HEMATOLOGY, PLASMA CHEMISTRY, AND SEROLOGY OF THE FLIGHTLESS CORMORANT (*PHALACROCORAX HARRISI*) IN THE GALÁPAGOS ISLANDS, ECUADOR

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ABSTRACT: The flightless cormorant (*Phalacrocorax harrisi*) is an endemic species of the Galápagos Islands, Ecuador. Health studies of the species have not previously been conducted. In August 2003, baseline samples were collected from flightless cormorant colonies on the islands of Isabela and Fernandina. Seventy-six birds, from nestlings to adults, were evaluated. Genetic sexing of 70 cormorants revealed 37 females and 33 males. Hematology assessment consisted of packed cell volume ($n=19$), leukograms ($n=69$), and blood smear evaluation ($n=69$). Microscopic evaluation of blood smears revealed microfilaria in 33% (23/69) of the cormorants. Plasma chemistries were performed on 46 cormorants. There was no significant difference in chemistry values or complete blood counts between male and female cormorants or between age groups. Based on a serologic survey to assess exposure to avian pathogens, birds ($n=69$) were seronegative for West Nile virus, avian paramyxovirus type 1 (Newcastle disease virus), avian paramyxovirus types 2 and 3, avian influenza, infectious bursal disease, infectious bronchitis, Marek's disease (herpes), reovirus, avian encephalomyelitis, and avian adenovirus type 2. Antibodies to avian adenovirus type 1 and *Chlamydophila psittaci* were found in 31% (21/68) and 11% (7/65) of flightless cormorants respectively. *Chlamydophila psittaci* was detected via polymerase chain reaction in 6% (2/33) of the cormorants. The overall negative serologic findings of this research suggest that the flightless cormorant is an immunologically naïve species, which may have a reduced capacity to cope with the introduction of novel pathogens.

Key words: Chemistry, flightless cormorant, Galápagos Islands, health survey, hematology, microfilariae, *Phalacrocorax harrisi*, serology.

INTRODUCTION

The Galápagos archipelago is located in the Pacific Ocean, on the equator, 1,000 km west of continental Ecuador. Galápagos avifauna is comprised of 58 resident species, of which nearly half are endemic. The flightless cormorant (*Phalacrocorax harrisi*) is an endemic species restricted to two western islands of the archipelago, Isabela and Fernandina. The cormorants prefer the eastern coast of Fernandina and the western coast of Isabela, where there is an upwelling of water due to the cold Cromwell current and consequently an abundance of fish (Houvenaghel, 1984). The species is considered endangered due to its small distributional range and the extreme fluctuations in the number of mature birds (BirdLife International, 2000). Since 1998, the numbers of flightless cormorants have been increasing; their census numbers were 727 in 1998 (Vargas and Wiedenfeld, 2003) and 1,411 in 2004 (Vargas and Wiedenfeld, 2004). With a census representing 83% of the total population (Valle, 1994), the 2004 population size is estimated to be at 1,700 individuals (Vargas and Wiedenfeld, 2004). While no avian species have become extinct from the Galápagos Is-

lands (Wikelski et al., 2004) and the flightless cormorants have had a mild population increase in the last few years, there is concern that disease could devastate the population. Hawaii is a striking example of an island ecosystem that has suffered great losses in endemic avian populations, several at least partly attributable to the introduction of exotic diseases (Warner, 1968; Van Riper et al., 1986).

Endemic avian species of the Galápagos Islands have been exposed to introduced pathogens, but these introductions have not been directly correlated to population decline. A large, ongoing, comprehensive avian health study in the Galápagos Islands has been in place since 2001. Since then, domestic chickens have been found seropositive for avian adenovirus type I, avian encephalomyelitis virus, avian paramyxovirus type 1 (Newcastle disease virus), infectious bronchitis virus, infectious bursal disease virus, reovirus, *Mycoplasma* sp. and *Chlamydophila psittaci* (Gottdenker et al., 2005). Antibodies to avian adenovirus type I and avian encephalomyelitis virus have been reported from waved albatrosses (*Phoebastria irrorata*) endemic to Española Island (Padilla et al., 2003). On several islands in the Galápagos archipelago, endemic Galápagos doves (*Zenaida galapagoensis*) have been positive for a *Haemoproteus* sp. and for *C. psittaci*, while introduced rock doves (*Columba livia*) have been positive for *Trichomonas gallinae* (Padilla et al., 2004). Furthermore, avipoxviruses have been detected in endemic Galápagos finches (*Geospiza fortis*), Galápagos mockingbirds (*Nesomimus parvulus*), and yellow warblers (*Dendroica petechia*) on Santa Cruz Island, representing two variants of canarypox virus, while isolates from chickens represent a distinct fowlpox virus (Thiel et al., 2005). Chickens sampled in 1995 from inhabited islands of the Galápagos were infected with Marek's disease virus (Vargas and Snell, 1997).

Flightless cormorants were evaluated in August 2003 as part of the ongoing avian health study in the Galápagos Islands. The aim of the study was to determine hematology and biochemistry parameters, exposure to avian pathogens, and overall health status. This is the first time this information has been compiled and baseline data for the endangered free-ranging flightless cormorant is reported.

MATERIALS AND METHODS

Study area and sample collection

Flightless cormorants are found on the islands of Isabela (0°25′30″S, 91°7′W) and Fernandina (0°22′0″S, 91°31′20″W) in the Galápagos Islands. All sampling procedures were in accordance with Saint Louis Zoo institutional animal care and use committee standards, and the project was a collaboration between the Saint Louis Zoo, the University of Missouri–Saint Louis, the Charles Darwin Research Station, the Galápagos National Park, and the University of Oxford. Over 4 days in August 2003, 76 cormorants were visually evaluated to determine health status. These animals were classified as 8% (6/76) nestlings, 32% (24/76) juveniles, and 61% (46/76) adults. The animals were aged by plumage and eye color (Snow, 1966; Harris, 1979). The cormorants were relatively sedentary on land, facilitating net or hand catches, and they were sampled in a small dinghy or on land. Seventy-three flightless cormorants were manually restrained for a brief physical examination, measurement of morphometric parameters, transponder placement, and sample collection. Morphometric measurements of bill length, width, and depth were taken with a caliper and body weights were measured to the nearest 50 g with a hand-held 5-kg spring scale (Pesola®, Baar, Switzerland) (Vargas, unpubl. data). A transponder (AVID Microchip, Folsom, Louisiana, USA) was placed subcutaneously over the left dorsal midphalangeal area and the skin defect was sealed with tissue glue (3M Vetbond, St. Paul, Minnesota, USA). Venipuncture of the right jugular vein was performed with 20–22-ga needles and 6 ml of blood was collected per bird ($n=69$). Blood was immediately placed in lithium heparin (Vacutainer PST gel, Becton Dickinson, Franklin Lakes, New Jersey, USA) after collection ($n=47$), except on the fourth day, when samples were placed in serum separator tubes (Vacutainer SST gel and clot activator, Becton Dickinson) and 0.2 ml was placed in lithium heparin (Microtainer gel, Becton Dickinson) ($n=22$). A single sterile swab

(Copan Diagnostics, Corona, California, USA) per bird ($n=70$) was used on conjunctival, choanal, and cloacal regions. The swab was collected from the sites in the order listed and stored in cryogenic vials (Nalge Nunc International, Rochester, New York, USA). Ectoparasites were visualized on the feathers, manually removed, and placed in cryogenic vials.

Sample processing

Using the whole-blood samples in lithium heparin, two thin blood smears were prepared, air dried, and fixed with methanol. Two microhematocrit tubes were prepared per individual ($n=19$) from whole blood, centrifuged (Mobilespin Model 128, Vulcon Technologies, Grandview, Missouri, USA) for 20 min, and the packed cell volumes (PCV) determined. Two to three drops of blood were added to cryogenic vials containing a lysis buffer preservative solution (Longmire et al., 1988) and held at ambient temperature for polymerase chain reaction (PCR) tests for sex determination. The remaining whole blood was centrifuged for 20 min, and separate cryogenic vials were used to preserve the plasma or serum and the packed red blood cells (RBC). Ectoparasites were preserved in 95% ethanol. The plasma, serum, swabs, and packed RBC samples were stored in liquid nitrogen while on the boat, at the Charles Darwin Research Station (CDRS), and for transport to the United States.

Sample and data analysis

A modified Wright-Giemsa stain (JorVet Dip-Quick, Jorgensen Laboratories, Loveland, Colorado, USA) was applied to the blood smears to estimate white blood cell (WBC) counts (AVL Veterinary Laboratory, Saint Louis, Missouri, USA), and to perform differential counts, thrombocyte evaluation, and hemoparasite examination (J.M.). The leukocyte-estimate-from-smear technique (Fudge, 2000a) was used to determine total white blood cell counts, and the differentials were achieved by identifying and classifying 100 white blood cells. Hemoparasite evaluations were performed on each blood smear ($n=69$) by scanning for 5 min at $10\times$ and then counting 200 oil immersion fields at $1,000\times$ magnification. Forty-six plasma samples were submitted for plasma chemistry analysis (Antech Diagnostics, Alsip, Illinois, USA) using the Hitachi 911 analyzer (Boehringer Mannheim Corporation, Indianapolis, Indiana, USA). Genetic testing ($n=70$) for sex determination (Fridolfsson and Ellegren,

1999) was performed at the University of Missouri–Saint Louis (UMSL). The presence of *C. psittaci* antibody was tested via elementary body agglutination (EBA) on plasma ($n=43$) (Grimes et al., 1994) and via direct complement fixation (DCF) on serum ($n=22$) (Grimes, 1985), while *C. psittaci* antigen was tested via PCR on conjunctival–choanal–cloacal swabs ($n=33$) (Sayada et al., 1995) (Texas Veterinary Medical Diagnostic Laboratory, College Station, Texas, USA). Testing for West Nile virus (WNV) was done with plaque reduction neutralization (Animal Health Diagnostic Center, Cornell University, Ithaca, New York, USA). Plasma or serum samples ($n=69$) were twofold serially diluted from $1:10$ to $1:320$ in a 0.1-ml volume of cell culture medium (CCM) (minimum essential medium with Earle's salts [MEME], Gibco-Invitrogen, Grand Island, New York, USA), 10% fetal bovine serum (FBS), and 10 µg/ml ciprofloxacin hydrochlorine (Bayer, Kankakee, Illinois, USA). Approximately 200 plaque-forming units of WNV were added in a volume of 0.1 ml CCM containing 10% guinea pig complement (Colorado Serum Company, Fort Collins, Colorado, USA) to each dilution and incubated for 1 hr at 37 C in a 5% CO_2 incubator. One hundred microliters of the virus–serum suspension was overlaid onto a confluent monolayer of Vero cells and incubated for another hour under the conditions described above. Cell monolayers were overlaid with CCM containing 2% FBS and 1% low melting-point agarose (Invitrogen, Carlsbad, California, USA). Assays were incubated for 3 days as described above. Monolayers were stained overnight by adding three drops of CCM containing 3 mg neutral red/ml (Gibco, Grand Island, New York, USA). Plaques were counted on day 4 and wells were scored positively for neutralization if the number of plaques was less than or equal the average plaque count at a $1:10$ dilution of input virus.

Additional serologic testing was conducted at the National Veterinary Services Laboratory (US Department of Agriculture, Ames, Iowa, USA) using the methods described (Swayne et al., 1998). Hemagglutination inhibition (HI) testing was used for avian paramyxovirus types 1–3, and infectious bronchitis virus (strains IB41, IB46, IB99, JMK). Agar gel immunodiffusion (AGID) testing was used to test for antibodies to avian influenza virus, infectious bursal disease virus, Marek's disease virus, avian adenovirus types 1 and 2, and avian encephalomyelitis virus. Testing for reovirus antibody was done by immunofluorescent antibody. Ectoparasite identification (UMSL) was done by microscopy.

A statistical software package (NCSS®, Kaysville, Utah, USA) was used for data analysis. Data were tested for normality using a Shapiro-Wilk *W*-test, and Mann-Whitney *U*-tests were used on samples where normality was rejected. A *t*-test with separate variance estimates was used when the assumption of equal variances was not met, and this test was used to compare hematology and chemistry results between genders, age classes, and disease states. Significance was determined if $P \leq 0.05$, but a Bonferroni-corrected P value less than or equal to 0.004 (0.05/13) was used for the 13 plasma chemistry values. Because two microhematocrit tubes were prepared per cormorant ($n=19$), a mean PCV was initially determined per individual using these two values. Then an overall mean PCV was determined for the studied cormorants and a standard error of the mean (SEM) recorded.

RESULTS

All flightless cormorants examined were alert and appeared healthy. Genetic sexing of 70 individuals revealed 37 females (53%) and 33 males (47%). Body weight and hematology results are provided in Table 1. A significant difference ($P<0.0001$) was determined between female and male body weights. No difference was found in WBC counts or PCV between males and females or between adults and subadults. Microscopic evaluation of blood smears revealed an adequate number of thrombocytes per individual cormorant ($n=69$), and a mean eosinophil count of 21% on differential (SD=11%, $n=60$). Microfilariae were detected in 33% (23/69) of blood smears, but intraerythrocytic parasites were not observed. The mean WBC of filarid-positive flightless cormorants (6.5×10^9/l, SD=2.2×10^9/l, $n=23$) was statistically lower ($P<0.002$) than that of filarid-negative birds (8.7×10^9/l, SD=2.9×10^9/l, $n=43$). The mean PCV of filarid-positive birds (51.0%, SEM=1%, $n=14$) was statistically higher ($P<0.02$) than filarid-negative birds (44%, SEM=3%, $n=5$).

Results of plasma chemistries are provided in Table 2; there was no significant difference between female ($n=25$) and male ($n=21$) results or between adults and subadults. Potassium values from the August 2003 sampling trip are omitted from Table 2. The potassium values were elevated, with an overall mean of 11.94 mmol/l (mEq/l) (SD=8.69, range 3.5 to 37.1 mmol/l, $n=46$). Because the cormorants had transponders and the same colonies were sampled the following year, the potassium values could be rechecked. The recheck potassium mean was 4.13 mmol/l (mEq/l) (SD=0.55, range 3.3 to 4.8 mmol/l, $n=6$).

All flightless cormorants were seronegative except for 31% (21/68) avian adenovirus type I and 11% (7/65) *C. psittaci* seropositivity (Table 3). There were two different antibody testing methods for *C. psittaci*; no birds were seropositive (0%, 0/43) via elementary body agglutination (EBA), while seven of 22 birds (32%) were seropositive by direct complement fixation (DCF). Of the 33 combination swabs for *C. psittaci* PCR, two were positive (6%, 2/33). These PCR-positive birds also had seropositive DCF results.

Ectoparasites collected in 2003 were unidentifiable due to storage difficulties. However, ectoparasites collected from adult and juvenile flightless cormorants ($n=12$) in the same colonies on Fernandina and Isabela Islands in spring 2004 were identified as the genus *Pectinopygus*. These are most likely *Pectinopygus nannopteri* (Ewing), family Philopteridae, which is the only louse species reported from this host (Price et al., 2003). Ectoparasite specimens were retained at the UMSL.

DISCUSSION

Mean PCV values (49%) for flightless cormorants were significantly higher ($P<0.009$) than those reported for captive double-crested cormorants (*Phalacrocorax auritus*) (International Species Information System [ISIS], 2002), black-faced cormorants (*Phalacrocorax* (*Leucocarbo*) *fuscescens*) (Melrose and Nicol, 1992), and

TABLE 1.   Weight and hematology results for free-ranging flightless cormorants, all ages combined.

Parameter	Mean±SD	Range	n
Female weight (kg)	2.6±0.3	1.95–3.65	37
Male weight (kg)	3.5±0.5	2.7–4.35	32
Packed cell volume (%)	49.2±1.3[a]	35–58	19
Estimated leucocyte count ($\times 10^9$/l)	8.0±2.8	2.80–14.8	69
Heterophils (%)	36.6±15.8	5–71	60
Lymphocytes (%)	40.0±16.1	8–76	60
Monocytes (%)	2.2±2.0	0–12	60
Eosinophils (%)	20.8±10.5	3–52	60
Basophils (%)	0.5±0.9	0–3	60

[a] Standard error of the mean (SEM) was used instead of SD.

common cormorants (*Phalacrocorax carbo*) (Balasch et al., 1974). These differences are likely not clinically important. Differences may have been attributable to low sample sizes and the age of the birds included in the studies (Melrose and Nicol, 1992). Significant differences were not detected between flightless cormorant mean WBC counts and reported WBC counts for captive double-crested cormorants (ISIS, 2002) or black-faced cormorants (Melrose and Nicol, 1992), although the technique of establishing a total WBC count was likely not uniform. The leukocyte differential for flightless cormorants was comparable with published ranges for similar species, except for an apparent elevation of eosinophils (21%). The avian eosinophil is not considered a reliable indicator of parasitism, but eosinophilia can occur in parasitized birds (Fudge and Joseph, 2000). Significant differences were not found in eosinophil percentages between flightless cormorants with and without ectoparasites or microfilarids. Further expansion of the hematology database for flightless cormorants may help determine if what appears to be an eosinophilia is normal for the species. The observations of lower mean WBC and higher mean PCV in filarid-positive birds were not determined to be clinically significant, and the PCV data should be interpreted with caution due to low sample size.

Hemoparasites are common worldwide and are present in the avifauna of the Galápagos archipelago (Padilla et al., 2003). One third of the flightless cormorants evaluated (33%) were positive for

TABLE 2.   Plasma chemistry for free-ranging female ($n=25$) and male ($n=21$) flightless cormorants, all ages combined.

Parameter (SI units)	Female mean±SD	Female range	Male mean±SD	Male range
Aspartate aminotransferase (U/l)	523.2±265	308–1517	388.6±131.1	150–653
Total protein (g/l)	40.4±3.7	34–47	39.2±5.24	31–51
Albumin (g/l)	16.4±1.6	14–20	17.3±2.3	14–23
Globulin (g/l)	24±2.8	20–31	22±3.5	16–28
Phosphorus (mmol/l)	4.2±2.7	0.9–7.9	4.7±2.3	0.9–8.2
Calcium (mmol/l)	2.2±0.3	1.5–2.8	2.3±0.3	1.5–2.7
Glucose (mmol/l)	7±2.3	3.8–10.4	7.3±2.1	36.1–11
Sodium (mmol/l)	150.2±7.5	130–158	150.7±5	138–160
Chloride (mmol/l)	109.6±4.4	99–116	108.4±3.4	102–115
Cholesterol (mmol/l)	5.9±0.9	4.3–7.7	6.7±0.9	4.8–9.0
Creatine kinase (U/l)	2,588.9±1,649.7	1,237–9,118	2,076.8±8,40.6	764–3,636
Uric acid (mmol/l)	1.4±0.5	0.5–2.1	1.3±0.6	0.4–2.4

TABLE 3.   Test results for flightless cormorants from the Galápagos Islands, Ecuador.

Disease agent	Method[a]	Prevalence[b]	% Positive
Microfilariae	Microscopic blood-smear evaluation	23/69	33.3
Chlamydophila psittaci	Elementary body agglutination	0/43	0
Chlamydophila psittaci	Direct complement fixation	7/22	31.8
Chlamydophila psittaci	Polymerase chain reaction[c]	2/33	6.1
Avian adenovirus 1	Agar gel immunodiffusion (AGID)	21/68	30.9
Avian adenovirus 2	AGID	0/68	0
Avian influenza	AGID	0/69	0
Infectious bursal disease	AGID	0/68	0
Marek's disease (herpes)	AGID	0/68	0
Avian encephalomyelitis	AGID	0/69	0
Avian paramyxovirus 1	Hemagglutination inhibition (HI)	0/69	0
Avian paramyxovirus 2	HI	0/69	0
Avian paramyxovirus 3	HI	0/69	0
Infectious bronchitis	HI	0/69	0
Reovirus	Immunofluorescent assay	0/68	0
West Nile Virus	Plaque reduction neutralization	0/69	0

[a] Sample was plasma or serum unless indicated.

[b] Number positive/number tested.

[c] Combined conjunctival, choanal, cloacal swabs.

microfilariae. Filarid nematodes and microfilariae have previously been detected in cormorant species (Cleland, 1915; Mackerras, 1962). Microfilarids are often incidental and, in most situations, considered apathogenic (Greiner and Ritchie, 1994); therefore, the significance of the flightless cormorant microfilariae is unknown. Further attempts to identify the species of filarial worm will be pursued.

This is the first baseline plasma biochemistry information for the flightless cormorant. Some of the values were higher than expected, such as uric acid and phosphorus. Marked elevations of uric acid occur in birds after a high protein meal (Phalen, 2000); a fasted sample is difficult to obtain in free-ranging birds. The elevated uric acid may be postprandial or a baseline for this population, while phosphorus levels are known to vary widely (Fudge, 2000b). The potassium results from August 2003 showed severe hyperkalemia compared with common cormorants (Balasch et al., 1974), fledgling black-faced cormorants (Melrose and Nicol, 1992), and double-crested cormorants (ISIS, 2002). Repeat flightless cormorant

sampling in 2004 allowed blood to be submitted for potassium determination (Antech) on cormorants matched by microchip. These follow-up tests yielded normal potassium values. Sample handling or laboratory error in 2003 was most likely. However, the samples were not grossly hemolyzed, and samples with normal potassium values were obtained, centrifuged, and stored in the exact same manner as samples with elevated potassium.

Flightless cormorants were seronegative except for avian adenovirus type 1 and C. psittaci. The negative results should be interpreted with caution because most bird serology has been validated in domestic chickens. The overall seroprevalence for avian adenovirus type 1 (31%, 21/68) was composed of 14/47 (30%) positive plasma samples and 7/21 (33%) positive serum samples. Avian adenovirus seropositive cormorants were found on both Isabela and Fernandina Islands. As mentioned, Galapagos Islands waved albatross and domestic chickens have shown seropositivity for avian adenovirus. Avian adenoviruses are distributed worldwide

and are often asymptomatic (Ritchie, 1995). The presence of antibodies does not confirm active infection or disease, but latent infections can become activated due to stress or immunocompromise (Ritchie, 1995). There were no differences in hematology or chemistries between adenovirus seropositive and seronegative birds.

Chlamydophila psittaci is endemic worldwide and is well adapted to avian hosts. Infections often are asymptomatic, but severity depends on strain virulence and host species susceptibility (Gerlach, 1994). Direct complement fixation (DCF) on serum detects the presence of longer acting IgG, while EBA on plasma detects acute IgM. Direct complement fixation is preferred for population serosurveys (Andersen and Vanrompay, 2003), and seven cormorants (32%, 7/22) were seropositive for *C. psittaci* via DCF, with titers ranging from 16 to 64. None of the 43 cormorants were positive by EBA. Because these EBA seronegative birds could not be further tested by DCF because serum was not available, it is difficult to determine the overall seroprevalence of *C. psittaci* in the flightless cormorant population. Thirty-three combination swabs for chlamydial PCR included seven DCF-seropositive cormorants, 18 EBA-seronegative cormorants, one DCF-seronegative bird, two birds not serologically tested, and five anticomplementary DCF results. Two out of 33 (6%) swabs were antigen positive and both positive samples were DCF seropositive. Antigen negative, seropositive results may reflect a previous infection in a recovered bird, an asymptomatic carrier, or a false positive due to cross-reacting antibodies (Vanrompay, 2000).

It is unknown if flightless cormorants are susceptible to WNV, but morbidity and mortality caused by WNV has been confirmed in double-crested and guanay cormorants (*Phalacrocorax bougainvillii*) (http://www.cdc.gov/ncidod/dvbid/west-nile/birdspecies.htm). At least one known competent mosquito vector for WNV, *Culex pipiens quinquefasciatus*, is present in the Galápagos Islands (Peck et al., 1998; Turrell et al., 2001). Although our study provides no evidence that WNV has arrived in the Galápagos, continued vertebrate and mosquito surveillance is needed, as well as strict quarantine and inspection at the main port of entry. On Isabela Island, where part of the flightless cormorant population resides, backyard chickens and broiler chicken farms are found and antibodies to Newcastle disease virus (NDV) have been reported (Gottdenker et al., 2005). Mortality associated with NDV has occurred in double-crested cormorants in North America (Glaser et al., 1999), and it is uncertain how naïve flightless cormorants would respond to infection. Furthermore, other poultry diseases detected in the islands (Gottdenker et al., 2005) could affect the cormorants. Direct or indirect contact between the cormorants and the chickens or chicken products should be monitored and minimized.

This is the first baseline health information for the endangered flightless cormorant population. Although we found no health impacts associated with antibodies or antigen to adenovirus and *C. psittaci*, or the presence of microfilariae, long-term studies would be necessary to fully assess potential effects of these disease agents. Even though the flightless cormorant population is currently on the rise, the threat of infectious disease cannot be discounted. The naïve status of this population for potentially important avian viral diseases, such as WNV and NDV, as well as bacterial or parasitic diseases, supports further monitoring of this population.

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