A preliminary survey for avian pathogens in Columbiform birds on Socorro Island, Mexico

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To assess the potential disease risks posed by resident Columbiformes to the reintroduction of the Socorro Dove *Zenaida graysoni* to Socorro Island, Mexico, the endemic Socorro Ground Dove *Columbina passerina socorrensis* and the recently arrived Mourning Dove *Zenaida macroura*, were screened for ecto- and endoparsites, haemosporidia, *Trichomonas gallinae*, *Chlamydophila psittaci* and avian pox. All of the Mourning Doves and Socorro Ground Doves sampled appeared healthy upon capture. We detected *Haemoproteus* spp. in 88% of Mourning Dove and 30% of Socorro Ground Dove samples using microscopy. Two polymerase chain reaction (PCR) DNA amplification methods detected either *Haemoproteus* spp. or *Plasmodium* spp. Pooling results from both tests yielded positives in 100% of the Mourning Doves and 52% of the Socorro Ground Doves. A nested PCR detected *Leucocytozoon* spp. in 94% of the Mourning Doves and 61% of the Socorro Ground Doves sampled. Thus, at least two genera of haemosporidia are present in columbids of Socorro Island. Microscopy for *T. gallinae* yielded positives in 33% of Mourning Dove and 30% of Socorro Ground Dove samples. *C. psittaci* was not detected using PCR on either cloacal swab samples or tissue samples from tested Mourning Doves or Socorro Ground Doves. Necropsies revealed neither lesions indicative of the wet form of avian pox, nor internal lesions associated with trichomoniasis. These results suggest that Socorro Doves selected for reintroduction should be screened carefully to evaluate potential immunological challenges by native haemosporidians and to avoid introduction of other diseases apparently absent from native Columbiformes on Socorro Island.

Key words: Avian pathogens, Disease risk, Socorro Dove, Reintroduction, Socorro Island

INTRODUCTION

THE use of captive breeding in species recovery has increased substantially in recent years. However, a major criticism of captive breeding is that, for the purpose of conservation, it cannot be considered a success until reintroduction from captivity is achieved (Snyder *et al.* 1996) and the reintroduced species is able to establish a self-sustaining and viable population (Ebenhard 1995). Baseline disease screening of existing avian populations before reintroducing a species is important for identifying epizootics that may prevent or hinder its re-establishment, as well as mitigate the risk of disease transmission between re-introduced and indigenous species. Thus, baseline sampling for pathogens provides essential information for implementing management practices to protect both the native avifauna and the species being reintroduced.

The medium-sized Socorro Dove *Zenaida graysoni* (body length 26.5–34.0 cm; Gibbs, *et al.* 2001), formerly endemic to Socorro Island in the Revillagigedo Archipelago, Mexico (Fig. 1), is currently categorized as "extinct in the wild" (IUCN 2002). Habitat destruction, introduced predators, and hunting are all thought to have contributed to its extirpation (Jehl and Parkes 1982). The last written account of the species describes how several doves were killed without apparent reason in 1972 (Velasco-Murgía 1982). Credible testimonials suggest that the endemic dove was still present by 1975 (Captain J. Durán Hernández pers. comm.). It is likely that the final decline and extinction of the species in the wild occurred simultaneously with development of military infrastructure including an airstrip and main road on the island in the late 1970s.

A captive population of the Socorro Dove, established in several European zoological gardens, will soon be used as the source for reintroduction efforts on Socorro Island (Martínez-Gómez *et al.* 2003). These doves are direct descendants of the live doves taken in 1925 by E. W. Gifford during the third expedition to the Revillagigedo Archipelago by the California Academy of Sciences (Hanna 1926). The prospects for the species have improved, thanks to a joint partnership between the Island Endemics Foundation and the Mexican Navy, which enabled the construction of a breeding station and small laboratory on Socorro Island to house captive bred Socorro Doves for acclimatization prior to reintroduction (Horblit *et al.* 2005; Stadler *et al.* 2005; Stadler 2006). Additionally, habitat restoration in the severely degraded southern and south-eastern portions of Socorro Island can move forward now that
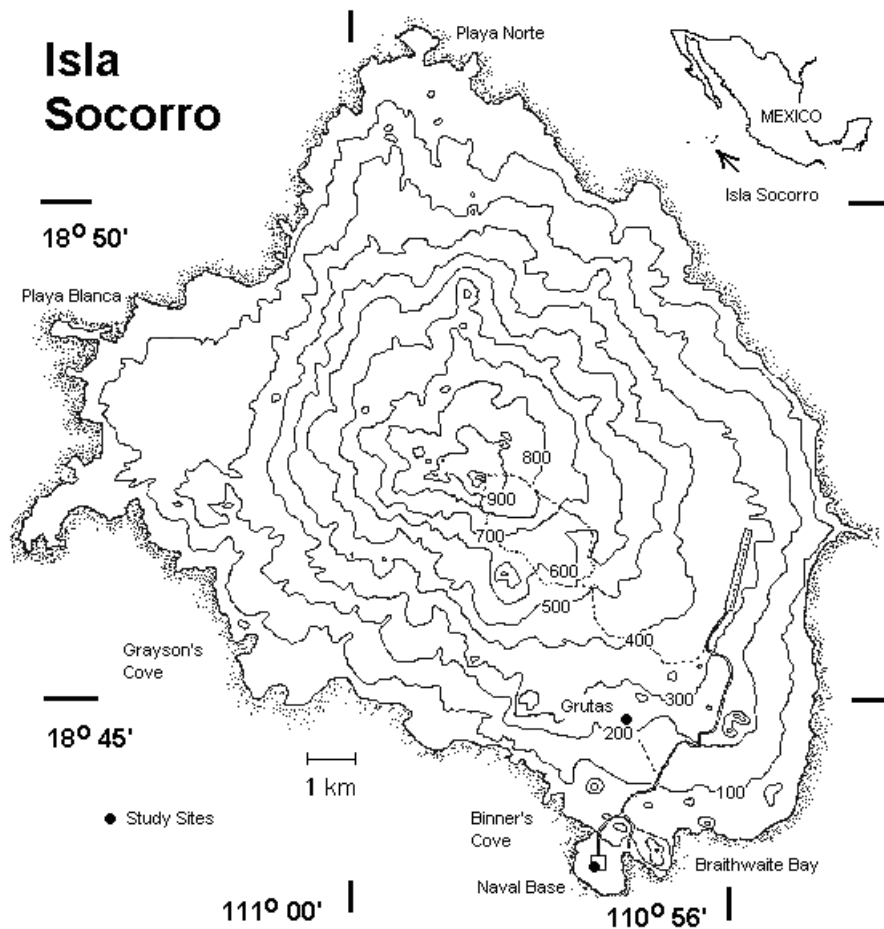
Fig. 1. Map of Socorro Island and Mexico. Note location of the Mexican Naval Base at the southern tip of the island and *Grutas* (caverns) due North of the Naval Base.

non-native sheep are being successfully removed from the island.

The IUCN/SSC Guidelines for Reintroduction call for the evaluation of the reintroduction site, including the "identification and elimination or reduction to a sufficient level, of previous causes of decline" (IUCN 2002). Although disease was not likely a factor in the extinction of the Socorro Dove from the wild, it has been a factor in hampering the restoration of other avifauna, such as with *Trichomonas gallinae* infections in the Pink Pigeon *Columba mayeri* (Bunbury *et al.* 2008) of Mauritius and the on-going problems of avian pox and malaria facing native Hawaiian avifauna (Atkinson and LaPointe 2009). Viggers *et al.* (1993) discuss the effects of infectious diseases on the success of reintroduction programmes. Captive bred animals have different immunological challenges and pathogen exposure than their wild counterparts and therefore may have very different disease expression to closely related wild species when released into the same environment. Genetic resistance and variation may also be reduced due to inbreeding and a lack of natural selection (Cunningham 1996).

Hemosporidian malaria parasites have been implicated in the decline of avian populations. *Plasmodium relictum* is known to cause high mortality in both captive and wild susceptible bird populations and is thought to have contributed to the decline of native forest birds in the Hawaiian Islands (Warner 1968; Van Riper *et al.* 1986; Atkinson 1999; Atkinson and LaPointe 2009). *Haemoproteus columbae* is the most commonly identified blood parasite in the Mourning Dove *Zenaida macroura* (Godfrey *et al.* 1990), while *Leucocytozoon marchouxi*, rarely occurs in this dove species (Conti 1993). The impact of the latter two hemosporidia on avian populations is generally thought to be less severe than that of *Plasmodium*, although there are several examples of deaths in wild birds from *Haemoproteus* and *Leucocytozoon* (Valkiûnas 2005).

Both avirulent and virulent strains of the protozoan *Trichomonas gallinae* occur in nature and circulate within bird populations (Cole 1999). Mourning Doves have been reported to carry the virulent strains without developing lesions, making them carriers of lethal strains

and possible sources of infection to susceptible birds (Conti 1993). Virulent strains of the protozoan have caused major mortality events or epizootics in Mourning Doves in the south-eastern United States and Band-tailed Pigeons *Columba fasciata* in the western United States (Conti 1993; Cole 1999).

Chlamydiosis, caused by *Chlamydophila psittaci*, is a common bacterial infection of domestic and feral pigeons worldwide and is a serious disease in domestic turkeys in the U.S. and domestic waterfowl in central Europe (Franson 1999). Kaleta and Taday (2003) have reported the occurrence of the disease in 30 orders of birds including migratory species of waterfowl, gulls, terns, shorebirds, pigeons and passerines. Franson (1999) reports the disease occurring in at least 159 species of birds, with psittacines (Parrots) commonly affected. Among caged birds, chlamydiosis occurs frequently in pigeons, doves and mynahs. Among wild birds, waterfowl, herons and pigeons are most commonly affected (Franson 1999). *C. psittaci* is also a zoonotic pathogen. Some strains not normally pathogenic to wild avian hosts can be highly virulent for domestic fowl and humans (Hubalek 2004, OEI 2003).

Avian pox, or avian diphtheria, is caused by a virus of the avipox group, a subgroup of pox-viruses (Hansen 1999). Three common strains of avipox have been identified: fowl pox virus, pigeon pox virus and canary pox virus (Hansen 1999). At least ten species of avipoxvirus have been described in about 232 avian species, many of which are migratory. Among these migratory species, six are in the Columbiformes, including the Mourning Dove (Bolte *et al.* 1999). An increasing frequency of this disease, including its appearance in previously unaffected avian species, suggests that avian pox is an emerging avian viral disease (Hansen 1999). Like avian malaria, avian pox is believed to be a factor in the decline of forest bird populations in Hawaii (Warner 1968; Van Riper 2002; Atkinson and LaPointe 2009). Carrier birds of the disease may spread avian pox among local populations, such as between feeding stations and along migratory routes used by various bird species (Hansen 1999). Mosquitoes are the principal vectors of avipoxes (Hansen 1999).

Two columbid species currently exist on Socorro Island: the Socorro Ground Dove *Columbina passerina socorrensis*, an endemic subspecies that exists in small numbers at lower elevations throughout the island (Wehtje *et al.* 1993), and the Mourning Dove, a migratory species that was not recorded on Socorro Island prior to 1978 but has since become established (Jehl and Parkes 1983). The Mourning Dove and Socorro Dove are genetically closely related

(Johnson and Clayton 2000). *Haemoproteus* sp., *Trichomonas* and *Chlamydophila* sp. were all detected in a closely-related island species, the Galapagos Dove *Zenaida galapagoensis* (Padilla *et al.* 2004). The goals of this study were to screen for the presence of gastrointestinal parasites, Haemosporidia (*Plasmodium*, *Haemoproteus* and *Leucocytozoon*), *Trichomonas gallinae*, *Chlamydophila psittaci* and avian pox in the two extant species of doves on Socorro Island, to assess the disease risks posed by resident doves to the reintro-duction of Socorro Doves, and to formulate management recommendations to mitigate those disease risks.

MATERIALS AND METHODS

Study site and trapping

Socorro Island is in the Revillagigedo Archi-pelago, located approximately 700 km West of the port city of Manzanillo, Colima, Mexico (Fig. 1). Socorro Island has a tropical semi-dry climate zone from 0 to 400 metres above sea level and a subtropical sub-humid climate zone between 400–1150 metres above sea level with occasional summer rains (Valero *et al.* 2001). Generally, the island is dry with a mean annual precipitation of 600 mm/year (Valero *et al.* 2001).

We collected samples on Socorro Island, Mexico from 6–15 January, 2004 at two sites (Fig 1): the naval base and Las Grutas (the caverns). Walk-in traps and mist nets were used to capture Socorro Ground Doves but only mist nets were used to trap Mourning Doves. Mist-netting procedures employed were similar to those described by the USDA Forest Service (1993). All captured doves were placed in cotton bags until processing and, in the case of Socorro Ground Doves, until their release at the site of capture. We followed appropriate protocols for research on wild birds (AOU 1988) and all procedures were in accordance with approved guidelines by the California State University, Sacramento, Institutional Animal Care Use Committee (IACUC).

Physical examination and disease screening

Captured doves were examined for overall health and subjected to close examination for oral lesions of trichomoniasis and/or cutaneous lesions of avian pox, as well as for visible external parasites. External parasites were collected using tweezers and placed in plastic snap-on containers containing 95% ethanol.

We collected faecal samples opportunistically and later analysed these samples for the presence of gastrointestinal parasites. We also took cloacal swabs for *Chlamydophila* PCR and crop swabs for *Trichomonas* detection. While

swabbing crops, we examined birds for lesions indicative of trichomoniasis. Crop swabs were rolled onto microscope slides, air-dried and then placed in slide boxes for later staining with Wrights–Giemsa stain and examination for *Trichomonas gallinae* as described by Hendrix (1998).

We collected approximately 0.5 ml of blood from the brachial vein using a tuberculin syringe with 27.5 or 29 gauge needles. Whenever possible, at least two blood smears were made from each blood sample collected using the slide method described by Benjamin (1978). Blood smears were stored in slide boxes until they could be stained with Wright-Giemsa stain (Surestai™, Fisher Diagnostics) to detect the presence of blood parasites (Benjamin 1978). We placed several drops of blood in plastic Eppendorf tubes containing 1.0 ml Longmire's lysis buffer (100 mM Tris p 8.0, 100 mM EDTA, 10 mM NaCl, 0.5% SDS) for molecular analysis.

Mourning Doves were euthanized using tracheal constriction (AOU 1988). During necropsies of Mourning Doves, we routinely collected sections of tissues and organs (breast muscle, heart, liver, lungs, trachea, pancreas, spleen, gonads, kidneys, adrenal glands, oesophagus, crop, gizzard, small intestines and large intestines) from each bird and placed these samples in screw cap plastic containers with 70% alcohol. Museum skins from all necropsied specimens were prepared and later deposited in the *Colección Nacional de Aves at the Instituto de Biología of the Universidad Nacional Autónoma de México* (UNAM) in Mexico City. One of the captured Socorro Ground Doves expired after capture and was subsequently necropsied.

Stained blood smears were evaluated for the presence of *Plasmodium* spp., *Haemoproteus* spp. and/or *Leucocytozoon* spp. using a light microscope (400x and 1000x). Microscopic identification of blood parasites followed the criteria of Aiello (1998) and was limited to the genus level. Stained crop smears were examined under a light microscope (400x and 1000x) for the presence of *Trichomonas gallinae* using diagnostic criteria of Hendrix (1998).

We used light microscopy to examine faecal samples for the presence of gastrointestinal parasite eggs, particularly nematodes, using direct smear and concentration methods, as described in Hendrix (1998), Zajak (1994) and McCurnin (1994). The concentration method, which requires a larger amount of faeces, has the advantage of being able to detect low parasite loads. For the concentration method, we employed a flotation procedure and used a commercially available flotation kit (Fecalyzer™, EVSCO Pharmaceuticals) that uses sodium nitrate as a flotation solution (Fecaso™, EVSCO Pharmaceuticals).

On captured birds we searched for external cutaneous lesions pathognomonic to avian pox as well as scarring which might indicate recovery from previous pox infection. During necropsies we searched for necrotic lesions on the mucous membranes of the mouth and upper digestive and respiratory tract indicative of the diphtheric form of the disease.

Molecular methods of disease screening

We used a commercially available kit (Qiagen DNeasy® kits, Valencia, California) to extract genomic DNA from blood, cloacal swabs, and lung and spleen tissues. The DNA was then used for Polymerase Chain Reaction (PCR) amplification. PCR is a useful tool to detect avian pathogens, by selectively amplifying pathogen DNA (Richard *et al.* 2002).

To confirm the successful extraction of avian DNA, we used the protocol described by Sehgal and Lovette (2003) to amplify the avian Brain-derived Neuotrophic Factor (BDNF) gene from all tested birds. Prior to performing diagnostic PCR for hemosporidia on field samples, we performed PCR on a positive hemosporidian control obtained from a Hawaiian honeycreeper, the Common amakihi *Hemignathus virens*, using the recommended conditions for the HaemF and HaemR2 primers described by Bensch *et al.* (2000). This positive control was used in all subsequent diagnostic PCR tests. Additional positive controls for *Plasmodium* or *Haemoproteus* were derived from our samples that yielded positive microscopy results. For negative controls, we used purified water in place of DNA template, or else samples that were consistently void of parasites as confirmed by microscopy and PCR.

We used two different PCR protocols to amplify a segment of the *cyt b* gene to detect the presence of *Plasmodium* and/or *Haemoproteus* as described in Bensch *et al.* (2000) and Szymanski and Lovette (2005), respectively. Amplified PCR products were visualized on 1% agarose gels using ethidium bromide under ultraviolet light. Bands with lengths of approximately 478-bp or 498-bp were considered indicators of positive infection (Bensch *et al.* 2000; Richard *et al.* 2002; Szymanski and Lovette 2005).

Since the above two PCR protocols only detected *Plasmodium* or *Haemoproteus*, to detect *Leucocytozoon*, we performed an additional nested PCR which amplified a portion of the parasites' *cyt b* gene. We followed the nested PCR protocol described by Sehgal *et al.* (2006). We ran amplified PCR products on a 2.0% agarose gel using 1×TBE and used ethidium bromide stain under ultraviolet light to visualize the resulting 865-bp PCR products. In addition, to test for the presence of *Chlamydophila psittaci* in the DNA

extractions from claocal swabs, we performed PCR on the samples at the Center for Vector-borne Diseases at the School of Veterinary Medicine, University of California in Davis, California using their standard PCR protocol for *C. psittaci*. The protocol, modified from Sykes *et al.* (1997), detects part of the *omp A* gene of *C. psittaci*. A band on a 1% agarose gel corresponding to a 1094-bp fragment was considered positive (Sykes *et al.* 1997). We used the same protocol and primers on DNA extracted from spleen and lung tissue.

RESULTS

A total of 33 Mourning Doves were captured at *Las Grutas*, most of which were caught during their dawn departure and dusk return flights to the site. Twenty-three Socorro Ground Doves were captured; 10 at *Las Grutas* and 13 at the Naval base. Based on overall appearance, feather condition and weight, all doves appeared to be in good health when captured. We did not observe gross lesions of either trichomoniasis or avian pox on any of the captured birds. Internal lesions associated with the diphtheric or wet form of avian pox were not observed in any of the 33 necropsied Mourning Doves or in the one necropsied Socorro Ground Dove (Table 1). We observed the Pigeon Louse Fly *Pseudolychia canariensis* on three (9%) of the Mourning Doves (Table 1).

We collected sufficient faecal samples to run both a direct smear and faecal flotation from 13 (39%) mourning doves and 13 (56%) Socorro ground doves (Table 1). We found eggs of *Ascaridia* spp., a nematode, in five (38 %) of the 13 fecal samples from the mourning doves. We found *Ascaridia* spp. eggs on only one (8 %) of the 13 fecal samples from Socorro ground doves. We did not identify any other gastrointestinal nematodes.

We demonstrated the presence of avian DNA using PCR in all blood and tissue samples. The 56 cloacal swab samples were not tested for avian DNA until several months after diagnostic PCR for *Chlamydophila psittaci* was run. Avian DNA was identified in only 25 (45 %) of the cloacal swabs, and of these, 17 (51 % of 33 samples) were from Mourning Doves and 8 (35 % of 23 samples) were from Socorro Ground Doves.

We detected blood parasites in 29 (88%) of our 33 Mourning Dove blood smears using light microscopy, while pooled results from the three PCR amplifications yielded at least one positive in each of the 33 blood samples (Table 2). The blood smears revealed the presence of only *Haemoproteus*. There was some disagreement in the results of the three PCR techniques. For detecting *Plasmodium* and/or *Haemoproteus*, the HaemF/HaemR2 primer pair yielded 33 (100%) positives, while the L1583/H15730 primers yielded 19 (57%) positives. For *Leucocytozoon*, the nested PCR using the LeucoF/LeucoR primers detected 31 (94%) positives. Given these results, we can conservatively state that *Haemoproteus* and *Leucocyotozoon* are present in Mourning Doves on Socorro Island and the incidence of both hemosporidia in the local population may be high.

Light microscopy revealed the presence of hemosporidia in seven (30%) of the 23 Socorro Ground Dove blood smears (Table 3). Pooling results from the PCR tests yielded 15 (65%) positives for hemosporidia (Table 3). As above, there was some disagreement in results between the PCR tests. In the tests for *Plasmodium* or *Haemoproteus*, the HaemF/HaemR2 primer pair yielded 10 (43%) positives, while the L1583/H15730 primers yielded 6 (26%) positives. For *Leucocytozoon*, the LeucoF/LeucoR primers detected 14 (61%) positives. No blood parasites were detected by any method in 8 (35%) of the Socorro Ground Doves samples. Given these results, we can conservatively state that *Haemoproteus* and *Leucocyotozoon* are present in Socorro Ground Doves on Socorro Island, but not all

Table 1. Results of screening samples of Mourning Doves and Socorro Ground Doves for select pathogens/diseases.

Pathogen/ Parasite/ DiseaseScreen	Mourning Dove (n=33)			Socorro Ground Dove (n=23)		
	(+) samples	(-) samples	No. Tested	(+) samples	(-) samples	No. Tested
Pigeon Louse Fly[1]	3	30	33	0	23	23
Gastrointestinal Parasites[2]	5	8	13	1	12	13
Avian Pox						
Physical Exam for Lesions	0	33	33	0	23	23
Necrospsy – Lesions of wet form	0	33	33	0	1	1
Trichomonas gallinae						
Physical Exam for Lesions	0	33	33	0	23	23
Crop swab	11	22	33	7	16	23
Chlamydophila psittaci						
PCR[3] (cloacal swabs)	0	33	33	0	23	23
PCR[3] (tissue samples)	0	33	33	0	1	1

[1]Physical examination
[2]Faecal Flotation; *Ascaridia spp.*
[3]Sykes *et al.* 1997

Table 2. Results of screening tests for hemosporoidia in morning dove samples using blood smears and PCR. See listed references for PCR primer descriptions.

Blood Smear	HaemF/HaemR2[1]	PCR Primers Used L1583/H15730[2]	LeucoF/LeucoR[3]	No. of Samples
Yes	Yes	No	Yes	11
Yes	Yes	Yes	No	1
Yes	Yes	Yes	Yes	17
No	Yes	No	No	1
No	Yes	No	Yes	2
No	Yes	Yes	Yes	1
			Total	33

[1]Bensch et al. 2000
[2]Szymanski and Lovette 2005
[3]Perkins and Schall 2002

Table 3. Results of screening tests for hemosporoidia in Socorro Ground Dove samples using blood smears and PCR. See listed references for PCR primer descriptions.

Blood Smear	HaemF/HaemR2[1]	PCR Primers Used L1583/H15730[2]	LeucoF/LeucoR[3]	No. of Samples
Yes	No	Yes	Yes	1
Yes	Yes	No	Yes	1
Yes	Yes	Yes	No	1
Yes	Yes	Yes	Yes	4
No	No	No	No	8
No	No	No	Yes	4
No	Yes	No	Yes	4
			Total	23

[1]Bensch et al. 2000
[2]Szymanski and Lovette 2005
[3]Perkins and Schall 2002

doves in this endemic subspecies may be infected.

Eighteen (32 %) of the 56 cloacal swabs we collected were positive for *Trichomonas gallinae* (Table 1). Of these 18 positives, 11 were from Mourning Doves (33 %, 11/33) and seven were from Socorro Ground Doves (30%, 7/23). Of the 33 Mourning Doves and a single Socorro Ground Dove necropsied, none exhibited the cheesy, yellowish lesions in the oral cavity, pharynx, oesophagus, crop, proventriculus or sinuses indicative of trichomoniasis (Table 1).

Neither the DNA extracted from 56 cloacal swabs (33 Mourning doves and 23 Socorro Ground Doves) nor from 34 tissue samples (33 Mourning Doves and one Socorro Ground Dove) tested positive for *Chlamydophila psittaci* using PCR (Table 1). However, we were unable to amplify avian DNA from the cloacal swabs extracts in 15 of the 22 Socorro Ground Doves.

DISCUSSION

Assessment of pathogen presence

None of the captured Mourning Doves or Socorro Ground Doves exhibited overt physical signs of illness or even physical injury, suggesting that both species on Socorro Island are in relatively good condition compared to island populations of Columbiformes elsewhere which show overt signs of avian pox and other ailments, e.g. Zebra Doves *Geopelia striata* in Hawaii (Bell, unpublished obs.)

We detected *Ascaridia* spp. in both species of doves on Socorro Island. Although large accumulations of this nematode may be fatal to individual birds if they cause intestinal obstruction or rupture, infestations of this parasite do not appear to impact Mourning Dove populations to a significant degree (Conti 1993). Overall, the risk of gastrointestinal nematodes to any reintroduced Socorro Doves would most likely be at the individual, rather than population level.

Avian pox was not detected neither during physical examination of captured birds nor during necropsy of the Mourning Doves and the single Socorro Ground Dove. Thus, the current risk of exposure to pox among columbids on Socorro Island appears to be low. However, the risk posed by the transmission of this disease via migratory species is unknown and warrants further attention.

The presence of the Pigeon Louse Fly on some of the captured Mourning Doves is significant because it is a known vector and intermediate host for the Columbiform protozoan blood parasite *Haemoproteus columbae* (Aiello 1998; Soulsby 1968; Kern 2003; Valkiûnas 2005) and may also transport the Pigeon Louse *Columbicola columbae* (Dranzoa et al. 1999) Klei and DeGiiusti (1975) documented the seasonal occurrence of *Haemoproteus columbae* and its vector in Rock Doves Columba livia in Detroit, Michigan and found that the greatest prevalence of the protozoan occurred during fall and winter

and was lowest during spring, corresponding to changes in the vector population. Although Pigeon Louse Flies were not found on any the Socorro Ground Doves, it may occur on this species since Socorro Ground Doves and Mourning Doves overlap in some locations on Socorro Island. Socorro Doves reintroduced to the island would face similar transmission risk to *Haemoproteus* spp. via this vector. Other vectors, if present, could provide further routes of transmission. For instance, the Common Ground Dove *Columbina passerina* was recently discovered to be a new target species for the Louse Fly *Microlynchia pusilla* (Tella et al. 2002).

Of the hemosporidia, we identified *Leucocytozoon* spp. *and Haemoproteus* spp. in both species of dove on Socorro Island, but it does not appear that *Plasmodium* spp. was present in our samples. This situation is similar to Galapagos Doves where *Haemoproteus* is common, but *Plasmodium* has not been detected (Padilla *et al.* 2004).

Haemoproteus spp. may have limited impact on its avian host (Atkinson 1999; Conti 1993). Padilla *et al.* (2004) considered finding *Haemoproteus* in Columbiformes of the Galapagos Islands "incidental" in terms of overall population risk, although Santiago-Alarcon *et al.* (2008) noted that its prevalence of up to 100% and associated parasitemias of up to 12.7% in island populations of the Galapagos Dove warranted further attention. Mortalities due to this parasite have been reported (Resende et al. 2001; Valkiûnas 2005) and it has been shown to affect overall fitness of migrating birds (Gicick 2001). *Leucocytozoon* infections may be more problematic. While one study of captive and free-living Pink Pigeons showed no significant difference in survival between birds infected with *L. marchouxi* and uninfected birds (Swinnerton *et al.* 2005), another showed that pathogenicity was greater in susceptible juveniles (Peirce *et al.* 1997) and yet another revealed that Pink pigeons infected with this hemosporidia had lower survival than uninfected birds to 90 days post-sampling (Bunbury *et al.* 2007).

Given the presence of at least one blood parasite vector and at least two genera of hemosporidia, there is a potential disease risk to any Socorro Doves returned to the island if they have not been previously exposed to these blood parasites. Since neither *Haemoproteus* spp. nor *Leucocytozoon* spp. appears to be responsible for population-wide infections, the risk level may be low.

The absence of gross lesions of trichomoniasis in the birds examined suggests the sampled strain is probably avirulent. This is not to say that this avirulent strain could not be replaced by a more virulent one at some future point in time. The risk of this protozoan to reintroduced

Socorro Doves is uncertain. Conti (1993) reported that Mourning Doves may carry virulent strains without developing lesions, making them carriers of lethal strains and possible sources of infection to susceptible birds. Bunbury *et al.* (2008) reported trichomoniasis to be the most common cause of mortality over a five year period in the free-living population of the Pink Pigeon. The origin of *T. gallinae* on Socorro Island is of interest, as it is unknown whether the Socorro Ground Doves acquired it from the Mourning Doves or already had the protozoan prior to the latter's arrival on the island in the 1970s. Rock Doves, a known carrier of *T. gallinae*, were present on the naval base until the early 1990's when the whole population was removed from the island (Martínez-Gómez and Curry 1996). Domestic Chickens *Gallus gallus* have been present at various times on the island, especially in the vicinity of the naval base, where cross-contamination between human food remains and birds and their vectors is common. In 1994 they were euthanized because of an epidemic outbreak in the local farm (Admiral P. León Herrera pers. comm.). Chickens were again briefly introduced to the island in 2003–2004 and later removed.

Although we were unable to detect *Chlamydophila psittaci* in any of our samples, it does not necessarily mean that the pathogen is not present on Socorro Island. The fact that we could not amplify avian DNA is some of our Socorro Ground Dove samples suggests that some of the claocal swabs may have been degraded. Alternatively, it may have gone undetected because our sample protocols did not include swabbing the conjunctiva or choana or our sample sizes for both species were too small. For example, Padilla *et al.* (2004) isolated *C. psittaci* from only six of their 102 Galapagos Dove samples. Another factor may be intermittent shedding of the pathogen by the host. Raso *et al.* (2002) found that only 44% of captive amazon parrots sampled at 48-hour intervals for *C. psittaci* antigen tested positive at both sampling intervals. Given the health significance of this pathogen and its ease of spread, its risk to the Socorro Dove reintroduction effort requires further attention.

The baseline information obtained here on the two species of dove that currently exist on Socorro Island suggests that reintroduced Socorro Doves may face at the very least an immunological challenge by native hemosporidian and trichomonas parasites.

Future research and management implications

Our findings and recommendations have implications for the reintroduction of the Socorro

Dove as well as other island reintroduction efforts. We therefore recommend continuing a disease surveillance programme on Socorro Island and expanding it to include additional pathogens, intermediary and avian hosts, and the quantification of parasitemias. Screens for new and emerging diseases, such as West Nile Virus, a mosquito-borne flavivirus that is spreading in North America, (CDCP 2003), should be employed.

Determining prevalence of pathogens requires knowledge of host population sizes. The Socorro Ground Dove was common around the naval base and its capture at *Las Grutas* suggests that its population size and distribution is extensive, even in the degraded areas of southern Socorro. The number of Mourning Doves on the island is roughly estimated at one thousand birds, concentrated primarily in the Southern half of the island (J.E. Martínez-Gómez, unpublished field notes), but its exact population size is unknown. Future studies involving these two species should include island-wide systematic censuses to better assess pathogen prevalence and risks to the reintroduction of Socorro Doves.

Surveys for arthropod vectors on Socorro Island should be undertaken. We have identified the Pigeon Louse Fly which may transmit *Haemoproteus* spp. and other vector borne diseases and/or ectoparasites. We observed mosquitoes on the island but did not sample them to determine if they carry *Plasmodium* spp. Although we do not know if Black Flies *Similium* spp. are present on the island, detection of *Leucocytozoon* spp. suggests the presence of this vector.

We recommend the use of both microscopy and PCR for disease screening of blood parasites. Both methods have advantages (Valkiûnas et al., 2008), although the slide method can in some cases underestimate the prevalence of blood parasites (Waldenström *et al*. 2004; Bensch *et al*. 2000; Richard *et al*. 2002; Feldman *et al*. 1995) and nested PCR may underestimate mixed infections of hemosporidian parasites (Valkiûnas et al. 2006). Use of a nested-PCR, such as that described by Hellgren *et al*. (2004) should be employed to simultaneously screen for *Leucocytozoon* spp. along with *Haemoproteus* spp. and *Plasmodium* spp. The use of PCR and microscopy detects only active infections of hemosporidia (Fallon *et al*. 2003). Serological techniques should be undertaken to detect antibodies and thus previous epizootics.

Analysis of hemosporidian DNA sequences will provide information on the evolutionary relationships among these parasites and their relationships to mainland and other island species. For example, work by Santiago-Alarcon *et al*. (2008) on doves of the Galapagos Islands revealed many lineages of haemosporidian parasites. Comparing parasite lineages from the Socorro Island birds to those of the Galapagos should uncover patterns of dispersal and colonization of haemosporidian parasites.

The spread of the pathogens such as *T. gallinae* and *C. psittaci* on Socorro Island among doves may be prevented by eliminating their access to kitchen wastes and garbage on the naval base. During the Socorro Dove reintroduction effort it is imperative to employ standard aviculture practices such as frequent decontamination of platforms, perches and other surfaces to lessen pathogen transmission in the aviaries and at feeding stations. Socorro Island has had numerous instances where cage birds and domestic fowls have been brought the island. It is vital to enforce the ban on importation of all non-indigenous birds to the Revillagigedo Archipelago. These preventive measures may help minimize the spread of pathogens among birds and humans as well.

Lastly, captive-bred Socorro Doves selected as candidates for reintroduction should be screened carefully and undergo appropriate quarantine to avoid introduction of additional diseases such as *C. psittaci* and avian pox, which are apparently absent from native Columbiformes on Socorro Island.

ACKNOWLEDGEMENTS

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BOOK REVIEW

Sharks and Rays of Australia

Second Edition (2009); Hardback, 656 pages
P. R. Last and J. D. Stevens, eds
CSIRO Publishing, Collingwood
H/b: ISBN: 9780643094574
RRP: AU$120.00

HARRY F. RECHER

THE first edition of *Sharks and Rays of Australia* was published in 1994. This second edition is fully revised, with descriptions of 322 species of sharks, skates, rays, and chimaerids. Since 1994, the Class Chondrichthyes has been extensively revised and the second edition of *Sharks and Rays of Australia* includes 26 species that have been formally described since the first edition, with formal classification of 97 species that were previously given temporary names. Names of other species have also been revised in light of new knowledge of the systematics within this important group of fish.

Sharks and Rays of Australia is superbly illustrated in full colour, with clear, easy-to-follow keys and sufficient detail for each to species that identification by non-ichthyologists should be relatively straight forward. I experienced no difficulties in using the keys and illustrations to "identify" the species of sharks and rays I encounter while fishing and exploring the Hawkesbury River north of Sydney. There are several features to this book that make it especially easy to use (or even just to enjoy the diversity of sharks and rays found in Australian waters). Each species is illustrated and the species account highlights key identifying features, the keys themselves are fully illustrated with arrows indicating important distinguishing characteristics, and, best of all, the book concludes with a full set of 91 colour plates of closely related species shown together. Each illustration on the plates identifies whether the individual shown is a male, female or juvenile. For some, as with the Saddled Swellshark *Cephaloscylium*

variegatum, male, female, and juvenile are all illustrated. Interestingly, for this species, male/female differences are not mentioned in the species account, although it is indicated that the juveniles are differently coloured from the adults. Also, males, females, and juveniles are not separately identified in the keys, but I doubt this would cause confusion given the straightforward simplicity of the keys for species identification.

Species accounts emphasize identification, but include comments on habitat and biology where available. I suspect we actually know little about the biology and ecology of most of Australia's sharks and rays. Each account includes a map of the species' distribution, comments about systematics, and references to the literature. There is a comprehensive list of references, a checklist of species, and indices to both scientific and common names that makes finding species (including alternative names) and moving between plates and species accounts easy.

All in all this is a superb book. Anyone interested in Australia's fish (or fishing) will find it both useful and informative. Given the high level of commercial exploitation of sharks and rays globally and in Australian waters, it is important that the systematics and biology of this group being as fully understood as possible so as to achieve the best possible management and conservation of species. This includes species, such as the smaller skates, shovelnose rays, and stingrays, which I commonly see being taken and dispatched as unwanted bycatch by recreation anglers. *Sharks and Rays of Australia* makes a highly commendable contribution to the appreci-ation, understanding, and conservation of this group of fishes and I want to thank the editors, Peter Last and John Stevens, and CSIRO Publications for a job well-done.

¹The Australian Museum, 6-8 College St., Sydney 2000, NSW, Australia.