Journal of Zoo and Wildlife Medicine 35(1): 60–64, 2004

SURVEY FOR *HAEMOPROTEUS* SPP., *TRICHOMONAS GALLINAE*, *CHLAMYDOPHILA PSITTACI*, AND *SALMONELLA* SPP. IN GALAPAGOS ISLANDS COLUMBIFORMES

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Abstract: Endemic free-ranging Galapagos doves (*Zenaida galapagoensis*) and introduced rock doves (*Columba livia*) were surveyed in several islands of the Galapagos archipelago to establish sample prevalence of hemoparasites, *Trichomonas gallinae*, *Chlamydophila psittaci*, and *Salmonella* species. A *Haemoproteus* sp., the only hemoparasite identified, was found in 89% of the Galapagos doves sampled but not in the rock doves. *Trichomonas gallinae* was detected by polymerase chain reaction in 44% of rock doves from San Cristobal but in none of the Galapagos doves. *Chlamydophila psittaci* was detected from cloacal swabs in 6% of the Galapagos doves but in none of the rock doves sampled. All positive cases of *C. psittaci* occurred on Española, where the crude sample prevalence was 24%. A polymerase chain reaction–based *Salmonella* test failed to show evidence of this organism from any birds sampled.

Key words: *Columba livia*, *Zenaida galapagoensis*, *Chlamydophila psittaci*, *Trichomonas gallinae*, *Haemoproteus* sp., Galapagos Islands.

INTRODUCTION

Introduced avian diseases are a growing concern and conservation priority in the Galapagos Islands, based on their negative impact on native and endemic species in other archipelagos.[17] Determining baseline disease prevalence in free-ranging populations is essential to the recognition of epizootics. Furthermore, it provides objective data for implementing sound management practices that protect the endemic biodiversity of a region and minimize the risk of disease transmission from introduced species.

The Galapagos dove (*Zenaida galapagoensis*) is an endemic species present on most of the Galapagos Islands.[15] The rock dove (*Columba livia*) was introduced to the human-inhabited islands in the early 1970s[14] and is the only other member of the Columbidae found currently in the archipelago.[15] The taxonomic relationship between these two columbids suggests a shared susceptibility to pathogens. The goal of this study is to establish a crude prevalence of four pathogens in the two columbids found on the Galapagos Islands.

Haemoproteus spp., *Trichomonas gallinae*, *Chlamydophila psittaci*, and *Salmonella* spp. occur in many columbiform species around the world[22] and have been associated with varying degrees of pathogenicity and epizootic potential. Of these four pathogens, *T. gallinae* and *C. psittaci* are of particular interest in the Galapagos Islands, because of a rising concern that these could have been introduced by domestic avian species. *Trichomonas gallinae* was presumptively introduced to North America by European settlers[6] and has been considered to cause the most important disease of wild doves in the continent. A high percentage of infected birds can be asymptomatic carriers,[6] but epizootics of trichomoniasis have been associated with major mortality events in wild mourning doves (*Zenaida macroura*)[18] and band-tailed pigeons (*Columba fasciata*).[6]

Feral rock doves are the species most commonly associated with *C. psittaci* infections worldwide,[3] although all avian species are susceptible.[4] The prevalence of *C. psittaci* in feral rock doves has been reported as high as 30% by serology in a survey from 20 countries, and rock doves are considered potential carriers of *C. psittaci* to wild bird populations.[3,4] Infections have been associated with at least one high-mortality event in free-ranging white-winged doves (*Zenaida asiatica*)[13] and are a concern whenever feral rock doves may be in contact with wild species.

MATERIALS AND METHODS

All procedures were in accordance with approved guidelines by the University of Missouri–Saint Louis Institutional Animal Care Use Committee. Wild Galapagos doves ($n = 105$) were collected by either mist or hand nets on the islands of Santa Cruz ($n = 27$), Santa Fe ($n = 25$), Española

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(n = 25), Santiago (n = 26), and San Cristobal (n = 2) between the months of May and July 2002 in the Galapagos Islands, Ecuador. Individuals were identified with a single, colored, numbered plastic leg band to prevent resampling, and were released near the site of capture upon completion of biological sample collection. Feral rock doves (n = 18) were trapped by local farmers on the island of San Cristobal in June 2002. Rock doves (n = 10) were also acquired from a single farm in Santa Cruz during July 2001, before the eradication of this species from the island. After sample collection, rock doves were humanely euthanatized with pentobarbital sodium (Euthasol, 390 mg/ml, Delmarva Laboratories Inc., Midlothian, Virginia 23113, USA; 85.0 mg/kg, i.v.) as part of Galapagos National Park rock dove eradication efforts.

Fresh, thin smears were prepared from blood collected by ulnar or jugular venipuncture after manual restraint in all Galapagos doves. Blood was collected by cardiocentesis in rock doves after being anesthetized with xylazine (Xylazine-20, 20 mg/ml, The Butler Co., Columbus, Ohio 43228, USA; 1.0 mg/kg, i.m.), before euthanasia. Coagulation precluded blood smear preparation in one Galapagos dove from Española. Blood smears were allowed to dry, fixed in methyl alcohol (Dip Quick fixative, Jorgensen Laboratories Inc., Loveland, Colorado 80538, USA) and examined for presence or absence of hemoparasites.

Two cloacal swabs were collected from each bird using a sterile Dacron® tipped-applicator (Copan Diagnostics, Corona, California 91719, USA) with the exception of two Galapagos doves from Santa Cruz. Esopharyngeal swabs were collected from all birds using the same type of applicators, with the exception of two Santa Cruz Galapagos doves and the Santa Cruz rock doves, from which none was collected. Cloacal and esopharyngeal swabs were placed in separate screw-cap nalgene cryotubes (Nalge Nunc International, Rochester, New York 14625, USA) with no media and frozen at −20°C or less within 24 hr of collection. Samples remained frozen until submission for laboratory analysis. Esophageal swabs were submitted to a commercial veterinary laboratory (Healthgene Corporation, Toronto, Ontario M6M 3Z4, Canada) and tested for presence or absence of *T. gallinae* by polymerase chain reaction (PCR). As a positive control, an esophageal swab from a juvenile wild mourning dove (*Z. macroura*) from St. Louis, Missouri, USA, affected with clinical trichomoniasis and diagnosed by direct microscopy, was submitted. Cloacal swabs were submitted to the Infectious Diseases Laboratory, University of Georgia College of Veterinary Medicine, Athens, Georgia 30602, USA, and tested for presence or absence of *C. psittaci* by PCR. All cloacal samples collected in 2002 were tested for presence or absence of *Salmonella* spp. by PCR at the same laboratory.

Crude sample prevalence was calculated for each disease as the number of positive individuals per total number of individuals sampled, without differentiating individuals by sex, age, or body mass.

RESULTS

Crude sample prevalence results of the four pathogens surveyed are presented in Table 1. *Haemoproteus* organisms were commonly observed on peripheral blood smear analysis of Galapagos doves but not seen in any of the rock doves. No other blood parasites were identified. *Trichomonas gallinae* was commonly seen in the rock doves sampled in San Cristobal but was not seen in Galapagos doves from any of the islands. *Chlamydophila psittaci* occurred commonly in Galapagos doves from Española but was not seen in birds from any other location.

DISCUSSION

A high sample prevalence of *Haemoproteus* organisms was observed in the Galapagos doves of five islands surveyed during the months of May and July 2002, but no hemoparasites were found in the introduced rock doves surveyed. Numerous ecologic[8,12] and evolutionary[2,19] implications are associated with the existence of *Haemoproteus* in different avian species. At least two species of *Haemoproteus* (*H. columbae* and *H. sacharovi*) have been identified in Columbiformes[7] but as many as seven have been proposed based on differences in taxonomic classifications.[1] As advanced molecular techniques are developed and refined, the ability to objectively differentiate between columbid species of *Haemoproteus* organisms will provide further insight into the host parasite evolution and the rate of speciation in island populations. Hippoboscid flies, often found on the doves in this study, are the presumptive vector in this species, although biting midges are vectors in some species.[7]

The finding of *Haemoproteus* sp. in blood smears is considered incidental, rather than an indication of the presence of a serious pathogen. The prevalence in some wild dove populations has been reported as high as 100%.[1] Heavily parasitized individuals may suffer from subclinical exercise intolerance,[21] which may indirectly affect host survival in the wild. Although not associated with high-mortality events, the effect of subclinical *Haemoproteus* infections is not known at the population level.

Table 1. Crude sample prevalence of four diseases in Galapagos doves (*Zenaida galapagoensis*) and rock doves (*Columba livia*) surveyed on the Galapagos archipelago.

Island	*Haemoproteus* sp.[a]		*Trichomonas gallinae*[b]		*Chlamydophila psittaci*[c]		*Salmonella* sp.[d]	
	Zenaida galapagoensis	*Columba livia*	*Zenaida galapagoensis*	*Columba livia*	*Zenaida galapagoensis*	*Columba livia*	*Zenaida galapagoensis*	*Columba livia*
Santa Cruz[e]	85% (23/27)	0% (0/10)	0% (0/25)	n/a[f]	0% (0/25)	0% (0/10)	0% (0/25)	n/a
Española	88% (21/24)	n/p[g]	0% (0/25)	n/p	24% (6/25)	n/p	0% (0/25)	n/p
Santa Fe	88% (22/25)	n/p	0% (0/25)	n/p	0% (0/25)	n/p	0% (0/25)	n/p
Santiago	96% (25/26)	n/p	0% (0/2)	n/p	0% (0/25)	n/p	0% (0/25)	n/p
San Cristobal	100% (2/2)	0% (0/20)	0% (0/2)	44% (8/18)	0% (0/2)	0% (0/18)	0% (0/2)	0% (0/18)
Total	89% (93/104)	0% (0/30)	0% (0/102)	44% (8/18)	6% (6/102)	0% (0/28)	0% (0/102)	0% (0/18)

[a] *Haemoproteus* sp. detected by blood smear microscopy analysis.
[b] *Trichomonas gallinae* tested by polymerase chain reaction (PCR) analysis of esopharyngeal swabs.
[c] *Chlamydophila psittaci* tested by PCR analysis of cloacal swabs.
[d] *Salmonella* sp. tested by PCR analysis of cloacal swabs.
[e] *Columba livia* samples were collected in Santa Cruz during 2001.
[f] n/a, samples not collected.
[g] n/p, species is not present in this island.

At least one experimental manipulation of blue tits (*Parus caeruleus*) suggests that *Haemoproteus* organisms can have detrimental effects on host reproductive success.[16] Decreased reproductive success may have a significant impact on the overall status of a population, despite being considered an incidental finding in an individual. Establishing the prevalence and incidence over time may elucidate correlations between the disease and host population fluctuations, which in turn may have implications for ecosystem management and species conservation.

Trichomonas gallinae was identified in eight of 18 rock doves from San Cristobal but not in the 102 Galapagos doves surveyed from multiple islands. None of the birds showed gross lesions indicative of clinical disease. A 1987 report suggested a high occurrence of *T. gallinae* in introduced rock doves on Santa Cruz, San Cristobal and Isabela islands, with the presence of gross lesions suggesting significant pathogenicity in the strain present.[14] The authors identified *T. gallinae* in Galapagos doves from the island of Santa Cruz but not in Galapagos doves from three rock dove–free islands and hypothesized that interactions between the two species led to transmission of trichomoniasis.[14] Those findings contrast with the inapparent detection of any evidence of trichomoniasis in the Galapagos doves surveyed. The observation of an apparent decline in the Galapagos dove population on the island of San Cristobal, where *Trichomonas* was identified in rock doves in 2002, is notable, although other factors, including large numbers of feral cats and rats, are more likely implicated. Because rock doves were eradicated from Santa Cruz in 2001, the risk and rate of disease transmission between the two species on this island will not be known.

The importance of trichomoniasis in wild Galapagos doves as well as the virulence of strains reportedly infecting the species is not known. The PCR-based *T. gallinae* diagnostic test used in this study provides an objective, sensitive method to determine the presence of this parasite and is applicable in field situations. The technique facilitates the transport of samples and eliminates the need for immediate microscopic examination, allowing biologists to optimize field time. Furthermore, as molecular techniques are refined, the PCR diagnostic techniques offer the possibility for genetically typing and differentiating strains that may elucidate disease transmission dynamics.[5]

Chlamydophila psittaci sequences were identified in cloacal swabs from six Galapagos doves from the island of Española. Because of the inter-

mittent shedding of this organism, our results may underestimate the true prevalence of this pathogen in the Galapagos dove population. A study in which cloacal samples from captive Amazon parrots were tested for *Chlamydophila* antigen by direct immunofluorescence at 48-hr intervals showed that only 44% of birds were positive on both sampling days.[20] This study suggests that there are limitations in using a single cloacal swab, although specific data on shedding frequency is not available for doves or pigeons. The PCR diagnostic tests can detect very small numbers of *Chlamydophila* organisms because it amplifies a portion of a nucleotide sequence,[11] but the increased sensitivity could also result in false positives from sample contamination and is still limited by the intermittent shedding of the organism. Because all the tests currently available for the detection of *Chlamydophila* have inherent limitations and there is no standard method of detection, establishing the true prevalence of this disease is difficult.

The finding of *C. psittaci* sequences in the Galapagos dove population suggests that this pathogen is present in the islands, although the ecological implications are not known. The occurrence of *C. psittaci* only in Galapagos doves sampled on the island of Española may reflect numerous host-specific factors or be related to ecological factors unique to this island. Multispecies congregations of colonial nesting seabirds on the island of Española may play a role in the transmission of this disease. In addition, long distance migrants that interact with South American continental birds before returning to nest on Española may serve as carriers of *C. psittaci*. In North America, waterfowl, herons, and pigeons are considered the most commonly infected groups of free-ranging wild birds,[9] and at least one epizootic in gulls has been associated with chlamydophilosis.[10] Multispecies studies of *C. psittaci* in wild birds are limited,[3] but subclinical carriers may be disease reservoirs in free-ranging populations. Determination of the prevalence of *C. psittaci* in the different species that congregate on Española island and the ecologic implications of differences in host susceptibility, strain virulence, seasonality of infection, and impacts on demographics may yield valuable insights into the transmission of the disease in wildlife populations and serve as a model for studying the ecology and epidemiology of other infectious diseases. The absence of *C. psittaci* in the feral rock doves sampled on the Galapagos archipelago does not rule out its role as a significant pathogen in the species but reflects the limitations of the testing procedures.

No *Salmonella* sequence was identified in any birds tested. A PCR-based diagnostic test was used to eliminate the inherent difficulties of culturing the organism from samples collected under field conditions. The methodology used does not discriminate between *Salmonella* species or strains and should have identified any *Salmonella* organisms being shed. Because salmonellosis may be associated with acute fatalities or short disease duration, antigen surveillance of subclinical carriers may not be indicative of its importance at the population level. Serological surveys as well as active monitoring during clinical epidemics may elucidate its role in the Galapagos Islands' bird populations.

This is the first study to estimate pathogen ocurrence in columbids in the Galapagos Islands. Calculating population prevalence necessitates a known or estimated population size,[23] which was not feasible in this case. The nonrandomized sampling technique used in this study maximized the number of samples collected in a short period of time. Although this sampling technique may bias results,[23] it provides general information on the presence or absence of pathogens in the immediate geographic vicinity where sampling occurred. The reported sample prevalence is indicative of relative frequency of occurrence of these pathogens in the two species sampled, although the absolute numbers are not equivalent to population prevalence estimates. Knowing relative frequency of occurrence of these pathogens in the two species of columbids is essential for proper epidemiological study design, disease modeling, and basic science surveys that can subsequently be used as tools for species conservation programs.

Acknowledgments: We thank the Des Lee Collaborative Vision in Zoological Research and the Saint Louis Zoo, which provided financial support for this disease survey. In addition, field work was partially supported by a grant from the International Center for Tropical Ecology (ICTE) to one of the authors (DS-A). We thank the Charles Darwin Research Station and the Galapagos National Park for providing logistic and travel support for the field collections; we thank Gustavo Jimenez, Noah Whiteman, and Jennifer Bollmer for invaluable assistance in field logistics, rock dove acquisition, sample collection and processing of samples. Special thanks to Dr. Tim Walsh, Dr. Mary Duncan, Dr. Theresa Thiel, Julia Thiel, and Jessy Rabenold for assistance in various aspects of this study.

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Received for publication 29 March 2003