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Diseases of poultry and endemic birds in Galapagos: implications for the reintroduction of native species

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Abstract

Reintroductions are increasingly utilized for the conservation of endangered avian species. To avert disease-related failures, studies to determine disease risks should be performed prior to the implementation of any avian reintroduction program. The presence, and prevalence, of disease-causing agents in both the source population and in birds at the site of reintroduction may help better direct reintroduction programs. In this study, we determined the prevalence of parasitic and pathogenic agents in chickens and wild birds on Floreana Island prior to the reintroduction of the critically endangered Floreana mockingbird *Mimus trifasciatus*. We investigated avian diseases on Floreana in 175 chickens and 274 wild birds. In addition to a number of clinical abnormalities, chickens tested positive for antibodies to paramyxovirus-1 (30%), adenovirus (11.3%) and seven other pathogens of concern for both domestic and wild birds. Wild birds on Floreana had antibodies to paramyxovirus-1 (3.0%) and adenovirus (2.4%). This is the first report of possible spillover of disease from domestic to wild birds in the archipelago. Based on these findings, and the lack of disease exposure documented in the source mockingbird population, we recommend improved poultry biosecurity measures on Floreana, and that mockingbirds only be reintroduced in areas on the island far from poultry and human presence and following further prerelease analyses. This study provides valuable data for the reintroduction of this iconic bird species and serves as a template for other avian reintroduction programs.

Introduction

Reintroduction may be defined as the intentional movement of an organism into a part of its native range from which it has disappeared or become extirpated in historic times (IUCN, 1998). Reintroductions of endangered species have been increasingly utilized in conservation to avert animal extinctions. For example, avian translocations as a conservation tool include upwards of 2327 translocation events involving 198 species at 749 sites recorded in the past two decades (Lincoln Park Zoo, http://www.lpzoo.org/artd/index.php). However, many of these programs have been compromised by disease (Cooper, 1993; Work *et al*., 2000, 2010).

Although disease risks are often appreciated as significant threats to the success of reintroduction plans (Viggers, Lindenmayer & Spratt, 1993; Woodford, 1993; Cunningham, 1996; Leighton, 2002), few reintroductions have implemented studies to minimize disease-related risks (Fontenot *et al*., 2006; Mathews *et al*., 2006). Prior to any animal movements, studies should be conducted to determine the prevalence of relevant disease-causing agents in the source population and in sympatric species at the reintroduction site (Woodford, 1993; Cunningham, 1996; Mathews *et al*., 2006). Importantly, both domestic and wild species may need to be targeted, as disease spillover from domestic to wild animals is often documented and may complicate conservation efforts (Cleaveland, Laurenson & Taylor, 2001; Fiorello *et al*., 2004).

In the Galapagos, there is growing concern for the long-term survival of endemic bird species due to habitat modification, climate change and introduced parasites and pathogens (Deem *et al*., 2008; Wiedenfeld & Jiménez-Uzcátegui, 2008; Parker, 2009), with the mangrove finch *Camarhynchus heliobates*, medium tree finch *Camarhynchus pauper*, and Floreana mockingbird *Mimus trifasciatus* among the rarest bird species in the world (O'Connor *et al*., 2009; Fessl *et al*., 2010a; Hoeck *et al*., 2010). Reintroductions may be necessary for the long-term survival of the mangrove finch and Floreana mockingbird (Charles Darwin Foundation, 2008; Fessl *et al*., 2010b).

The Floreana mockingbird, along with its three allopatric congeners in Galapagos (*Mimus parvulus*, *Mimus macdonaldi*, *Mimus melanotis*), are some of the most important birds in the history of science due to their pivotal role in triggering Darwin's theory on the evolution of species by natural selection (Darwin, 1859). Extirpated from Floreana Island over 125 years ago, the Floreana mockingbird now inhabits two small satellite islands, Champion ($n = 20$–53) (Grant, Curry & Grant, 2000) and Gardner-by-Floreana ($n = 200$–500) (P. E. A. Hoeck and L. F. Keller 2009, unpubl. census data). This species was listed as critically endangered by the International Union for the Conservation of Nature in 2008 due to the limited geographic range, fragmented distribution and small size of these two populations (http://www.iucnredlist.org/). The probable causes of extirpation of the Floreana mockingbird from Floreana in the late 1880s were the invasion of the island by goats that ate the birds' favorite food, *Opuntia* cactus, and predation by black rats (Grant *et al.*, 2000). Currently, the extinction threats include loss of genetic variation, environmental stochasticity (e.g. climate change), the possible introduction of invasive species on to the islets (e.g., black rats, cats), and disease (Hoeck *et al.*, 2010; Deem *et al.*, 2011). To avert extinction, the Floreana Mockingbird Reintroduction Plan was formulated (Charles Darwin Foundation, 2008). Determining the health status of birds on Floreana was indicated as a top priority within this plan.

Poultry broiler farms and backyard production have increased on Floreana in recent years, to supply both the resident human population and the growing tourist trade on the island, with some of the meat exported to other islands in the archipelago (Deem, pers. obs). There are seven commercial broiler farms in the highlands with an average flock size of approximately 100 birds. In the town of Puerto Velasco Ibarra, most households have backyard chickens with an average of 2–12 birds per household (Deem, pers. obs.). Replacement of chickens on Floreana are mostly by birth on the islands, although day-old chicks from the Ecuadorian mainland were imported starting in 2008 with an estimated 200 chicks legally imported that year [Servicio Ecuatoriano de Sanidad Agropecuaria (SESA)-Galapagos, unpubl. data]. Poultry vaccines, which are illegal in Galapagos, are not used on Floreana, and bio-security measures, including veterinary care, are limited (Deem, pers. obs.).

We predicted disease risks for the Floreana mockingbird reintroduction to include exposure to pathogens associated with the poultry industry on Floreana and avian poxvirus and *Philornis downsi*, both present on Floreana and known to cause disease in mockingbirds in Galapagos (Vargas, 1987; Curry & Grant, 1989; Fessl & Tebbich, 2002; Thiel *et al.*, 2005; Kleindorfer & Dudaniec, 2006; O'Connor *et al.*, 2009). Our hypotheses were that prevalence of parasitic and infectious agents would be (1) higher in chickens than wild birds, (2) higher in wild birds near chickens compared with those in the Galapagos National Park (GNP) and (3) higher in birds on Floreana than in Floreana mockingbirds previously tested on islets near Floreana (Deem *et al.*, 2011). Therefore, the primary objective of this study was to evaluate potential parasitic and infectious disease agents in poultry and wild birds at different locations on Floreana prior to the reintroduction. An additional objective was to provide a template for avian reintroduction programs in Galapagos and globally.

Methods

We visited Floreana Island in April–May 2008 and July 2008. Floreana (1°28′ S 90°48′ W) comprises 17 000 ha, with 98% of its surface within the Galápagos National Park (Fig. 1). Approximately 120 people and 1200 chickens live on Floreana in the town of Puerto Velasco Ibarra and seven farms. The first Galapagos Island colonized by humans, Floreana has sustained great loss in biodiversity and has been modified extensively by agriculture and the introduction of invasive plants, invertebrates and vertebrates (Curry, 1986; Grant *et al.*, 2000; Charles Darwin Foundation, 2008).

Prior to sample collection, permission to sample chickens and wild birds was obtained from Floreana residents and the Galapagos National Park. Chickens received a physical examination. Blood samples were collected by ulnar or jugular venipuncture using a 22-g needle and 6-mL syringe. Approximately 50 μL blood was stored in lysis buffer preservative (Longmire *et al.*, 1988) for hemoparasite identification, and the remainder of the blood was placed in a red top vacutainer and kept cool until centrifugation. A combined swab sample was collected from the conjunctiva, choana and cloaca of each chicken, transferred to cryotubes, and stored in a –20°C freezer on Floreana. After approximately 4 hours, red top tubes were centrifuged for 10 min, and sera samples were subsequently frozen in 1.8 mL cryogenic vials at –20°C while on Floreana and then at –80°C until analyzed.

Using mist nets and Potter traps baited with crackers, wild birds were captured on farms, in town and at sites in the GNP (Table 1 and Fig. 1). All wild birds were handled for less than 30 min from time of capture to release. Each bird was banded. Body weights, using a spring scale, and standard measurements were recorded, and physical examinations performed with inspection of nares and non-feathered areas to detect avian poxvirus-like lesions and evidence of previous *P. downsi* infestations.

Blood samples (< 1% of body weight) were collected from the ulnar vein using a 25 or 26-g needle by pricking the vein and then filling 1–2 heparinized capillary tubes. Blood smears were immediately prepared and approximately 50 μL blood was stored in lysis buffer preservative for hemoparasite identification. All remaining capillary tubes were sealed with clay and kept cool while in the field. Later that day, capillary tubes were centrifuged for 10 min and plasma decanted and subsequently frozen in 0.4 mL cryotubes at –20°C on Floreana and –80°C in the laboratory. A microtip swab was collected from the cloaca of each passerine, transferred to cryotubes, and stored at –20°C freezer while on Floreana. Fecal samples were collected opportun-

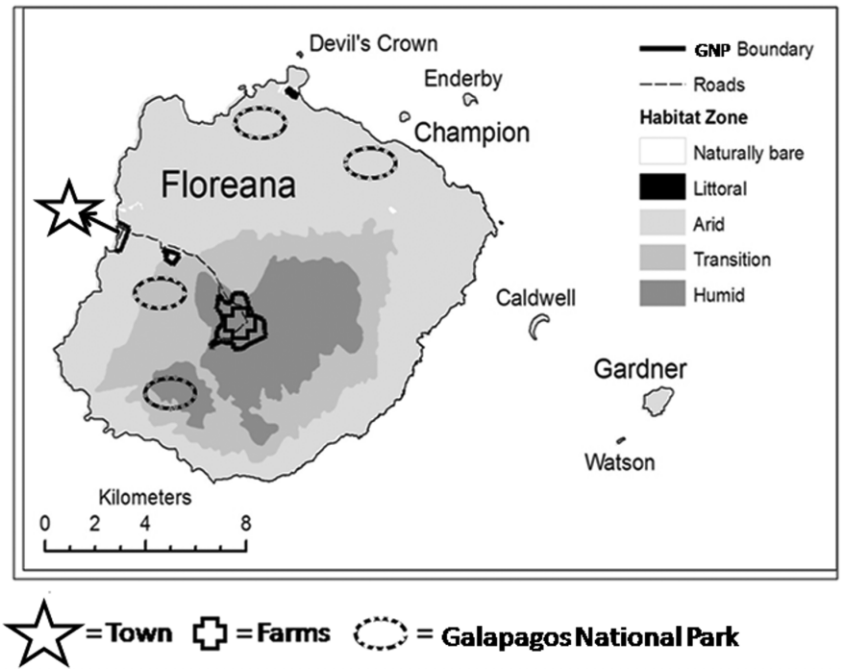

Figure 1 Floreana Island, Galapagos, Ecuador with sites of sample collection from chickens and wild passerines in town, farms and in the Galapagos National Park are indicated.

Table 1 Number of individuals of each species of wild bird evaluated on Floreana Island, Galapagos as part of the Floreana Mockingbird reintroduction plan

Bird species	Town	Farms	Galapagos National Park
Dark-billed cuckoo *Coccyzus melacoryphus*	5	1	
Small tree finch *Camarhynchus parvulus*		4	6
Medium tree finch *Camarhynchus pauper*		4	
Yellow warbler *Dendroica petechia*	15	6	11
Medium ground finch *Geospiza fortis*	39	1	
Small ground finch *Geospiza fuliginosa*	36	49	61
Cactus finch *Geospiza scandens*			2
Galapagos flycatcher *Myriarchus magnirostris*	8	13	13

istically and preserved in 10% buffered formalin. All birds were released when hemostasis was confirmed.

Suspected pox lesions were sampled by either taking cutaneous scrapings stored in ethanol or by puncturing nodules with a sterile needle and collecting the exudate in lysis buffer. DNA extraction was performed using a phenol-chloroform method (Sambrook & Russell, 1989). Tissue samples stored in ethanol were dried, homogenized, and stored in lysis buffer prior to DNA extraction. Samples were imported to the US at room temperature (slides, blood in lysis buffer) or frozen on dry ice (swabs, sera, plasma).

Tissue samples were tested for avian poxvirus DNA by polymerase chain reaction (PCR) (Thiel *et al.*, 2005). Chicken serologic tests were performed at the University of Georgia Poultry Diagnostic Research Center in Athens, GA. Antibody titers to avian paramyxovirus-1 (PMV-1) (positive cut off value used by laboratory to detect exposure to pathogen (CO) > 64), *Mycoplasma gallisepticum* (CO > 1076), infectious bursal disease virus (IBD) (CO > 400), avian encephalomyelitis virus (AEV) (CO > 400), avian reovirus (CO > 400) and infectious laryngotracheitis virus (ILT) (CO > 1076), were determined using enzyme-linked immunosorbent assays (ELISA). The hemagglutination inhibition test (CO > 64) was employed to evaluate titers to infectious bronchitis virus. Exposure to avian influenza type A virus, group 1 avian adenovirus and Marek's disease virus (MDV) were determined using agar gel precipitin tests (AGP) for positive or negative results. Tube agglutination (TA) tests were used to evaluate exposure to *Salmonella typhimurium* and *Salmonella pullorum* (CO > 10).

Because of the limited volume of blood safely collected from wild birds, analyses of their sera were prioritized based on serological findings from chickens. Wild bird serologic tests were performed at the Veterinary Medical Diagnostic Laboratory, University of Missouri – Columbia, Columbia, MO. Antibody titers to avian PMV-1 (CO > 396), adenovirus-2 (CO > 2000), and *M. gallisepticum* (CO > 1076) were determined using ELISA.

Swabs were submitted, for detection of *Chlamydophila psittaci* DNA sequencing by PCR, to the Infectious Diseases Laboratory, College of Veterinary Medicine, University of Georgia, Athens, GA and to the College of Veterinary Medicine, North Carolina State University, Raleigh, NC for detection of *M. gallisepticum* DNA sequencing by PCR.

To determine the presence of any Haemosporidian parasites, molecular tests were conducted at the University of

Missouri – St. Louis, St. Louis, MO. DNA was extracted from blood using a standard phenol chloroform extraction protocol, and PCR was used to amplify a region of the parasite mitochondrial cytochrome *b* gene (Perkins & Schall, 2002; Waldenström *et al*., 2004), and amplification was detected by gel electrophoresis.

Fecal samples were analyzed by flotation, using a saturated sugar solution, and a semi-quantitative McMaster fecal test was performed at the Laboratory of Epidemiology, Genetics, and Pathology, Puerto Ayora, Galapagos.

Prevalence was defined as the proportion of tested birds with clinical signs or positive laboratory test results, with 95% confidence intervals provided (Thrusfield, 2007). Chi-squared test or Fisher's exact test were used to compare findings between poultry and wild birds, and between different sites on the island (town, farms, GNP). Comparisons of poultry and wild birds with Floreana mockingbirds (Deem *et al*., 2011) were evaluated by Fisher's exact test. Results were analyzed using a commercial statistical software package (NCSS, Kaysville, UT, USA).

Results

We evaluated 175 chickens (116 on farms and 59 in town) and 274 wild birds representing eight different species (Table 1). Eleven of 175 chickens (prevalence; 95% confidence interval) (6.3%; 3.5–10.9%) had clinical evidence of poor health (e.g., skin lesions, thin, respiratory signs, diarrhea). Sixteen of 274 wild birds (5.8%; 3.6–9.3%) had signs of poor health; five of these birds (1.8%; 0.8–4.2%) had pox-like lesions, and five (1.8%; 0.8–4.2%) had nares deformation consistent with past *P. downsi* infestation (Galligan & Kleindorfer, 2009).

Three of the five wild birds exhibiting possible poxvirus infections tested positive by PCR. The two samples that tested negative were exudate samples. All wild birds with pox-like lesions (e.g., nodules and hypertrophic skin on extremities) were *Geospiza fuliginosa* (three in town and two on farms), and all birds with malformation of the nares were *G. fuliginosa* (three on farms and two in the GNP). The six other clinical signs noted in wild birds included a *G. fuliginosa* found in the GNP with a sunken eye and hypertrophic skin over the face, plus five birds in town (one *Myriarchus magnirostris* with extremely poor feathering, two *G. fuliginosa*, one with digital fractures and deformities bilateral and another which appeared dehydrated and pale, and two *Geospiza fortis*, one with a large ulcerative lesion on the pectoral region and the other with abnormally colored diarrhea). There was no significant difference between numbers of chickens and wild birds on Floreana with lesions (chi-squared test; $P = 0.85$). Additionally, there were no significant differences in number of chickens with lesions on farms (6/116) and in town (5/59) (Fisher's exact test; $P = 0.51$), or between wild birds with lesions on farms (5/78), in town (7/101), or in the GNP (4/95) (Chi-squared test; $P = 0.70$). There was a significant difference in the number of birds with lesions on Floreana (27/449) and Floreana mockingbirds on Champion and Gardner-by-Floreana (1/235) (Fisher's exact test; $P = 0.0001$) (Table 4) (Deem *et al*., 2011).

Infectious and parasitic agent test results are presented in Table 2 (chickens), Table 3 (wild birds), and Table 4 (chickens, wild birds and Floreana mockingbirds). Chickens were seropositive to IBD, AEV, reovirus, IBV (Mass), IBV (Conn), ILT, PMV-I, MDV, adenovirus-1 and *M. gallisepticum*. Chickens in town had a higher seroprevalence for IBD, IBV (Mass) and IBV (Conn) than chickens on farms, whereas chickens on farms had a higher seroprevalence to PMV-1 than chickens in town (Table 2). Wild birds were seropositive to PMV-1 and adenovirus-2 (Table 3). There were no significant differences in seroprevalence among wild birds sampled in the three sites (town, on farms, in the GNP). Seroprevalence to PMV-1 differed between chickens (53/177) and wild birds (6/197) on Floreana (Chi-squared test; $P < 0.0001$) and between all birds on Floreana (59/374) and Floreana mockingbirds on Champion and Gardner-by-Floreana (0/86) (Fisher's exact test; $P < 0.0001$) (Table 4) (Deem *et al*., 2011). Seroprevalence to adenovirus differed between all birds on Floreana (21/218) and Floreana mockingbirds on Champion and Gardner-by-Floreana (0/81) (Fisher's exact test; $P = 0.002$) (Table 4) (Deem *et al*., 2011). All of the 175 chickens and 274 wild birds tested were negative for *C. psittaci* DNA, and none of the 15 chickens or 37 wild birds tested was positive for *M. gallisepticum* DNA.

All chickens tested were negative for Haemosporidian blood parasites (59 from town, 34 from farms). However, among 223 endemic passerine birds tested from seven species (nine *Camarhynchus parvulus*, five *C. pauper*, 32 *Dendroica petechia*, 38 *G. fortis*, 102 *G. fuliginosa*, two *Geospiza scandens*, 35 *Myiarchus magnirostris*), three tested positive (one *C. parvulus* and two *G. fuliginosa*). The parasite in *C. parvulus* was typed as *Haemoproteus* sp. Two of the infections were in the GNP (*C. parvulus* and one *G. fuliginosa*), while the second *G. fuliginosa* was on a farm.

Of the 63 wild bird fecal samples evaluated for gastrointestinal parasites, a coccidian agent, identified as an *Isospora* sp. based on morphology (McQuistion & Wilson, 1989) was found in six birds (9.8%; 4.6–19.8%), and an unidentified egg was found in two other individuals. The *Isospora* sp. eggs were detected in two *D. petechia* in the GNP, one *G. fuliginosa* on farms and two in the GNP, and one *C. parvulus* on a farm. One unidentified egg was found in a *D. petechia* in town and a *G. fuliginosa* on a farm. There was no significant difference between coccidian prevalence in wild birds on Floreana (6/63) and Floreana mockingbirds on Champion and Gardner-by-Floreana (1/33) ($P = 0.4160$) (Table 4) (Deem *et al*., 2011).

Discussion

The initiation of reintroduction plans to expand the geographical distribution of critically endangered populations may minimize the risks of extinction in the short term. In this study, we documented a number of health risks for the reintroduction of the Floreana mockingbird. Risks may be

Table 2 Pathogen, diagnostic tests performed, number of positives/number tested, and percent positive; 95% confidence intervals in the evaluation of select infectious and parasitic agents in domestic chickens *Gallus gallus domesticus* on Floreana Island, Galapagos

Disease or agent (test)	All chickens	Town	Farms	*P*-value[a]
Infectious bursal disease (ELISA)	122/177	49/61	73/116	0.017
	69%; 61.8–75.3%	80.3%; 68.7–88.4%	63%; 54–71.2%	
Avian encephalitis (ELISA)	115/177	36/61	79/116	NS
	65%; 57.8–71.6%	59%; 46.5–70.5%	68.1%; 59.2–75.9%	
Reovirus (ELISA)	90/177	25/61	65/116	NS
	51%; 43.5–58.1%	41%; 30–53.5%	56%; 47–64.7%	
Infectious bronchitis virus (Mass) (H1)	64/176	37/61	27/115	<0.001
	36.4%; 30–43.7%	61%; 48.1–72%	23.5%; 16.7–32%	
Infectious bronchitis virus (Conn) (H1)	33/176	18/61	15/115	0.0077
	19%; 13.7–25.2%	30%; 20–41.9%	13%; 8.1–20.4%	
Infectious laryngotrachitis (ELISA)	56/166	17/60	39/106	NS
	34%; 27–41.2%	28.3%; 18.5–40.8%	36.8%; 28.2–46.3%	
Paramyxovirus-1 (ELISA)	53/177	10/61	43/116	0.004
	30%; 23.7–37.1%	16%; 9.2–27.6%	37.1%; 28.8–46.1	
Mareks (AGP)	27/177	10/61	17/116	NS
	15.3%; 10.7–21.3%	16%; 9.2–27.6%	15%; 9.4–22.2%	
Adenovirus-1 (AGP)	20/177	4/61	16/116	NS
	11.3%; 7.4–16.8%	6.6%; 2.6–15.7%	14%; 8.7–21.2%	
Mycoplasma gallisepticum (ELISA)	3/176	0/61	3/115	NS
	1.7%; 0.6–4.9%	0%; 0–5.9%	2.6%; 0.9–7.4%	
Avian influenza (AGP)	0/177	0/61	0/116	NS
	0%; 0–2.1%	0%; 0–5.9%	0%; 0–3.2%	
Salmonella typhimurium (Tube agglutination)	0/73	0/35	0/38	NS
	0%; 0–5.0%	0%; 0–9.9%	0%; 0–9.2%	
Salmonella pullorum (Tube agglutination)	0/73	0/35	0/38	NS
	0%; 0–5.0%	0%; 0–9.9%	0%; 0–9.2%	
Chlamydophila psittaci (PCR swab)	0/146	0/51	0/95	NS
	0%; 0–2.6%	0%; 0–7.0%	0%; 0–3.9%	
Mycoplasma gallisepticum (PCR swab)	0/15	0/4	0/11	NS
	0%; 0–20.4%	0%; 0–49%	0%; 0–26%	
Haemosporidian parasites (PCR)	0/93	0/59	0/34	NS
	0%; 0–4.0%	0%; 0–6.1%	0%; 0–10.2%	

[a]Chi-square test (or Fisher's exact when values < 5) for prevalence between chickens sampled from town and on farms.
ELISA, enzyme-linked immunosorbent assay; HI, hemagglutination inhibition; AGP, agar gel precipitation; PCR, polymerase chain reaction; NS, non-significant.

Table 3 Pathogen, diagnostic tests performed, number of positives/number tested, and percent positive; 95% confidence intervals in the evaluation of select infectious and parasitic agents in wild birds on Floreana Island, Galapagos

Disease agent (test)	Totals	Town	Farms	Galapagos National Park
PMV-1 (ELISA)	6/197[a]	4/72	0/60	2/65
	3.0%; 1.4–6.5%	5.6%; 2.2–13.4%	0%; 0–6.0%	3.1%; 0.8–10.5%
Mycoplasma galliespticum (ELISA)	0/45	0/17	0/12	0/16
	0%; 0–7.9%	0%; 0–18.4%	0%; 0–24.2%	0%; 0–19.4%
Adenovirus-2 (ELISA)	1/41[b]	1/15	0/14	0/12
	2.4 %; 0.4–12.6%	6.7%; 1.2–21.8	0%; 0–21.5%	0%; 0–24.2%
Chlamydophila psittaci (PCR swab)	0/180	0/70	0/58	0/52
	0%; 0–2.1%	0%; 0–5.2%	0%; 0–6.2%	0%; 0–6.9%
Mycoplasma gallisepticum (PCR swab)	0/37	0/16	0/6	0/15
	0%; 0–9.4%	0%; 0–19.4%	0%; 0–39.0%	0%; 0–20.4%
Haemosporidian parasites (PCR)	3/223	0/85	1/64	2/74
	1.3%; 0.4–3.9%	0%; 0–4.3%	1.6%; 0.3–8.3%	2.7%; 0.7–9.3%

[a]Of the 6 birds positive for PMV-I, both birds away from humans were G. *fuliginosa* and 3/4 in town were *G. fuliginosa*. One *G. fortis* was PMV-I positive in town.
[b]The one adenovirus-2 positive bird was a *G. fuliginosa*.
PMV-1, Paramyxovirus-1; ELISA, enzyme-linked immunosorbent assay; PCR, = polymerase chain reaction.

Table 4 Pathogen, diagnostic tests performed, number of positives/number tested, and percent positive; 95% confidence intervals in the evaluation of select infectious and parasitic agents in wild birds and chickens on Floreana Island and of Floreana mockingbirds on Champion and Gardner-by-Floreana islands, Galapagos

Disease or agent (test)	Totals of chickens	Totals of wild birds on Floreana	Totals of all birds on Floreana	Totals of Mockingbirds on Champion and Gardner[a]
PMV-1 (ELISA)	53/177[b]	6/197[b]	59/374[b]	0/86[b]
	30%; 23.7–37.1%	3.0%; 1.4–6.5%	15.8%; 12.2–19.9%	0%; 0–4.2%
Mycoplasma galliespticum (ELISA)	3/176	0/45	3/221	0/88
	1.7%; 0.6–4.9%	0%; 0–7.9%	1.4%; 0.2–3.9%	0%; 0–4.1%
Adenovirus[c] (ELISA)	20/177	1/41	21/218[b]	0/81[b]
	11.3%; 7.4–16.8%	2.4 %; 0.4–12.6%	9.6%; 6.1–14.3%	0%; 0–4.5%
Chlamydophila psittaci (PCR swab)	0/146	0/180	0/326	0/43
	0%; 0–2.6%	0%; 0–2.1%	0%; 0–1.1%	0%; 0–8.2%
Mycoplasma gallisepticum (PCR swab)	0/15	0/37	0/52	n/a
	0%; 0–20.4%	0%; 0–9.4%	0%; 0–6.8%	
Haemosporidian parasites (PCR)	0/93	3/223	3/316	0/46
	0%; 0–4.0%	1.3%; 0.4–3.9%	0.9%; 0.2–2.7%	0%; 0–7.7%
Clinical signs	11/175	16/274	27/449[b]	1/235[b]
	6.3%; 3.5–10.9%	1.8%; 0.8–4.2%	6.0%; 4.0–8.6%	0.4%; 0–2.4%
Coccidian GIT parasite	n/a	6/63	6/63	1/33
		9.8%; 4.6–19.8%	9.8%; 4.6–19.8%	3%; 0–16.2%

[a]Data from Deem *et al.* (2011).

[b]Chi square test (or Fisher's exact if numerators < 5) results were significantly different ($P < 0.05$).

[c]An ELISA to detect adenovirus-1 antibodies in the chickens and adenovirus-2 in the wild passerines and Floreana mockingbirds was used.

PMV-1,Paramyxovirus-1; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction; n/a, not applicable.

associated with poultry production and may include spillover to wild birds on the island. These health data are instrumental for the proper implementation of the Floreana mockingbird reintroduction plan, and provide a template for disease studies applicable in other endangered avian reintroduction programs.

Results from this study supported some of the original hypotheses with the seroprevalence of PMV-1 higher in chickens than wild birds on Floreana and in all birds on Floreana when compared with the Floreana mockingbirds on Champion and Gardner-by-Floreana. Additionally, birds on Floreana had a higher seroprevalence to adenovirus than Floreana mockingbirds and tended to have significantly more clinical abnormalities. However, there were no significant differences in the other pathogen data or between any of the measures in wild birds on Floreana based on site of sample collection.

Approximately six percent of chickens and wild birds on Floreana were rated in poor health based on clinical signs. One chicken had respiratory signs and two had severe diarrhea. The chicken with respiratory signs (e.g., dyspnea and respiratory stidor) was positive for IBV (Mass) and IBV (Conn). Both chickens with diarrhea had PMV-1 antibodies. Since birds with clinical signs are more likely to be actively shedding disease agents, these three chickens may have been infectious at the time of sampling (Saif *et al.*, 2003).

There was no significant difference between the number of chickens and wild birds with clinical lesions; however the clinical health of Floreana mockingbirds on Champion and Gardner-by-Floreana was significantly higher than all birds on Floreana. Although not all pox-like lesions are caused by pox virus, since other possible diagnoses exist, such as trauma, bacterial or fungal infections (van Riper & Forrester, 2007; Parker *et al.*, 2011), the presence of pox-like lesions in Floreana wild birds (1.8%), and the absence of lesions in Floreana mockingbirds (Deem *et al.*, 2011) suggest that mockingbirds will have higher exposure to pox virus upon reintroduction back to Floreana. The high susceptibility of Galapagos mockingbirds and the naïve status of the Floreana mockingbirds on Champion and Gardner-by-Floreana suggest that the presence of avian pox virus on Floreana may cause morbidity and mortality in reintroduced Floreana mockingbirds. This one infectious agent has the potential to hinder the reintroduction effort as has previously occurred during the reintroduction of the Hawaiian goose *Branta sandvicensis* (Kear, 1977).

Clinical signs of past *P. downsi* infestation were present in wild birds on Floreana, but no Floreana mockingbirds on Champion or Gardner-by-Floreana had evidence of exposure (Deem *et al.*, 2011). *Philornis downsi* is recognized as the primary threat, which led to the listing of medium tree finch as critically endangered (O'Connor *et al.*, 2009). In contrast, *P. downsi* appears to be present on Champion and Gardner-by-Floreana at low prevalence (Jiménez-Uzcátegui, 2008). Therefore, it is likely that Floreana mockingbirds reintroduced on to Floreana will have a higher exposure to *P. downsi*.

Based on data from this study and previous work in Galapagos, poultry may serve as reservoirs of infectious and parasitic diseases at the domestic – wild bird interface, and may spillover to wild birds in the archipelago (Gottdenker *et al.*, 2005; Soos *et al.*, 2008). The disease threats associated

with introduced poultry in Galapagos will increase as the poultry industry continues to grow, both for the residential population and the expanding tourist trade (González *et al*., 2008; Soos *et al*., 2008). In this study, evidence of a variety of viral and bacterial diseases in chickens on Floreana was demonstrated. Antibodies were detected for 10 of the 13 pathogens tested and eight of these were detected in over 15% of chickens tested.

Sixty-nine per cent of the chickens were seropositive to IBD, a Birnavirus that causes necrosis of the lymphoid tissues resulting in immunosuppression, which may lead to the emergence of viral, bacterial and fungal infections. In addition to possible IBD virus spillover from poultry to wild birds, secondary infections in chickens positive for IBD may also be pathogenic for the wild birds on Floreana.

Paramyxovirus-1 antibodies were identified in 30% of the chickens, two with clinical signs (diarrhea), and 3% of the wild birds. Paramyxovirus-1 has caused large-scale die-offs in wild bird populations and may spill over into introduced Floreana Mockingbirds when reintroduced on to the island (USGS, 1999; Leighton & Heckert, 2007). Of the other pathogens to which the chickens were seropositive, *M. gallisepticum* and adenovirus are of grave concern. Although our data show a low prevalence of *M. gallisepticum* on Floreana, this bacterium causes serious disease in passerine species in North America and therefore may be pathogenic to introduced mockingbirds (Dhondt, Tessaglia & Slothower, 1998). Additionally, adenoviruses are known to cause hepatitis, enteritis, and respiratory signs in a number of avian species and the presence of antibodies in both poultry and wild birds on Floreana suggests that introduced mockingbirds may be exposed (Ritchie, 1995).

Antibodies to PMV-1 and adenovirus-2 were also present in endemic passerines on Floreana. The finding of 3.0% of the wild birds positive to PMV-1 is the first time wild birds in Galapagos have tested positive to PMV-1 and may be due to spillover from the chicken population. Four of these six birds were located in the town where there is history of an epiornitic 4 years prior to sampling in which 90% of the chickens died within hours. Unfortunately, no diagnostics were performed at that time. The two positive birds that were in the GNP were located at Cerro Pajas, which, although in the GNP and away from human habitation, is located less than 2 km from farm land and a site where feral chickens may be present. None of the Floreana mockingbirds tested were positive for PMV-1 (Deem *et al*., 2011). This finding is highly suggestive of disease spillover from chickens (30% seroprevalence) to passerines (3% seroprevalence), although PMV-1 is commonly found in wild bird populations and thus may not be related to chicken farming on Floreana (USGS, 1999). Virus isolation and sequence comparison is necessary to determine the relationship between the chicken and wild bird PMV-1 viruses.

No Haemosporidian blood parasites were detected in the chickens on Floreana, although a number have been found in Galapagos (Padilla *et al*., 2004, 2006; Levin *et al*., 2009; Santiago-Alarcon *et al*., 2010; Valkiunas *et al*., 2010). Three of 223 wild Floreana passerines tested were positive by PCR with one confirmed *Haemoproteus* sp. and two not yet typed to genus. Continued monitoring of poultry and wild birds for these parasites is important, because *Culex quinquefasciatus*, a vector of avian malaria and avian pox, has been introduced (Whiteman *et al*., 2005).

Six wild birds were positive for a coccidian agent, which we identified as an *Isospora* sp. based on morphology (McQuistion & Wilson, 1989). Egg counts were low in all these birds. *Isospora* sp. has been detected previously in finches on Floreana and in Floreana mockingbirds on Champion and Gardner-by-Floreana (Dudaniec, Hallas & Kleindorfer, 2005; Deem *et al*., 2011), and thus we do not believe this agent poses additional risks to the reintroduction plan.

Limitations to this study include the use of sera for antibody testing of pathogens in chickens and plasma for passerines, and the testing of antibodies to adenovirus-1 in chickens and adenovirus-2 in passerines. Additionally, it is possible that some of the chickens were seropositive to certain pathogenic agents due to vaccination and not previous or current infection. However, we feel this possibility is very low as vaccination is illegal in Galapagos and all poultry owners stated that they do not vaccinate their chickens. The intention was to test all birds for adenovirus-1 based on past studies in Galapagos (Padilla *et al*., 2003). The use of plasma for wild birds was logistically easier with the small blood samples, and there should be no difference in antibody detection between sera and plasma samples. Lastly, we were unable to test the wild birds for all of the pathogens tested in the chickens due to the small amount of blood that could be safely collected from each passerine. Therefore, we prioritized which agents to test after having results from the chickens and based on known disease-causing agents of concern in wild birds.

We recommend further health evaluations are conducted and that more sensitive and specific molecular techniques for disease testing are incorporated to compare viral strains circulating in chickens and wild birds on Floreana. Additionally, domestic cats *Felis catus* were present throughout the island and threaten the success of the reintroduction plan due to their predatory habits and possible *Toxoplasma gondii* transmission, as we have found evidence of *T. gondii* exposure in other avian species in Galapagos (Gottdenker *et al*., 2005; Deem *et al*., 2010). Removal of feral cats from the island should be addressed prior to any Floreana mockingbird reintroductions, as suggested in the reintroduction plan (Charles Darwin Foundation, 2008).

The high seroprevalence noted for many pathogens in chickens in the town support possible exposure to Floreana mockingbirds if a captive breeding center or soft release site is constructed near the town as currently suggested in the reintroduction plan (Charles Darwin Foundation, 2008). We recommend that this be reconsidered and located at a site in the GNP and far from human and chicken presence. Additionally, Cerro Pajas has been slated as one of the better sites for mockingbird release. The finding of two of the six PMV-1 seropositive wild birds located at Cerro Pajas gives concern for this site for mockingbird release.

Data are now available on the genetics and health status of the source Floreana mockingbird populations (Hoeck *et al*., 2010; Deem *et al*., 2011); the genetic data support the reintroduction of birds from both Champion and Gardner-by-Floreana, since unique alleles are present in both populations. However, diseases and exposure to disease-causing agents documented in the poultry and wild birds of Floreana should be taken into consideration. Prior to any mockingbird reintroduction, we recommend measures, including vaccination of poultry, improved biosecurity on poultry farms, and locating sites for mockingbird introduction far from humans and poultry within the GNP, are taken to mitigate negative impacts of diseases. Additionally, population viability and disease risk analyses should be performed based on diseases of concern, home range sizes of poultry and wild birds on Floreana and Floreana mockingbirds on the islets, better data on poultry production on Floreana, and the risk of extinction for the Floreana mockingbird with and without reintroduction efforts (Lacy, 1993; Deem, 2012). In addition to these pre-reintroduction recommendations, long term monitoring following the reintroduction should be performed to assess the likelihood of new or additional disease risks (Viggers *et al*., 1993; Woodford, 2001; Sutherland *et al*., 2010).

Currently, two endemic Galapagos bird species, Floreana mockingbird and mangrove finch, are slated for translocation and reintroduction plans (Charles Darwin Foundation, 2008; Fessl *et al*., 2010b). In addition to providing important data for the Floreana mockingbird reintroduction, this study may serve as a template for other translocation efforts in Galapagos, such as the mangrove finch, and for avian species globally.

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