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A SURVEY FOR SELECTED VIRAL, CHLAMYDIAL, AND PARASITIC DISEASES IN WILD DUSKY-HEADED PARAKEETS (*ARATINGA WEDDELLII*) AND TUI PARAKEETS (*BROTOGERIS SANCTITHOMAE*) IN PERU

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ABSTRACT: Thirty-eight free-ranging dusky-headed parakeets (*Aratinga weddellii*) and 13 tui parakeets (*Brotogeris sanctithomae*) were caught and released in Parque Nacional del Manu in southeastern Peru from 19 July to 5 August 1993. Blood and fecal samples were collected and sera were evaluated for titers to Pacheco's disease herpesvirus, psittacine polyomavirus, paramyxovirus-1, and *Chlamydia psittaci*. Fecal samples were examined for evidence of ascarid or coccidial infection by fecal flotation, and blood smears were examined for hemoparasites. Five (50%) of 10 *A. weddellii* serum samples tested by complement fixation (CF) for psittacine polyomavirus antibodies were positive, and three (19%) of 16 *A. weddellii* samples tested by virus neutralization (VN) for psittacine polyomavirus antibodies were positive, yielding a total of 8 (38%) of the 21 *A. weddellii* samples positive for psittacine polyomavirus. Based on CF for herpesvirus, four (11%) of 38 *A. weddellii* samples had antibodies against herpesvirus. All *B. sanctithomae* were negative for psittacine polyomavirus and psittacine herpesvirus. Thirty-five of the *A. weddellii* tested were negative for *Chlamydia psittaci* by CF, latex agglutination, and elementary body agglutination, and all *B. sanctithomae* were negative for *Chlamydia psittaci* by the CF test. Nine *A. weddellii* and eight *B. sanctithomae* evaluated for paramyxovirus-1 titers by the hemagglutination inhibition test were negative. All fecal samples were negative for ascarids or coccidia by fecal flotation, and all blood smears were negative for hemoparasites by direct microscopic examination. This is the first known description of psittacine polyomavirus and psittacine herpesvirus in free-ranging parrots. Serologic evidence of Pacheco's disease herpesvirus in wild *A. weddellii* is interesting in light of the fact that *Aratinga* spp. are considered to be possible carriers of this virus in captivity.

Key words: Psittaciformes, parrot, dusky-headed parakeet, *Aratinga weddellii*, tui parakeet, *Brotogeris sanctithomae*, polyomavirus, herpesvirus, *Chlamydia psittaci*.

INTRODUCTION

The avian order Psittaciformes, or the parrots, comprises more than 330 extant species ranging worldwide, primarily in the tropical regions of the southern hemisphere (Forshaw, 1989). Eighteen species are considered endangered, and 42 others are listed as rare or vulnerable (Groombridge, 1994). The growing awareness of the endangered status of parrots in the wild, and the consensus that many of these populations will require some degree of management if they are to remain viable, has prompted an increased interest in the biology and ecology of wild parrot populations (Beissinger and Snyder, 1992).

The need for an increased effort in research on disease in threatened and endangered avian populations was emphasized by a symposium on disease and threatened birds held during the XIX World Conference of the International Council for Bird Preservation (ICBP) in June, 1986 (International Council for Bird Preservation, 1989). The ICBP members resolved to encourage health monitoring of threatened avian species in light of increasing evidence that disease has an adverse impact on avian populations. Indeed, epizootics resulting in significant morbidity and mortality in wild populations of the endangered whooping crane (*Grus americana*), sandhill crane (*Grus cana-*

densis), bald eagle (*Haliaeetus leucocephalus*), California condor (*Gymnogyps californianus*), and several endangered native Hawaiian species have been reported (Van Riper et al., 1986; Friend and Thomas, 1992).

While the diseases and medical management of captive parrots is a rapidly expanding specialty in veterinary medicine, diseases of free-ranging parrots have received relatively little attention. Burnet (1935) diagnosed psittacosis (*Chlamydia psittaci*) in several wild Australian parrot species. Evidence of psittacine beak and feather disease (PBFD) infection has been found in wild Australian populations of sulfur-crested cockatoos (*Cacatua galerita*) (McOrist et al., 1984), galahs (*Eolophus roseicapillus*) (Pass and Perry, 1985), and two species of corellas (*Cacatua tenuirostris* and *C. sanguinea*) (Raidal et al., 1993). Johnson et al. (1986) did not detect paramyxovirus-1, the causative agent of Newcastle Disease, in several native parrot species in the Philippines.

There are several published reports of disease surveys conducted at quarantine stations. Most survey efforts at quarantine stations have been directed either at diseases known to be important to the poultry (e.g., Newcastle Disease) or pet bird (e.g., PBFD) industries, or at zoonotic diseases (e.g., chlamydiosis) (Meyer and Eddie, 1934; Rigby et al., 1981; Alexander et al., 1982; Senne et al., 1983). It is difficult to interpret these reports in terms of the role these pathogens may play in wild parrot populations: the data are complicated by the stress of capture, transport and species mixing, all of which may exacerbate subclinical infections or allow for spread of disease.

Given that captive parrots are susceptible to a variety of viruses and parasites, and that many of these diseases occur in quarantined birds, we hypothesized that these diseases may exist in wild parrot populations. Thus, our objective was to evaluate a wild population of parrots for serologic evidence of exposure to diseases

known to affect captive birds, specifically paramyxovirus-1, psittacine herpesvirus, psittacine polyomavirus, *Chlamydia psittaci*, ascarids, coccidia, and hemoparasites. Parque Nacional Manu was chosen as a field site specifically because many of the parrots flock routinely and predictably to sites along the Manu River where they forage for clay. This behavior is observable at close range, and provides a rare opportunity to capture adult wild parrots with relative ease.

MATERIALS AND METHODS

Parque Nacional Manu lies in the western Amazon basin of southeastern Peru (11°50'S, 71°26'W). The park encompasses a section of lowland tropical rainforest which has been deemed perhaps the most pristine rainforest habitat remaining in the world (Terborgh, 1983).

Data collection took place from 19 July to 5 August, 1993. Adult dusky-headed parakeets (*Aratinga weddellii*) and tui parakeets (*Protophygia sanctithomae*) were caught in nylon mist nets (Avinet Inc., Dryden, New York, USA) placed approximately 3 to 5 m in front of clay banks known to be used by these two species. Nets were placed prior to the birds' visits, and all birds were caught as they flew off the banks of their own accord. Birds were removed from the nets immediately, placed in wire mesh cages, and transported back to the field station for sample collection.

Birds were manually restrained and examined for body condition, external lesions and ectoparasites. Blood samples were collected by jugular venipuncture. Blood smears were made from each sample and stained using a modified Wright's and Giemsa stain (Campbell, 1994); the remainder of the blood sample was centrifuged, and the serum frozen in liquid nitrogen. Fecal samples were collected from plastic sheets placed under the transport cages and preserved in 5% formalin. Prior to release, each bird was marked with indelible black ink on the breast feathers to avoid re-sampling of the bird. All birds were released within 1 to 2 hr of capture.

Serum samples were evaluated for antibodies to psittacine herpesvirus by complement fixation (CF) (Casey, 1965) at the Texas Veterinary Medical Diagnostic Laboratory (TVMDL), College Station, Texas, USA; to psittacine polyomavirus by both CF (Casey, 1965) at TVMDL and virus neutralization (VN) (Phalen et al., 1993) at the School of Veterinary Medicine, Texas A&M University, College Station, Texas; to paramyxovirus-1 by hemagglutination inhi-

TABLE 1. A serosurvey for viruses and *Chlamydia psittaci* in wild *Aratinga weddellii* and *Brotogeris sanctithomae* in southeastern Peru, July to August 1993.

Serologic tests	<i>Aratinga weddellii</i>	<i>Brotogeris sanctithomae</i>
<i>Chlamydia psittaci</i>		
Complement fixation	0/17 (0) ^a	0/13 (0)
Latex agglutination	0/35 (0)	not tested
Elementary body agglutination	0/35 (0)	not tested
<i>Herpesvirus</i>		
Complement fixation ^b	4/38 (11)	0/13 (0)
<i>Polyomavirus</i>		
Complement fixation ^c	5/10 (50)	0/3 (0)
Virus neutralization ^d	3/16 (19)	0/11 (0)
<i>Paramyxovirus-1</i> (HI)		
Hemagglutination inhibition	0/9 (0)	0/8 (0)

^a Number positive/total tested (percent positive).

^b Based on Pacheco's Disease herpesvirus.

^c Based on a budgerigar psittacine polyomavirus isolate.

^d Based on a non-budgerigar psittacine polyomavirus isolate.

bition (HI) (Hansen, 1980) at the California Veterinary Diagnostic Laboratory, Davis, California (USA); and to *Chlamydia psittaci* by CF, latex agglutination (LA), and elementary body agglutination (EBA) (Grimes et al., 1993, 1994) at TVMDL. These serologic tests are routinely used by avian practitioners, and vary in their sensitivity and specificity. The number of serologic tests performed on each bird was determined by the amount of serum obtained.

Fecal samples were centrifuged; the 5% formalin was decanted and the pellet was examined by fecal flotation using a commercially available kit (Ovatector®, BGS Medical Products Inc. Venice, Florida, USA). Blood smears were examined via direct microscopy at 40× and 100× magnification.

RESULTS

Thirty-eight *Aratinga weddellii* and 13 *Brotogeris sanctithomae* were captured (Table 1). All birds were free of obvious external lesions or ectoparasites and were in good body condition. Psittacine herpesvirus titers in the *A. weddellii* ranged from 1:8 to 1:32. Psittacine polyomavirus titers as measured by CF in the *A. weddellii* ranged from 1:8 to 1:64. Eight (38%) of 21 *A. weddellii* tested for polyomavirus by either or both serological tests were positive for polyomavirus. Of the 21 samples tested for polyomavirus antibody, five were evaluated using both the CF and VN tests: three were negative by both assays, one

was positive by VN and negative by CF, and one was positive by CF and negative by VN. All *B. sanctithomae* were negative for psittacine polyomavirus and psittacine herpesvirus. All *A. weddellii* were negative for *Chlamydia psittaci* by CF, LA and EBA, and all *B. sanctithomae* were negative for *Chlamydia psittaci* by CF. Nine *A. weddellii* and 8 *B. sanctithomae* evaluated for paramyxovirus-1 titers by HI were negative. All fecal samples were negative for ascarids and coccidia by fecal flotation, and all blood smears were negative for hemoparasites via direct microscopic examination.

DISCUSSION

Although both psittacine polyomavirus and psittacine herpesvirus infections have been reported in captive parrot species, neither virus is known to have been reported previously in a wild parrot. Titers to a papovavirus-like virus that was suspected to be psittacine polyomavirus were demonstrated in recently imported sun conures (*Aratinga solstitialis*) from Guyana in 1984 (S. Clubb, pers. comm.). Whether psittacine herpesvirus or psittacine polyomavirus have caused significant disease in the free-ranging *Aratinga weddellii* population in Manu is not known.

Polyomaviruses are small double-stranded DNA viruses belonging to the Papovaviridae family. The psittacine polyomavirus is the etiologic agent of budgerigar fledgling disease, an acute generalized viral infection of young budgerigars (*Melopsittacus undulatus*) (Müller and Nitschke, 1986). The virus also has been reported to cause disease in other parrot species (Jacobson et al., 1984; Pass et al., 1987). A persistent carrier state is possible after recovery from acute illness or with occult infection (Gerlach, 1994); indeed, chronic antigenic stimulation probably is responsible for the virus's ability to elicit production of antibody titers that are long-lived (Gaskin, 1989). Based on our data, *Aratinga weddellii* in Manu were exposed to the virus, and moreover, were possibly still infected. This raises the question as to whether a persistent low-grade infection of the population exists.

It is difficult to interpret the conflicting results of the VN and CF assays for psittacine polyomavirus in two of the *A. weddellii* samples. The VN assay is used to detect immunoglobulin M antibodies in addition to immunoglobulin G (IgG) antibodies, while the CF assay is used to detect IgG only; thus it is possible that the bird positive for psittacine polyomavirus by VN, but negative by CF, had experienced a more recent exposure to the virus.

Psittacine herpesviruses are associated with several disease syndromes in parrots; the best described is Pacheco's disease (Simpson et al., 1975). This disease usually causes a brief illness marked by lethargy, regurgitation, and diarrhea, but also can result in acute death with no clinical signs. The available serologic test for psittacine herpesvirus is used to detect antibodies to one serotype of the Pacheco's disease virus. A persistent carrier state is common with psittacine herpesvirus infections, and carriers probably shed periodically, especially during times of stress. Conures, a term applied to the group of parrots comprised of the genera *Aratinga*, *Nandayus*, and *Cyanoliseus*, are considered to be persistent

carriers of Pacheco's disease virus, and have been associated with several aviary epizootics (Gerlach, 1994). However, conures vary widely in their susceptibility to the virus: *Cyanoliseus patagonus* and *Nandayus nenday* often are very resistant to clinical disease in the face of an epizootic, despite evidence for antibodies, while the *Aratinga* spp. are variably susceptible to the virus (Gaskin, 1989). Our results support the idea that conures are herpesvirus carriers, and raise the possibility that *A. weddellii* in Manu may serve as a source of infection for other parrot species in the region.

Based on our data, *Chlamydia psittaci* exposure has not occurred in the Manu *A. weddellii* and *B. sanctithomae* populations. These results were surprising, considering that much of the data on diseases of quarantined parrots concern *Chlamydia psittaci* infections. The combination of the three assays for *Chlamydia psittaci* provided as thorough a serologic examination for this disease as is commercially available at this time. A fecal antigen assay may have resulted in additional information on the status of *Chlamydia psittaci* in the population, but was not available for use during this project. It is possible that there was a low prevalence of the organism in the population which was undetectable given the sample size.

Additionally, the fact that all birds tested for paramyxovirus-1 were negative for antibody cannot be interpreted as evidence for a lack of Newcastle Disease infection in these populations, given the small sample size.

The lack of parasite ova in fecal samples was somewhat surprising, as ascarids and coccidia have been identified in wild or recently-quarantined parrots (Ewers, 1973; Webster, 1982; Nixon and Weekes, 1985; Shinn-Shyong et al., 1992). It is possible that the study birds were parasitized and simply not shedding ova at the time of sampling. Also, it is possible that the findings of this study were related to what appears to be little opportunity for trans-

mission of enteric parasites in these species: both *A. weddellii* and *B. sanctithomae* are wholly arboreal, and feed on a variety of fruits and seeds (Hilty and Brown, 1986). They come into contact with the ground only at the claybanks (J. Gilardi, unpubl.), and there is little opportunity for ingestion of infective parasite ova, as there seems to be very little defecation occurring at this site (K. V. K. Gilardi, unpubl.). Thus, a lack of opportunity for parasite transmission may explain the absence of ascarids or coccidia demonstrated in fecal samples in this study. Additionally, based on analysis of the diets of several parrot species in Manu, the diets contain several secondary compounds (J. Gilardi, unpubl.) that may act as parasitocides (Janzen, 1978). There were not enough feces to conduct a complete enteric parasite examination on each sample; the possibility for cestode, trematode and other parasite infections remains.

Based on examination of the peripheral blood smears, there was no evidence for hemoparasite infection. Blood parasites have been reported in wild *Eclectus* sp. and *Trichoglossus* sp. in New Guinea (Ewers, 1973), in *Pionopsitta* spp. in Panama (Bennett and Borrero, 1976), in *Pionopsitta* spp. and *Pionus* sp. in Colombia (Galindo and Sousa, 1966), and in an *Ara* sp. in Mexico (Bennett et al., 1991). Our results were unexpected. In a review of the hematozoa of neotropical birds, White et al. (1978) noted a low but consistent prevalence of blood parasites across several avian families, including the Psittacidae. Parrots, however, were not as highly infected as some other avian families.

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