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WAS DISEASE INVOLVED IN THE DECIMATION OF GUAM'S AVIFAUNA?

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ABSTRACT: Between 1982 and 1986, 402 (290 live, 112 dead) exotic, migrant or native resident birds on Guam were surveyed for disease-causing agents to determine the role of disease in the decline of native forest bird populations on Guam. Traumatic injury, primarily from collisions with motor vehicles and predation, was the most prevalent (46%) cause of death. Thirty-eight percent of the carcasses examined were in poor body condition largely as a result of inadequate nutrition in captive native birds and poultry and adipose exhaustion in errant migrants. A variety of commensal or opportunistic bacteria, including *Salmonella* spp., were cultured from 220 birds, and nothing remarkable was found in 15 fecal samples. Lastly, no haematozoans, the suspected cause for the decline of the Hawaiian avifauna, were observed in blood slides examined from 260 birds. Based on the results of the survey and other lines of evidence presented in the discussion, we concluded there were no data implicating disease in the decline of Guam's avifauna.

Key words: Avian disease, endangered species, extinction, island avifauna, factors in population decline.

INTRODUCTION

The native forest avifauna of Guam has become endangered in recent decades. The 11 native species were distributed formerly throughout the island (Jenkins, 1983). Ten of the 11 species (the exception being the Island Swiftlet, *Aerodramus vanikorensis*), disappeared from Guam's southern ravine forests in the 1960's, and then populations progressively declined in the north (Jenkins, 1983; Savidge, 1986). In 1982, at the start of this survey, detectable populations of all 10 species were present only in the most northern limestone forests (Engbring and Ramsey, 1984). Currently, six of 11 forest species are listed federally as endangered and one is listed as a candidate endangered species (Federal Register, 1984, 1985); all 11 species are listed as endangered by the Government of Guam. These 11 species nest in a variety of locations (trees, caves, cliff potholes, ground) and include frugivores, omnivores, insectivores, and partial nectivores.

By 1982, preliminary data from earlier studies by other biologists tentatively had eliminated pesticides and competition from introduced avian species as possible causes of the declines (Maben, 1982; Grue, 1985). Substantial forest habitat remained in both southern and northern Guam (Savidge, 1984), and forests in northern Guam that had harbored birds a few years earlier now had virtually none. Several exotic predators were introduced to Guam and more recent data indicate that predation by the brown tree snake (*Boiga irregularis*) was probably responsible for the avian population declines (Savidge, 1987). However, in 1982, many biologists on Guam considered disease a cogent explanation for the population declines, especially because pox and malaria have been implicated in the decline of the native Hawaiian avifauna (Warner, 1968; van Riper et al., 1986). Exotic avian species that might have carried pathogens have been introduced to Guam and include the blue-breasted quail (*Coturnix chinensis*), Philippine turtledove (*Streptopelia bitorquata*), black francolin (*Francolinus francolinus*), chestnut mannikin (*Lonchura malacca*), Eurasian tree-sparrow (*Passer montanus*), black

drongo (*Dicrurus macrocercus*), and rock dove (*Columba livia*). The quail and turtledove were introduced before 1900, but the other species were introduced in the late 1950's and early 1960's. The black francolin, a game bird introduced from India in 1961, increased in numbers and expanded its range throughout the southern savanna on Guam while native birds declined (Guam Division of Aquatic and Wildlife Resources, unpubl. data). The number of arthropod disease vectors also has increased. The variety of mosquito species on Guam increased from five collected before World War II to 35 after the Vietnam War, largely a result of increased air traffic (Nowell, 1975). *Culex quinquefasciatus*, a known vector of avian malaria and pox with a feeding preference for birds, was first reported on Guam in 1936 and may have been present in 1911 (Swezey, 1942). Warner (1968) suggested that introduction of this mosquito in Maui in 1826 triggered massive avian extinctions in the Hawaiian Islands.

If disease was responsible for the population declines on Guam, it seemed possible that examination of birds, especially those from the northern limestone forests where populations were declining at the time of our study, might reveal the etiology. In this study we surveyed endangered species and species that could serve as disease reservoirs or indicators of an island-wide problem (migrants, introduced species including several that were also declining on Guam, and poultry) between 1982 to 1986 for pox, malaria and other parasites, bacteria or viruses that might be extirpating the forest avifauna.

STUDY AREA AND METHODS

Guam is located approximately halfway between Japan and New Guinea (13°30′N, 144°40′E). The climate is warm and humid. The northern half of the island is an uplifted limestone plateau dominated by second-growth forest, whereas the southern portion is largely of volcanic origin with savanna and ravine forest the main habitat types (Stone, 1970).

We captured live forest birds by mist netting with Japanese-style shelf nets concentrated in the northern limestone forest (n = 77) and on Cocos Island (n = 18), a 30.8-ha island 2.5 km south of Guam. Birds were either released after sampling or retained for captive propagation efforts. We also sampled Guam Rails (*Rallus owstoni*) born in captivity at Guam Division of Aquatic and Wildlife Resources (GDAWR) in central Guam (n = 17). These rails were part of the Division's captive propagation program and were kept outdoors, exposed to vectors. Live exotic psitticines, chickens, quail, ducks and domestic pigeons were sampled at local pet stores and private residences. Introduced free-living birds were mist-netted, shot by hunters, or found injured. We sampled injured birds whenever available, and we collected and examined by necropsy carcasses of all recently killed birds found dead on the road or in the field.

We obtained blood for smears from live birds by venipuncture or by clipping a claw. Blood was removed from the body cavity or heart of dead birds. Usually two or more blood slides for microscopic examination were made from each bird. Blood smears were air-dried and stained with either Diff-Quik Stain (Dade Diagnostics, Inc., Aguada, Puerto Rico 00602, USA) or Camco Quik Stain II (Cambridge Chem. Products, Inc., Fort Lauderdale, Florida 33310, USA). We examined each slide at 1,000× for 10 min.

Cloacal and tracheal swabs were obtained for bacterial disease or for Newcastle disease and influenza virus isolation procedures. We swirled swabs for viral tests in a transport medium (Hanks BSS containing antibiotics and gelatin; Docherty and Slota, 1988), and we then stored the medium in an ultralow freezer at −56.7 C. We used Amies transport media with swabs (Precision Dynamics Corp., Burbank, California 91504, USA) for bacteriological samples. These were sealed in whirl bags and frozen at −8 C. All swab samples were shipped on dry ice by air freight to the National Wildlife Health Research Center (NWHRC). Because of the long time in transit, an unrecorded number of these samples were thawed when unpacked but others were received frozen. It is unknown what effect thawing may have had on the viability of potential pathogens. Laboratory procedures included inoculation of blood agar, eosin-methylene blue media, and *Salmonella* spp. enrichment media for bacteriological tests. Embryonated chicken eggs and cell cultures of duck embryo fibroblasts were used for virus isolation procedures (Docherty and Slota, 1988). We stored and shipped fecal samples in 2% potassium dichromate.

The carcasses of 13 native forest birds were found in the field, and 13 (10 of which had been captured for artificial propagation efforts) died

in captivity. We examined 86 carcasses of other species. Initially, we shipped carcasses on dry ice to the NWHRC (n = 44), but because of artifactual changes in carcasses caused by freezing and an unrecorded number of instances of thawing during shipment, we necropsied the remainder (n = 68) on Guam. Necropsy protocol included body weight and condition (based on the quantity of adipose in normal storage depots and the degree of atrophy of muscle and viscera) and usually the collection of tissues for microscopic examination, blood from the heart for blood smears, and cloacal swabs for bacteriological and virological tests. Tissues for microscopic examination were fixed in 10% formalin, embedded in paraffin, sectioned at 5 μm, stained with hematoxylin and eosin, and examined by light microscope.

RESULTS

We conducted laboratory examinations of 763 biological samples and 112 necropsies for 402 live or dead birds representing 48 species of 20 families (Tables 1, 2). None of the live native forest birds captured in Northern Guam, on Cocos Island, nor the live rails sampled at GDAWR appeared sick or depressed. Trauma (46%) or emaciation (38%) were the significant findings in most of the carcasses (Table 2). A variety of secondary lesions were found, but there were no significant, consistent disease syndromes or pathogens in the carcasses. Hematozoans were not found in the blood smears and neither Newcastle, influenza, nor any other viruses were isolated from the swabs taken from live and dead birds.

Carcasses of the native forest species found in the field were of special interest because these carcasses were expected to provide a clue to the suspected disease process. Trauma (collision with automobiles or aircraft, gunshot, and snake predation) was the cause of death of all 13 (Table 2). Thirteen captive endangered forest birds succumbed to intra-specific strife, stress-related anorexia, heat prostration, accidental drowning in water bowls, or incorrect force-feeding. The liver and kidney of a native resident Mariana fruit-dove (*Ptilinopus roseicapilla*) that had been killed by a snake had small granules of hemosiderin-like pigment. However, no malarial organisms were detected in histological preparations from the bird. We detected no important secondary findings or concurrent diseases in any of the other carcasses. The only endangered nonforest species examined was a gallinule (*Gallinula chloropus*) that succumbed to trauma.

Trauma was also a common cause of death for 85 nonendangered, nonforest species examined (Table 2). Thirty-four (40%) collided with vehicles, or were killed by snakes, dogs, or hunters. Emaciation was the next most common (27%) finding for nonforest birds, and was diagnosed for captives, accidental migrants, and resident free-flying birds. Microbiological culture of the intestine of a domestic pigeon carcass with acute enteritis yielded *Salmonella newport*. Although a variety of secondary findings was observed in the carcasses of nonendangered species (Table 2), there was no consistent pattern. Microscopic examination revealed abundant hemosiderin-like pigment in the reticuloendothelial cells of the spleen of a lesser golden-plover (*Pluvialis dominica*) with enteritis, but again, no malarial organisms were found.

One live immature chicken had lesions morphologically consistent with poxvirus infection. Both orbits were occluded by swollen conjunctiva and thick masses of fibrinous exudate loosely adherent to the cornea. Unfortunately tissue samples from this chicken were lost at NWHRC and the diagnosis was not confirmed microscopially or virologically. Histopathological examination of eye lesions from a rock dove and another golden-plover revealed moderately severe conjunctivitis of undetermined etiology.

A variety of commensal or opportunistic bacteria, fungi, and yeasts of no apparent pathological significance was cultured from swabs or tissue samples from live and dead birds (Savidge, 1986, Appendix B). Potential pathogens were isolated from 11 nonforest birds: *Candida tropicalis* was isolated from a coturnix quail (*Coturnix coturnix*); *Salmonella waycross* was isolated from the intestine of a northern pin-

TABLE 1. Disease sampling regime on Guam. Numbers represent total birds sampled with number of living birds in parentheses.

	Migrants/ accidentals	Poultry	Exotic psitta- cines	Introduced birds	Native forest birds	Other native birds	Total
Necropsies	16	24	9	24	26	13	112
Swabs							
Bacterial	21(11)	55(38)	7(2)	97(15)	24(9)	16(7)	220(82)
Viral	20(2)	38(19)	9(1)	95(15)	26(10)	12(3)	200(50)
Histology	10	1	2	8	8	6	35
Parasitology	10	3	1	5	7	7	33
Fecal	6(6)	1(1)	1(1)	2(2)	5(5)	0	15(15)
Blood smear	24(22)	37(36)	2(1)	83(22)	105(95)	9(8)	260(184)

tail (*Anas acuta*), an accidental migrant on Guam, and from two native yellow bitterns (*Ixobrychus sinensis*); *Salmonella amager* was cultured from cloacal swabs obtained from four black francolin; and *S. oranienburg* from two black francolin. We saw no intestinal lesions or other evidence of salmonellosis in any of the francolin and all individuals appeared in good condition.

Parasitic problems included severe infestations of biting lice (*Neottiales fregate*) in two great frigatebirds (*Fregata minor*) and one case of severe verminous (trematode) hepatitis in a euthanatized cattle egret (*Bubulcus ibis*) with a fractured wing. One domestic chicken had numerous nematodes (*Tetrameres ihuilliere* and *Dispharynx nasuta*) in the proventriculus. A pintail duck had parasites, possibly one of the schistosomes, in many of the small parenchymal and subcapsular blood vessels of the adrenal gland. This same pintail had intestinal cestodes (*Diorchis* sp.) and unidentified nematode and trematode ova in its fecal sample. Nematode ova were found in fecal samples of two native Micronesian starlings (*Aplonis opaca*) and one native Mariana crow (*Corvus kubaryi*). The remaining 12 fecal samples were unremarkable.

DISCUSSION

This survey provided no evidence that infectious or parasitic diseases were involved in the extinctions and declines of the native forest birds on Guam. We recognize these data are cursory and preclude exoneration of disease in the bird population declines. On the other hand, these are the only data available thus far and provide no evidence for the disease role suspected by local biologists in 1982. If disease was involved in the decline, its elusiveness is perplexing. All carcasses of forest birds were free of disease, and all live forest birds that were sampled appeared healthy and in good flesh. Most of the native birds that had been killed by vehicular collisions were endangered Guam rails or yellow bitterns. The rail is flightless and the bittern is a relatively common bird that flies at low heights so a high prevalence of vehicular trauma may be expected.

Starvation was likely the cause of most of the emaciated carcasses, but starvation in wild birds, a premortem process, cannot be diagnosed reliably from postmortem material. Isolation of opportunistic *Salmonella* spp. from wild birds with no evidence of the disease is not unusual (Steele and Galton, 1971). While salmonellosis is reported occasionally in individual wild birds, reports of epizootics are rare and are limited to artificial concentrations of birds at animal feed lots, "backyard" bird feeders, and to situations where wild birds are exposed to human sewage (Steele and Galton, 1971; Wobeser, 1981). Interestingly, since 1975 Guam has experienced an increasing trend in numbers of human *Sal-*

TABLE 2. Summary of postmortem examinations and ancillary tests of 112 avian carcasses on Guam between 1982 and 1985. Scientific names of native forest species are in bold.

Family/Species	Number	Status[a]	Age[b]			Cause of death or significant finding[c]				Other results (secondary)
			J	A	U	E	T	U	Other	
Procellariidae										
Puffinus spp.	2	M	2			1	1			
Sulidae										
Sula sula	1	M	1			1				
Fregatidae										
Fregata minor	2	M	2			2			Acute dermatitis (2)[d], anemia (2)	
Ardeidae										
Bubulcus ibis	1	M	1				1			Verminous hepatitis
Ixobrychus sinensis	8	N	2	5	1	1	5	1	Pulmonary hemorrhage (1)	Bursal necrosis (1), hepatitis (1), nephritis (1)
Anatidae										
Anas acuta	1	M	1			1				Cestodiasis, circulatory parasite
Anser anser	1	P		1		1	1			
Phasianidae										
Chrysolophus pictus	2	P		2					Mycotic pneumonia (2)	Poor condition (2)
Coturnix coturnix	14	P	11	3		5	2	1	Enteritis (3), pulmonary edema (3), omphalitis (2), coccidiosis (1)	Poor condition (6), enteritis (1), intestinal hemorrhage (1)
Francolinus francolinus	4	I	2	2			4			Silicosis (1), myopathy (1), nephritis (1), poor condition (1)
Gallus gallus	7	P	5	2			2	1	Enteritis (1), pneumonia (1), myocarditis (1), suspect pox (1)	Parasitic proventriculitis (1), coccidiosis (1), peritonitis (1), poor condition (1)

TABLE 2. Continued.

Family/Species	Number	Status[a]	Age[b]			Cause of death or significant finding[c]				Other results (secondary)
			J	A	U	E	T	U	Other	
Rallidae										
Rallus owstoni	13	N	3	7	3		11		Asphyxiation (1), drowning (1)	Poor condition (3)
Gallinula chloropus	2	N		2		1	1			Peritonitis (1)
Charadriidae										
Charadrius mongolus	1	M	1					1		
Pluvialis dominica	6	M	1	5			5	1		Splenic hemosiderosis (1), enteritis (1)
Scolopacidae										
Numenius phaeopus	1	M	1				1			
Laridae										
Anous stolidus	1	N	1				1			Stomatitis, pancreatic, trematodiasis
Gygis alba	2	N	2			1		1		Nephrosis (1)
Sterna hirundo	1	M	1			1				
Columbidae										
Columba livia	7	I	3	1	3		3		Dermatitis (1), enteritis (1), cloacal impaction (1), mycotic pneumonia (2)	Hepatitis (1), enteritis (1), poor condition (2)
Ptilinopus roseicapilla	1	N		1			1			Hemosiderosis
Streptopelia bitorquata	3	I	2	1		2	1			
Alcedinidae										
Halcyon cinnamomina	4	N		4		2	2			

TABLE 2. Continued.

Family/Species	Number	Status[a]	Age[b]			Cause of death or significant finding[c]				Other results (secondary)
			J	A	U	E	T	U	Other	
Psittacidae										
Agapornis spp.	3	E	2	1		1		2		Nephritis (1), enteritis (1)
Cacatua spp.	3	E		3		1	1		Polyserositis (1), mycotic pneumonia (1)	
Eclectus roratus	1	E		1					Pneumo-airsacculitus	
Ramphastos toco	1	E		1		1				Visceral gout
Psittacus sp.	1	E		1		1				Myositis
Muscicapidae										
Myiagra freycineti	1	N		1		1				Dehydration
Rhipidura rufifrons	2	N		2		1		1		
Passeridae										
Passer montanus	7	I	4	3		2	4			Pulmonary edema (1), renal amyloidosis (1), ascites (1)
Sturnidae										
Aplonis opaca	5	N	2	2	1		2		Heat prostration (3)	
Dicruridae										
Dicrurus macrocercus	3	I	1	2		1	2			

[a] E, exotic psitticine; I, introduced or feral; M, migrant; N, native; P, poultry.
[b] A, adult; J, juvenile; U, unknown.
[c] E, emaciation; T, trauma; U, unknown. Multiple diagnoses were made for some carcasses.
[d] Number of individuals with the condition.

monella spp. infections (Haddock and Nocon, 1986). *Salmonella waycross*, a serotype found in three birds in our study and relatively rare elsewhere in the world, accounts for about 20% of the human cases on Guam (Haddock, 1983). Soil samples indicate *Salmonella* spp. bacteria are relatively widespread in Guam's urban environment.

Warner (1968) reported that avian pox was one of the factors that devastated the Hawaiian avifauna in the late 19th century. He quoted reports of naturalists of the period that attest to finding large numbers of diseased and badly debilitated birds in the field. Fowlpox occurs on Guam and is a common problem for Guamanians who keep chickens. Pox apparently has been present on Guam for many decades as a 1915 Guam Agricultural Experiment Station publication considered "chicken pox" the most widely disseminated poultry disease on the island. But, contradictory to the situation in Hawaii, avian pox has never been reported in a free-flying wild bird on Guam, even though professional and lay biologists have been aware and concerned about the demise of the avifauna since the 1960's.

Using a theoretical infection rate of 7.8% for malaria, since this is the detectable rate of infection in Hawaii where malaria is hypothesized to be a factor in the decline of native birds (van Riper et al., 1986), a sample size of 69 birds would be needed to detect malaria in native birds on Guam at the 0.05 level of significance (Sokal and Rohlf, 1981, p. 766). One hundred and five native forest birds and 155 other birds were sampled on Guam, and blood parasites were lacking in all, suggesting malaria was not present as an active disease. This observation is again contradictory to the situation in Hawaii, where Warner (1968) readily detected malaria in lowland populations of introduced passerines, and van Riper found one species of *Plasmodium* from sea level to tree line (van Riper et al., 1986).

Difficult sampling and logistics posed constraints to the efficacy of this survey. Although transport media are designed to protect infectious agents during shipment, it is approximately 13,400 km from Guam to NWHRC, and it is possible that some pathogens did not survive the trip to the laboratory. Carcasses and tissue samples sometimes had substantial postmortem changes caused by decomposition before collection or by thaw during air transport. Large sample sizes of endangered forest birds simply were not available. Even so, it seemed unlikely that a disease could be virulent enough to cause the extinction of one or more species without causing recognizable lesions in some of the individuals examined.

Other lines of evidence suggest that disease may not have been a factor in the decline of Guam's birds. Birds on Cocos Island, only 2.5 km south of Guam, are thriving despite development on much of the island (Savidge, 1987). If disease was a problem on Guam, we would expect the birds on Cocos to be similarly affected because bird interchange occurs between Cocos and Guam. A sentinel study exposing four species of birds to potential disease vectors was conducted on Guam in 1984 in the habitat of remaining forest birds (J. A. Savidge, unpubl. data). Chickens at one site developed lesions consistent with poxvirus, but the virus appeared to be host specific as none of the other sentinel species contracted the virus. No other infectious diseases were found. In addition, disease has not been a problem in the captive-rearing program for Guam rails initiated on Guam in 1983. Lastly, one might argue that disease reduced populations to a level that transmission could not occur, and hence the disease disappeared or occurred at levels too low to be detected. However, avian populations continued to decline throughout our study and in subsequent years indicating the cause of the problem was still present. Thus, considering the results of the present study and

the above, we conclude there are no data suggesting disease was responsible for the decline of the native forest birds on Guam.

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