológica, and Ecocentro Puerto Madryn for providing financial support; and Cenpat (Conicet) for institutional support.

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Received for publication 20 February 2002.