

## Health Evaluation of Free-Ranging Humboldt Penguins (*Spheniscus humboldti*) in Peru

Kristine M. Smith,<sup>AB</sup> William B. Karesh,<sup>A</sup> Patricia Majluf,<sup>C</sup> Rosana Paredes,<sup>D</sup> Carlos Zavalaga,<sup>E</sup> Almira Hoogesteijn Reul,<sup>F</sup> Mark Stetter,<sup>G</sup> W. Emmett Braselton,<sup>H</sup> Helena Puche,<sup>I</sup> and Robert A. Cook<sup>A</sup>

<sup>A</sup>Wildlife Conservation Society, 2300 Southern Boulevard, Bronx, NY 10460

<sup>C</sup>Centro para la Sostenibilidad Ambiental-Universidad Peruana Cayetano Heredia, Lima, Perú

<sup>D</sup>Department of Biology, Memorial University of Newfoundland, St. John's, Newfoundland and Labrador, Canada A1C 5B7

<sup>E</sup>Department of Biology and Marine Biology, University of North Carolina, 601 South College Road, Wilmington, NC 28403-5915

<sup>F</sup>Department of Human Ecology, Cinvestav IPN, Unidad Merida, Km 6 Antigua Carretera a Progreso, AP 73-Cordemex 97310 Merida Yucatan, Mexico

<sup>G</sup>Disney's Animal Programs, 1200 Savannah Circle, Lake Buena Vista, FL 32819

<sup>H</sup>Animal Health Diagnostic Laboratory, Michigan State University, East Lansing, MI 48824-1315

<sup>I</sup>Chicago Botanic Garden, 1000 Lake Cook Road, Glencoe, IL 60022

Received 17 July 2007; Accepted and published ahead of print 17 December 2007

**SUMMARY.** As part of ongoing ecological studies of Humboldt penguins (*Spheniscus humboldti*) at Punta San Juan, Ica Department, Peru, health surveys were conducted in November 1992, 1993, and 1994. In the three surveys, 98 birds in total were handled for examination, and blood was collected for laboratory analysis from 90 of these birds. All birds seemed to be in good condition. Body weights of females were significantly lower in 1994 than in the other years. Fleas (*Parapsyllus humboldti*) and ticks (*Ornithodoros amblus*) were found on the penguins and in their nests. Females had significantly higher plasma calcium and phosphorus levels, and they had lower weights than males. No other differences were found between the sexes. Hematology, plasma chemistries, and plasma mineral levels varied between years. Positive antibody titers for *Chlamydomydia psittaci* (62%), avian adenovirus (7%; 1994 only), paramyxovirus-2 (7%; 1993 only), and *Salmonella* Pullorum (7%) were found. Plasma chemistry and mineral levels differed between individuals testing positive *vs.* negative on serologic tests for avian adenovirus and *Salmonella* Pullorum. Serologic tests for antibodies to avian influenza A virus, avian encephalomyelitis virus, infectious bronchitis virus, avian reovirus, duck viral enteritis virus, equine encephalitis (eastern, western, and Venezuelan) viruses, infectious bursal disease virus, infectious laryngotracheitis virus, *Aspergillus* sp., and paramyxovirus-1 and -3 were negative. All chlorinated pesticide and polychlorinated biphenyl analyses were below detectable limits.

**RESUMEN.** Evaluación de la salud de pingüinos de Humboldt (*Spheniscus humboldti*) en el Perú.

Como parte de los estudios ecológicos que se llevan a cabo con los pingüinos de Humboldt (*Spheniscus humboldti*) en Punta de San Juan, departamento de Ica en el Perú, se realizaron encuestas sanitarias durante el mes de Noviembre de los años 1992, 1993 y 1994. En las tres encuestas se examinaron un total de 98 aves, obteniendo sangre para análisis de laboratorio a partir de 90 de estas aves. Todas las aves parecían estar en buenas condiciones. Los pesos de las hembras fueron significativamente menores en el año de 1994 que en los otros años. Tanto en los pingüinos como en sus nidos, se encontraron pulgas (*Parapsyllus humboldti*) y garrapatas (*Ornithodoros amblus*). Las hembras tenían niveles significativamente mayores de calcio y fósforo plasmático, y tuvieron menor peso que los machos. No se encontraron otras diferencias entre los sexos. Los resultados de hematología, química plasmática y los niveles de minerales en el plasma variaron entre los años. Se encontraron títulos positivos de anticuerpos contra *Chlamydomydia psittaci* (62%), adenovirus aviares (7% sólo para el año 1994), paramyxovirus-2 (7% sólo para el año 1993) y *Salmonella* Pullorum (7%). La química plasmática y los niveles de minerales difirieron entre los individuos encontrados positivos versus los negativos en la pruebas serológicas para adenovirus y *Salmonella* Pullorum. Las pruebas serológicas fueron negativas en la detección de anticuerpos contra los virus de influenza aviar tipo A, encefalomyelitis aviar, reovirus aviar, enteritis viral del pato, encefalitis equina (Venezolana, del este y del oeste), enfermedad infecciosa de la bolsa, laringotraqueítis infecciosa, paramyxovirus 1 y 3, lo mismo que del hongo *Aspergillus* sp. Todos los análisis para pesticidas clorinados y bifenilos policlorinados estuvieron por debajo de los límites de detección.

**Key words:** penguin, *Spheniscus*, disease, hematology, serology

**Abbreviations:** ANOVA = analysis of variance; HP = Humboldt penguins; IgG = immunoglobulin G; PCV = packed cell volume(s); PMV = paramyxovirus; WBC = white blood cell

Comprehensive health assessments of free-ranging avian species have rarely been reported in the literature. Studies on free-ranging animal health typically report a limited set of tests or pathogens. Previous studies have shown diseases to play a significant role in the reproductive success or population size of wild bird colonies in addition to other more obvious environmental variables such as food availability or weather conditions (7). Population health evaluations can often be easily integrated with ongoing biological field studies

such as those involving tagging, banding, or counting (26). Modern conservation efforts could be enhanced by the availability of comprehensive health information, which provides baseline data for populations (17).

A large amount of work has been published on a variety of health concerns in captive penguins, and some information is available on captive Humboldt penguins (HP; *Spheniscus humboldti*) (3,11,23). However, information on the health parameters of HP in the wild is extremely limited (3,28). In November 1992, 1993, and 1994, health surveys of free-ranging HP were undertaken at one of the main colonies remaining in Peru. This study was initiated to provide

<sup>B</sup>Corresponding author. E-mail: ksmith@wcs.org

a health assessment of HP in a protected colony that has been the focus of ecological studies for more than a decade (14,29). Specific objectives were to 1) establish baseline health indices, including hematology, plasma chemistries, metal and mineral levels, and selected toxic chemicals; 2) evaluate the serologic evidence of infectious diseases; and 3) determine variation in health parameters among years.

## MATERIALS AND METHODS

**Study period and site description.** In conjunction with ongoing ecological studies at Punta San Juan, Marcona, Department of Ica, Peru ( $15^{\circ}21'50''S$ ,  $75^{\circ}12'W$ ), evaluations of individual HP and sample collection for health analysis were conducted in November 1992, 1993, and 1994. The sampling period coincided with the second nesting season of the year and most birds were rearing chicks. This preceded the normal molting period by 2 to 3 mo. The penguin colonies are situated on gentle rocky slopes above the beaches and along the cliff edges of a 54-ha headland that is legally protected from human disturbance. The penguin colony increased from ~400 nesting pairs in 1990–91 to an estimated 1500 pairs by the 1994–95 breeding season. The total number of birds on the reserve was ~300,000 in November 1994 and included guanay cormorants (*Phalacrocorax bougainvillii*), Peruvian pelicans (*Pelecanus thagus*), band-tailed gulls (*Larus belcheri*), Peruvian boobies (*Sula variegata*), and Inca terns (*Larosterna inca*).

**Sample and data collection.** In 1992 and 1993, HP were captured at their nests. In 1994, birds of unknown breeding status were captured on the beach near the nesting area. In all cases, the birds were manually restrained for ~5 min for examination and sample collection, and then they were released. This work was done in the morning while the ambient temperature was still cool (15–20 C). The handling procedure included body measurements, notation of visual or palpable abnormalities, individual banding, and collection of 20 ml of blood from the jugular vein using a heparinized syringe and 20-gauge needle. Sex was determined by discriminant analysis of external measurements (bill length, width, and head width) as described by Zavalaga and Paredes (30). Ninety-eight adult HP were examined. Ectoparasite samples were collected from the birds when possible and from the nests and preserved in 90% isopropyl alcohol.

**Sample handling and storage.** Immediately after venipuncture, the heparinized blood was placed in serum separator tubes (Corvac; Sherwood Medical, St. Louis, MO) and maintained in an insulated box at ambient temperature. Tubes were maintained in an upright position to reduce blood contact with the rubber stopper and leaching of zinc from the vulcanized rubber into the sample (20). Initial processing of blood samples occurred within 2 hr of blood collection. Thin blood smears were fixed with 99% methanol. Packed cell volumes (PCVs) were determined after centrifugation in a portable 12-V centrifuge (Mobi-lespin; Vulcan Technologies, Grandview MO), and plasma total solids were measured using a hand-held refractometer (Schulco, Toledo, OH) calibrated at the site. White blood cell counts were completed using the eosinophil counting method (Unopette Test 5877; BD Biosciences Vacutainer Systems; BD Biosciences, Franklin Lakes, NJ) (4). The remaining blood was centrifuged for 10 min. Plasma was harvested and frozen in liquid nitrogen. Plasma samples were heat-treated in a water bath at 56 C for 2 hr in accordance with the U.S. Department of Agriculture regulations before importation to the United States.

**Sample and data analysis.** Blood smears were stained with a modified Wright-Giemsa stain (Hematology Three-step Stain; Accra Lab, Bridgeport, NJ), and smears were examined for blood parasites and white blood cell (WBC) differentials at the Wildlife Health Center, Wildlife Conservation Society in The Bronx, New York. Plasma chemistries and enzymes were processed on an automated analyzer (Ciba Corning Alliance 580 Auto Analyzer; Ciba Corning Diagnostics Corp., East Walpole, MA) at a commercial veterinary laboratory (Vet Research, Farmingdale, NY). The chemistry and enzyme panel included glucose, blood urea nitrogen, uric acid, creatinine, calcium, inorganic phospho-

rus, sodium, potassium, chloride, creatine kinase, cholesterol, total protein, albumin, globulin, and aspartate aminotransferase. Plasma samples were analyzed for aluminum (Al), boron (B), barium (Ba), copper (Cu), cobalt (Co), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mb), and zinc (Zn) by inductively coupled argon plasma emission spectroscopy as described by Stowe *et al.* (27). Plasma also was analyzed for polychlorinated biphenyls and the chlorinated pesticides aldrin;  $\alpha$ -BHC (lindane);  $\beta$ -BHC; O,P'-DDD; P,P'-DDD; P,P'-DDE; O,P'-DDT; P,P'-DDT; dieldrin; endrin; heptachlor; heptachlor epoxide; lindane ( $\gamma$ -BHC); and nonachlor using the method described by Price *et al.* (22).

Serologic antibody testing for infectious laryngotracheitis virus (indirect immunofluorescence); avian adenovirus type 127 (agar gel immunodiffusion); avian encephalomyelitis virus (agar gel immunodiffusion); avian influenza A virus (agar gel immunodiffusion); avian reovirus (indirect immunofluorescence); duck viral enteritis virus (serum neutralization); equine encephalitis (eastern, western, and Venezuelan) viruses (hemagglutination inhibition); infectious bursal disease virus (agar gel immunodiffusion); infectious bronchitis virus types Massachusetts 41 (IB41), Connecticut 46 (IB46), Arkansas 99 (IB99), and JMK (IBJMK) (hemagglutination inhibition); paramyxovirus (PMV)-1, -2, and -3 (hemagglutination inhibition); *Histoplasma capsulatum* (agar gel immunodiffusion); *Salmonella* Pullorum (microscopic agglutination); and *Chlamydomphila psittaci* (complement fixation) was conducted at the National Veterinary Services Laboratory in Ames, IA. Antibody titers for *Aspergillus* sp. (enzyme-linked immunosorbent assay) were determined by the Raptor Center, College of Veterinary Medicine, in St. Paul, MN. Table 1 summarizes the infectious disease serologic tests performed; the methods used; and where appropriate, the antibody titers defined as positive in this study.

All sets of data were tested for normality and homoscedasticity before performing analysis of variance (25). All variables were normally distributed with the exception of PCV, monocytes, eosinophils, uric acid, creatine kinase, and Fe. Different transformations were performed on those variables. However, after analysis of the residuals from the transformed data, the heterogeneity of the variance was not reduced with any transformation; therefore, we treated those variables as ordinal. A one-way analysis of variance (ANOVA) was performed on the normal data to detect differences among years and between sexes. A Kruskal-Wallis one-way ANOVA was conducted on the ordinal data to detect possible differences between years. A Mann-Whitney *U*-test was used on the ordinal data to determine differences due to sex. Differences in infectious disease serology results between the samples collected in 1993 and 1994 were determined using a Mann-Whitney *U*-test. A Mann-Whitney *U*-test was also used to determine whether the hematology, chemistry, or mineral test results differed between groupings of serology positive and negative birds. All statistical findings were considered significant at  $P < 0.05$  (25).

## RESULTS

All HP handled ( $n = 98$ ) were found to be in good physical condition. Of these 98 birds, 90 HP were successfully bled, 83 were successfully weighed, and 76 were successfully sexed. Mean body weights were  $4.49 \pm 0.51$  kg ( $n = 21$ ),  $4.52 \pm 0.47$  kg ( $n = 50$ ), and  $4.09 \pm 0.55$  kg ( $n = 12$ ); 8.9% and 9.5% decrease from 1992 and 1993, respectively) for 1992, 1993, and 1994, respectively. Overall, males had significantly higher ( $P < 0.0001$ ) body weights ( $4.75 \pm 0.41$ ;  $n = 47$ ) compared with females ( $4.04 \pm 0.33$ ;  $n = 28$ ). The male-to-female-to-unsexed ratios of birds handled were 11:8:2, 31:14:6, and 6:6:14 in 1992, 1993, and 1994, respectively. The male-to-female-to unsexed ratios of birds that were successfully weighed were 11:8:2, 31:14:5, 5:6:1 in 1992, 1993, and 1994, respectively. Average weights in 1992, 1993, and 1994 were  $4.74 \pm 0.52$  kg ( $n = 11$ ),  $4.78 \pm 0.37$  kg ( $n = 31$ ), and  $4.56 \pm 0.38$  kg ( $n = 5$ ) for males, and  $4.25 \pm 0.29$  kg ( $n = 8$ ),  $4.07 \pm 0.23$  kg ( $n = 14$ ), and 3.72

Table 1. Serologic test procedures, level of antibody titers defined as positive, and results for each test used for free-ranging HP in Peru.

Disease agent	Test procedure	Positive titer	No. positive/no. tested
Avian adenovirus (127)	Agar gel immunodiffusion	n/a <sup>A</sup>	4/61
Avian encephalomyelitis virus	Agar gel immunodiffusion	n/a	0/61
Avian influenza virus	Agar gel immunodiffusion	n/a	0/61
<i>Aspergillus</i> sp.	Enzyme-linked immunosorbent assay	n/a	0/32
Avian PMV-1	Hemagglutination inhibition	≥1:8	1/61
Avian PMV-2	Hemagglutination inhibition	≥1:8	4/61
Avian PMV-3	Hemagglutination inhibition	≥1:8	1/61
Avian reovirus	Indirect immunofluorescence	≥1:20	1/61
<i>C. psittaci</i>	Complement fixation	≥1:10	38/61
Duck viral enteritis virus	Serum neutralization	≥1:4	0/61
Equine encephalitis (eastern) virus	Hemagglutination inhibition	≥1:10	0/41
Equine encephalitis (western) virus	Hemagglutination inhibition	≥1:10	0/41
Equine encephalitis (Venezuelan) virus	Hemagglutination inhibition	≥1:10	0/41
Infectious bronchitis (41) virus	Hemagglutination inhibition	≥1:10	0/20
Infectious bronchitis (46) virus	Hemagglutination inhibition	≥1:10	1/20
Infectious bronchitis (99) virus	Hemagglutination inhibition	≥1:10	1/20
Infectious bronchitis (JMK) virus	Hemagglutination inhibition	≥1:10	1/20
Infectious bursal disease virus	Agar gel immunodiffusion	n/a	0/20
Infectious laryngotracheitis virus	Indirect immunofluorescence	≥1:10	0/61
<i>Histoplasma capsulatum</i>	Agar gel immunodiffusion	n/a	0/4
<i>Salmonella</i> Pullorum	Microscopic agglutination	≥1:20	4/61

<sup>A</sup>n/a = not applicable.

± 0.38 ( $n = 6$ ) for females, respectively. When male weights were compared between years, weights in 1994 were lower than for the other years; however, this difference was not significant ( $P = 0.52$ ). When weights of females were compared between years, females weighed significantly less in 1994 ( $P = 0.01$ ). Ectoparasites were occasionally found on birds, in the nest burrows, and in the soil or sand around the nests. Fleas were identified as *Parapsyllus humboldti* and the ticks as *Ornithodoros amblys*. Ninety of the 98 birds handled were successfully bled. No blood parasites were found during examination of blood smears ( $n = 90$ ). Results of hematology, plasma chemistry, enzyme, metal, and mineral analyses are provided in Table 2. Females had significantly higher ( $P = 0.011$ ) calcium levels ( $10.63 \pm 1.53$  mg/dl;  $n = 25$ ) than males ( $9.67 \pm 1.35$  mg/dl;  $n = 39$ ). Females also had higher ( $P = 0.004$ ) phosphorus levels ( $4.26 \pm 1.13$  mg/dl;  $n = 25$ ) than males ( $3.36 \pm 1.11$  mg/dl;  $n = 39$ ). No other test result differences were found between sexes. Levels for Al (<1.66 ppm), B (<1.66 ppm), Ba (<0.062 ppm), Co (<0.125 ppm), Mn (<0.062 ppm), and Mb (<0.250 ppm) were below accurate detectable limits. All chlorinated pesticide and polychlorinated biphenyl analyses were below detectable limits (0.001–0.007 ppm and 0.05 ppm, respectively).

Findings for positive and negative antibody titers for infectious agents are provided in Table 1. There was no significant difference in these values between years. Analyses of relationships between hematology or biochemistry values and serologic results did indicate statistically significant differences ( $P < 0.05$ ; Mann-Whitney  $U$ -test) among positive and negative groups (Table 3) and among years (Table 4).

## DISCUSSION

**Physical examination and parasites.** The apparently normal condition of the birds examined suggests that they were not under extremely adverse environmental or health conditions. Nonetheless, the significantly lower body weights of females in 1994 indicate that some factor or factors were resulting in a physiologic effect. Alternatively, the difference may have been due to the fact that these

birds were captured off the shore. Although proximate to the nesting grounds during nesting season, birds captured off the shore may or may not have been nesting at the time of assessment, whereas all birds sampled in the previous 2 yr were captured off their nests. The average male weight in 1994 was also lower than in 1992 and 1993,

Table 2. Hematology, plasma chemistry, enzyme, mineral, and metal values for free-ranging HP in Peru.

Test (units)	Mean ± SD	Range	$n$
Hematocrit (%)	47.2 ± 3.64	34–53	87
Total solids (g/dl) <sup>A</sup>	5.64 ± 0.67	4.2–7.6	87
WBC (cells/ml <sup>3</sup> × 10 <sup>3</sup> ) <sup>A</sup>	12.86 ± 11.5	1.8–40.9	84
Heterophils (%) <sup>A</sup>	45.6 ± 10.6	27–70	85
Lymphocytes (%) <sup>A</sup>	47.3 ± 10.0	26–70	85
Monocytes (%)	3.2 ± 2.4	0–12	85
Eosinophils (%) <sup>A</sup>	3.6 ± 4.0	0–18	85
Basophils (%)	0	0	85
Glucose (mg/dl) <sup>B</sup>	258.9 ± 46.0	120–433	83
Uric acid (mg/dl) <sup>A</sup>	13.6 ± 10.3	0.3–44.1	83
Creatinine (mg/dl)	0.60 ± 0.13	0.3–1.0	83
Total protein (g/dl) <sup>AB</sup>	5.22 ± 0.89	2.0–7.4	83
Albumin (g/dl)	1.87 ± 0.27	0.8–2.4	83
Globulins (g/dl) <sup>A,B,B</sup>	3.35 ± 0.71	1.2–5.1	83
Albumin/globulin ratio <sup>AB</sup>	0.57 ± 0.12	0.4–1.2	83
Aspartate aminotransferase (IU/L) <sup>A</sup>	208.1 ± 118.8	92–1036	83
Cholesterol (mg/dl)	193.9 ± 35.3	89–292	83
Creatine kinase (IU/L) <sup>AB</sup>	361.5 ± 172.9	80–1110	83
Calcium (mg/dl)	9.98 ± 1.35	4.1–15.0	83
Chloride (mEq/L)	108 ± 10.4	50.0–125.0	83
Copper (µg/ml)	0.6 ± 0.12	0.36–0.93	78
Iron (µg/ml) <sup>AB</sup>	2.60 ± 2.33	0.57–12.3	78
Magnesium (µg/ml)	27.67 ± 4.70	20.6–42.5	78
Phosphorus (mg/dl)	3.67 ± 1.25	0.9–7.2	83
Potassium (mEq/L) <sup>AB</sup>	2.82 ± 0.70	1.0–5.3	83
Sodium (mEq/L) <sup>A</sup>	149.6 ± 13.1	73.0–170.0	83
Zinc (µg/ml) <sup>A</sup>	2.33 ± 0.57	1.29–4.56	78

<sup>A</sup>Statistically different means among years are provided in Table 4.

<sup>B</sup>Statistically different means between antibody positive and negative birds are provided in Table 3.

Table 3. Mean and standard deviations ( $n$ ) for plasma chemistry values that differed among seronegative and seropositive HP in Peru ( $P < 0.05$ , Mann-Whitney  $U$ -test).

Test	Positive	Negative
<i>Salmonella</i> Pullorum (1993 and 1994 combined)		
Glucose (mg/dl)	211.5 $\pm$ 15.97(4)	261.39 $\pm$ 45.9 (56)
<i>Salmonella</i> Pullorum (1994 only)		
Potassium (mEq/L)	3.0 $\pm$ 0.36 (3)	2.4 $\pm$ 0.43 (17)
Iron ( $\mu$ g/ml)	6.33 $\pm$ 0.90 (3)	5.52 $\pm$ 0.67 (17)
Avian PMV-2		
Total protein (g/dl)	6.12 $\pm$ 0.54 (4)	5.08 $\pm$ 0.81 (56)
Globulin (g/dl)	4.25 $\pm$ 0.48 (4)	3.23 $\pm$ 0.65 (56)
Albumin/globulin ratio	0.45 $\pm$ 0.06 (4)	0.58 $\pm$ 0.12 (56)
Creatine kinase (IU/L)	178.7 $\pm$ 74.4 (4)	388.8 $\pm$ 178.0 (56)

but this finding was not significant. Yearly changes in body weight, which correlate with the Pacific El Niño currents, and decline in fish stocks have been noted for HP and pinnipeds living in this region (14,19). The El Niño of 1982–83 coupled with heavy commercial fishing was reported to result in a 65% decline in population of HP in Peru (14). A long El Niño event, lasting from late 1991 to mid-1995, and the resulting decline in fish stocks may have contributed to the decrease in body weights found during this study. It should be expected that the population size of this colony would have decreased in the face of a food scarcity (6). However, this penguin colony grew from ~400 nesting pairs in 1990–91 to an estimated 1500 pairs by the 1994–95 breeding season. Population size of the overall seabird colony increased from ~250,000 to ~350,000 during this time, and there was a similar increase in the HP colony from ~3000 to ~4000 individuals. Thus, it seems unlikely that food scarcity played a role in weight decline unless the population increase observed was due to influx of birds from surrounding areas in search of food. The lower female body weights observed in 1994 also could be attributed to reproductive status or overall size of the individuals sampled compared to individuals sampled in 1992 and 1993.

The lack of blood parasites seen in the free-ranging HP contrasts with the common finding of malaria (*Plasmodium elongatum*, *Plasmodium relictum*) in captive and free-ranging penguins (3,13). Because malaria organisms are very seldom found in circulating erythrocytes of infected animals, negative findings on blood smears cannot be relied on to indicate a lack of infection (13). The development of serologic tests for avian malaria antibodies, as

reported for other species of penguins, would be useful to determine infection prevalence in free-ranging penguins (13). At this study site, as in much of the undisturbed areas of coastal Peru, mosquitoes are not present; thus, malaria would not be expected.

The presence of ectoparasites is not uncommon in penguin colonies (3). The *P. humboldtii* and *O. amblus* found in this study have not been reported in reviews of penguin parasites (3). Since the early 1990s, a subjective observation of a decline in the number of ectoparasites on the birds and in the colony and nest substrates at this site has been made by the field staff handling these birds, including us. This coincides with the aforementioned growth of this HP colony and overall seabird colony over the previous 5 yr. In other colony-nesting seabirds in Peru, nest desertion and poor reproductive success were correlated with the abundance or density of *O. amblus* (5).

**Hematology, plasma chemistries, enzymes, minerals, and metals.** Mean PCVs for HP were higher than those typically reported in other studies of captive and free-ranging penguins, but they were similar to the findings in free-ranging HP in Chile (3,15,28). Mean total leukocyte counts were higher than values reported for captive and free-ranging penguins but with exception to year 3, they were not as high as the findings for free-ranging HP in Chile (3,15,28). Plasma chemistries, enzymes, minerals, and metals were not remarkably different from those reported for other HP (10,15). There was a significant rise in mean total leukocyte count, with a relative increase in heterophils and decrease in lymphocytes from 1993 to 1994. There was also a significant increase in creatinine kinase, uric acid, and aspartame aminotransferase from 1993 to 1994. The rise in WBCs, creatine kinase, and aspartame aminotransferase may be attributable to physical stress experienced by birds captured off the beach in 1994 compared with those captured off nests in 1992 and 1993. Birds captured off the beach required a brief chase for capture. The physiologic alterations also may have been due to presence of a disease for which the birds were not tested in this study. Establishing that polychlorinated biphenyls and selected chlorinated pesticides were below detectable levels in the study population provides a valuable baseline for comparison with other populations and for the study population over time.

**Serology.** It is important to note that none of the serologic assessments performed in this study have been validated for use in penguins, and sensitivity and specificity values even in poultry are not well established. Thirty-eight of the 61 HP (62%) tested for *C. psittaci* in this study had positive antibody titers. *Chlamydomphila*

Table 4. Mean and standard deviations ( $n$ ) for plasma chemistry values that differed among years for HP in Peru.

Test	1992	1993	1994
WBC (cells/mL <sup>3</sup> $\times$ 10 <sup>3</sup> )	8.54 <sup>A</sup> $\pm$ 3.26 (11)	5.81 <sup>AB</sup> $\pm$ 2.01 (49)	29.25 <sup>B</sup> $\pm$ 8.34 (24)
Heterophils (%)	44.2 <sup>A</sup> $\pm$ 6.0 (10)	40.6 <sup>A</sup> $\pm$ 8.3 (49)	55.7 <sup>B</sup> $\pm$ 8.7 (26)
Lymphocytes (%)	49.8 <sup>A</sup> $\pm$ 6.8 (10)	50.9 <sup>A</sup> $\pm$ 8.9 (49)	39.5 <sup>B</sup> $\pm$ 8.8 (26)
Plasma total protein (g/dl)	5.96 <sup>A</sup> $\pm$ 0.73 (10)	5.19 <sup>B</sup> $\pm$ 0.93 (49)	5.01 <sup>B</sup> $\pm$ 0.71 (25)
Albumin (g/dl)	2.01 <sup>A</sup> $\pm$ 0.30 (10)	1.80 <sup>B</sup> $\pm$ 0.25 (49)	1.96 <sup>A</sup> $\pm$ 0.26 (25)
Globulin (g/dl)	3.95 <sup>A</sup> $\pm$ 0.53 (10)	3.39 <sup>B</sup> $\pm$ 0.74 (49)	3.05 <sup>C</sup> $\pm$ 0.55 (25)
Albumin/globulin ratio	0.5 <sup>A</sup> $\pm$ 0.08 (10)	0.54 <sup>A</sup> $\pm$ 0.08 (49)	0.66 <sup>B</sup> $\pm$ 0.14 (25)
Uric acid (mg/dl)	10.7 <sup>A</sup> $\pm$ 4.1 (10)	10.1 <sup>A</sup> $\pm$ 8.7 (49)	21.7 <sup>B</sup> $\pm$ 10.4 (25)
Creatine kinase (IU/L)	271 <sup>A</sup> $\pm$ 127 (10)	343 <sup>A</sup> $\pm$ 179 (49)	433 <sup>B</sup> $\pm$ 153 (25)
Aminotransferase (IU/L)	163 <sup>A</sup> $\pm$ 31.2 (10)	187 <sup>A</sup> $\pm$ 71.8 (49)	258 <sup>B</sup> $\pm$ 187 (25)
Potassium (mEq/L)	3.64 <sup>A</sup> $\pm$ 1.03 (10)	2.75 <sup>B</sup> $\pm$ 0.57 (49)	2.63 <sup>B</sup> $\pm$ 0.56 (25)
Iron ( $\mu$ g/ml)	4.23 <sup>A</sup> $\pm$ 3.48 (9)	1.99 <sup>B</sup> $\pm$ 1.30 (49)	3.36 <sup>A</sup> $\pm$ 3.02 (20)
Zinc ( $\mu$ g/ml)	2.34 <sup>A</sup> $\pm$ 0.43 (9)	2.51 <sup>A</sup> $\pm$ 0.59 (49)	1.91 <sup>B</sup> $\pm$ 0.33 (20)

Means in the same row with different superscript letters (A, B, C) are significantly different ( $P < 0.05$ ) using a one-way ANOVA or Kruskal-Wallis one-way ANOVA (uric acid, creatine kinase, and iron).

infections are known to occur within a wide range of avian species and geographic areas, including captive and free-ranging penguins (3,24). Latent infections are common, and avian chlamydiosis is frequently associated with colony-nesting wild birds. Because the complement fixation test only assesses for presence of immunoglobulin F (IgG), acute or active infections in this colony may have been missed.

Antibody titers to *Salmonella Pullorum* were found in 7% of the HP in this study. Positive birds were found in 1993 and 1994 only. *Salmonella Pullorum* is not thought to be an important cause of illness and mortality in free-ranging birds (9). However, the detection of IgG suggests *Salmonella* sp. infection and immune system exposure in these HP at some point in time. Without culturing and identifying the organism within the feces, it is not possible to know with certainty which *Salmonella* sp./spp. is/are responsible for the titers found, and whether positive birds were actively infected. Plasma biochemistry differences were correlated with positive titers (Table 3). For 1993 and 1994 combined, mean plasma glucose levels were found to be significantly lower in individuals with positive antibody titers to *Salmonella Pullorum*. For 1994 alone, birds with positive titers had higher mean K and Fe levels than HP with no titers. However, given that these birds were not in poor condition upon examination and were, therefore, unlikely to be suffering from clinical disease, it is possible that these are incidental findings only.

Avian reoviruses have a worldwide distribution and wide host range (8), but the presence of reovirus antibodies has only been found once in penguins (16). The significance of one positive titer from 61 HP tested in this study is unknown. It is also not known whether the antibody response to the avian reovirus antigen used in this test was a cross-reaction due to antibodies to the reovirus reported to be present in the *O. amblyus* found in Peruvian seabird colonies (2).

Coronaviruses, which cause diseases such as infectious bronchitis, are generally considered to be pathogens of Galliformes (8). Antibody titers to infectious bronchitis serotypes have been reported for rockhopper penguins (*Eudyptes chrysocomes*) in Argentina (16), and they were found in one HP of 20 tested in this study. Hematology and plasma biochemistry values differed among individual rockhopper penguins that were seropositive and seronegative, suggesting that a physiologic response had taken place. No hematology or biochemistry findings differed in the HP showing a positive titer in this study.

Adenovirus infections have been reported to cause disease in Galliformes, pigeons, raptors, Psittaciformes, waterfowl, and rockhopper penguins in Argentina (8,16). Titers were found in 23% of rockhopper penguins, and seropositive birds had a lower mean plasma phosphorus level than seronegative birds (16). Biochemical differences were not found between seropositive and seronegative HP in this study; thus, it is uncertain whether the finding of 7% positive adenovirus antibody titers was of clinical significance in these birds.

In relative terms, avian paramyxovirus infections are the most described or well documented for penguins, including the little blue (*Eudyptula minor*), Adélie (*Pygoscelis adeliae*), king (*Apterodytes patagonica*), rockhopper, royal (*Eudyptes schlegeli*) and sphenisciform penguins (1,3). Infections reported in penguins range from subclinical with positive antibody response, to fatal mesogenic or velogenic infections seen with PMV-1 (8). The highest number (7%) of HP in this study had titers to PMV-2. The degree of clinical abnormalities in HP that occurs during active infection remains uncertain. Mean albumin-to-globulin ratios and creatinine kinase levels were lower and plasma total protein and globulin levels were higher in the HP that

had positive titers for antibodies to PMV-2 than the birds that did not have titers (Table 3). The higher globulin levels are consistent with active or recent viral infection. However, given that these birds did not show evidence of disease at the time of sampling, compounding factors may have played a role in these findings.

HP in this study tested negative for antibodies to avian influenza virus, but positive findings have been reported a number of times from other penguin species and migratory marine birds (3,12,21). Despite the negative findings during this study, monitoring for this disease agent is warranted because the study site is also a significant Peruvian breeding area for a variety of marine birds and pinnipeds that could be affected by the virus (18).

## REFERENCES

1. Austin, F. J., and R. G. Webster. Evidence of ortho- and paramyxoviruses in fauna from Antarctica. *J. Wildl. Dis.* 29:568–571. 1993.
2. Chastel, C. E. Tick-borne virus infections of marine birds. In: *Advances in disease vector research*, Vol. 5. K. F. Harris, ed. Springer-Verlag, New York. pp. 25–60. 1988.
3. Clarke, J. R., and K. R. Kerry. Diseases and parasites of penguins. *Korean J. Polar Res.* 4:79–96. 1993.
4. Dein, F. J., A. Wilson, D. Fischer, and J. Langenberg. Avian leucocyte counting using the hemocytometer. *J. Zoo Wildl. Med.* 25:432–437. 1994.
5. Duffy, D. C. The ecology of tick parasitism on densely nesting Peruvian seabirds. *Ecology* 64:110–119. 1983.
6. Forero, M. G., J. L. Tella, K. A. Hobson, M. Bertellotti, and G. Blanco. Conspecific food competition explains variability in colony size: a test in Magellanic penguins. *Ecology* 83:3466–3475. 2002.
7. Friend, M., R. G. McLean, and J. Dein. Disease emergence in birds: challenges for the twenty-first century. *Auk* 118:290–303. 2001.
8. Gerlach, H. Viruses. In: *Avian medicine: principles and application*. B. W. Ritchie, G. J. Harrison, and L. R. Harrison, eds. Wingers Publishing, Inc., Lake Worth, FL. pp. 862–948. 1994b.
9. Gerlach, H. Bacteria. In: *Avian medicine: principles and application*. B. W. Ritchie, G. J. Harrison, and L. R. Harrison, eds. Wingers Publishing, Inc., Lake Worth, FL. pp. 949–983. 1994a.
10. Ghebremeskel, K., G. Williams, I. F. Keymer, D. Horsley, and D. A. Gardner. Plasma chemistry of rockhopper (*Eudyptes crestatus*), Magellanic (*Spheniscus magellanicus*), and Gentoos (*Pygoscelis papua*) wild penguins in relation to molt. *Comp. Biochem. Physiol.* 92A:43–47. 1989.
11. Glünder, G. Zum vorkommen von *Plesiomonas shigelloides* bei wild- und zoovögeln. *Belin München. Tierärztliche Wochenschrift* 101:334–337. 1988.
12. Graves, I. L. Influenza viruses in birds of the Atlantic flyway. *Avian Dis.* 36:1–10. 1992.
13. Grazyck, T. K., M. R. Cranfield, J. J. Brossy, J. F. Cockrem, P. Jouventin, and P. J. Seddon. Detection of avian malaria infections in wild and captive penguins. *J. Helminthol. Soc. Wash.* 62:135–141. 1995.
14. Hays, C. Effects of the 1982–1983 El Niño on Humboldt penguin colonies in Peru. *Biol. Conserv.* 36:169–180. 1986.
15. International Species Inventory System. MedArks ISIS physiologic data. ISIS, Apple Valley, MN. 1991.
16. Karesh, W. B., M. M. Uhart, E. Frere, P. Gandini, W. E. Braselton, H. Puche, and R. A. Cook. Health evaluation of free-ranging rockhopper penguins (*Eudyptes chrysocomes*) in Argentina. *J. Zoo Wildl. Med.* 30:25–31. 1999.
17. Karesh, W. B., and R. A. Cook. Application of veterinary medicine to in situ conservation efforts. *Oryx* 29:244–252. 1995.
18. Kennedy-Stoskopf, S. Viral diseases in marine mammals. In: *CRC handbook of marine mammal medicine: health, disease, and rehabilitation*. L. A. Dierauf, ed. CRC Press, Inc., Boca Raton, FL. pp. 97–113. 1990.
19. Majluf, P., and J. L. Reyes. The marine mammals of Peru: a review. In: *The Peruvian upwelling ecosystem: dynamics and interactions*. D. Pauly, P. Muck, J. Mendo, and I. Tsukayama, eds. IMARPE, Callao, Peru. pp. 344–363. 1989.

20. Minnick, P. D., W. E. Braselton, G. L. Meerdink, and M. R. Slanker. Altered serum element concentrations due to laboratory usage of vacutainer tubes. *Vet. Hum. Toxicol.* 24:413–414. 1982.
21. Morgan, I. R., H. A. Westbury, I. W. Caple, and J. Campbell. A survey of virus infection in sub-Antarctic penguins on Macquarie Island, Southern Ocean. *Aust. Vet. J.* 57:333–335. 1981.
22. Price, H. A., R. L. Welch, R. H. Scheel, and L. A. Warren. Modified multiresidue method for chlordane, toxaphene and polychlorinated biphenyls in fish. *Bull. Environ. Contamin. Toxicol.* 37:1–9. 1986.
23. Ritchie, B. W., G. J. Harrison, and L. R. Harrison, eds. *Avian medicine: principles and application*. Wingers Publishing, Inc., Lake Worth, FL. 1994.
24. Shewen, P. E. Chlamydial infection in animals: a review. *Can. Vet. J.* 21:2–11. 1980.
25. Sokal, R. R., and F. J. Rohlf. *Biometry: the principles and practice of statistics in biological research*, 2nd ed. W. H. Freeman and Co., New York, NY. 1981.
26. Spalding, M. G., and D. J. Forrester. Disease monitoring of free-ranging and released wildlife. *J. Zoo Wildl. Med.* 24:271–280. 1993.
27. Stowe, H. D., W. E. Braselton, J. B. Kaneene, and M. Slanker. Multielement assays of bovine tissue specimens by inductively coupled argon plasma emission spectroscopy. *Am. J. Vet. Res.* 46:561–565. 1985.
28. Wallace, R. S., J. A. Teare, E. Deibold, M. Michaels, and M. J. Willis. Hematology and plasma chemistry values in free-ranging Humboldt penguins (*Spheniscus humboldti*) in Chile. *Zoo Biol.* 14:311–316. 1995.
29. Zavalaga, C., and R. Paredes. Humboldt penguins at Punta San Juan, Peru. *Penguin Conserv.* 10(1):6–8. 1997.
30. Zavalaga, C., and R. Paredes. Sex determination of adult Humboldt penguins (*Spheniscus humboldti*) using morphometric characters. *J. Field Ornithol.* 68:102–112. 1997.

#### ACKNOWLEDGMENTS

We thank the following individuals and institutions for the assistance and support without which this study could not have been completed: the Proyecto Especial de Promoción de Aprovechamiento de Abonos provenientes de Aves Marinas-PROABONOS and INRENA (the Peruvian National Natural Resources Institute) of the Peruvian Ministry of Agriculture and the Peruvian Marine Research Institute IMARPE for providing guidance and authorization for the project; Fabiola Leon Velarde, Cecilia Riva, and Luis Paz-Soldán for providing invaluable assistance in project logistics; Richard G. Robbins, Walter Reed Army Medical Center, for the identification of ectoparasites; Kirk Stuart, Michigan State Animal Health Diagnostic Laboratory, for in-depth analysis of samples; Roberta Wallace, Milwaukee County Zoo, for providing valuable bibliography material; and Susan Rosenberg and Ivan Llanes, Wildlife Conservation Society, for extensive laboratory and data entry work. We also thank the American Zoological Association Taxon Advisory Group for funding.