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COMPARISON OF PATHOGENS IN BROILER AND BACKYARD CHICKENS ON THE GALÁPAGOS ISLANDS: IMPLICATIONS FOR TRANSMISSION TO WILDLIFE

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Abstract.—As the human population and tourism increase in the Galápagos Islands, increased poultry production raises risks of pathogen spillover into native avian populations. Here, we characterize the disease risks to Galápagos avifauna of different types of poultry farming by comparing health status and serosurvey results between broiler and backyard chickens (*Gallus gallus domesticus*). Backyard chickens were more frequently diseased than broilers, and were more likely to be seropositive for several pathogens (*Mycoplasma gallisepticum*, infectious laryngotracheitis virus, infectious bronchitis virus, avian reovirus, and Marek's disease virus). Seroprevalence for other pathogens (avian paramyxovirus-1, infectious bursal disease, avian encephalomyelitis virus, and avian adenovirus) was relatively high among all chickens. Preliminary serological results from wild birds revealed no evidence of previous exposure to these diseases, which suggests that transmission of disease from poultry to wildlife is currently not detectable with the sample sizes and tests employed, and that wildlife are likely not the source of exposure to poultry. Our results suggest that backyard chickens may pose a greater threat to Galápagos avifauna because they are more likely to be infectious, have a high seroprevalence for numerous pathogens, and interact directly with wild birds or wild bird habitat, with no biosecurity measures employed. The broiler industry has greater potential for importation of pathogens into the islands and indirect transmission of diseases to wildlife (e.g., through use of poultry litter on agricultural land). Regulatory and management decisions should focus on minimizing the poultry–wildlife interface, reducing infectious diseases in backyard chickens, and preventing importation of poultry diseases. *Received 28 October 2006, accepted 22 August 2007.*

Key words: conservation, emerging infectious diseases, *Gallus gallus*, poultry–wildlife interface, risk, seroprevalence.

Comparación de Patógenos en Pollos de Criadero y Caseros en las Islas Galápagos: Implicancias para la Transmisión a la Vida Silvestre

Resumen.—A medida que aumenta la población humana y el turismo en las Islas Galápagos, el incremento en la producción de aves de corral eleva el riesgo de propagación de patógenos a las poblaciones de aves nativas. Aquí caracterizamos el riesgo de transmisión de enfermedades a la avifauna de Galápagos desde diferentes tipos de aves de corral, comparando el estatus de salud y los resultados serológicos entre pollos (*Gallus gallus domesticus*) de criadero industrial y pollos caseros Los pollos caseros presentaron enfermedades con mayor frecuencia que los de criadero industrial y presentaron mayor probabilidad de ser positivos en el examen serológico para varios patógenos (*Mycoplasma gallisepticum*, virus de laringotraqueitis infecciosa, virus de bronquitis infecciosa, reovirus aviar y virus de la enfermedad de Marek). La prevalencia de otros patógenos en el suero (paramixovirus-1 aviar, enfermedad bursal infecciosa, virus de encéfalo mielitis aviar y adenovirus aviar) fue relativamente alta entre todos los pollos. Los resultados serológicos preliminares de las aves silvestres no brindaron evidencia de exposición previa a estas enfermedades, lo que sugiere que la transmisión de las enfermedades desde las aves de corral a la vida silvestre no puede ser actualmente detectada con los tamaños de muestra y los exámenes empleados en

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este estudio. Adicionalmente, la vida silvestre no es probablemente la fuente de la exposición a las aves de corral. Nuestros resultados sugieren que los pollos caseros pueden representar una mayor amenaza a la avifauna de Galápagos porque tienen una mayor probabilidad de ser infecciosos, tienen una alta prevalencia en el suero para numerosos patógenos e interactúan directamente con las aves silvestres o con su hábitat sin el empleo de las medidas de bio-seguridad necesarias. La industria avícola tiene un potencial mayor de importación de patógenos a las islas y de transmisión indirecta de enfermedades a la vida silvestre (e.g., a través del uso de desperdicios de pollo en las tierras agrícolas). Las regulaciones y las decisiones de manejo deberían enfocarse en minimizar la interfase entre las aves de corral y la vida silvestre, reduciendo las enfermedades infecciosas en los pollos caseros y previniendo la importación de enfermedades de aves de corral.

The impact of human activities on the Galápagos Islands ecosystem has been increasing since the islands were first inhabited, despite the restriction of human habitation to 4 of the 13 main islands. Recent years have seen rapid expansion of both the human population and the tourism industry (Instituto Nacional de Estadística y Censos, 2001–2006). Poultry production has also increased, and increasingly, poultry farms are being established in areas used by native and endemic avian species. In 2003, 81,380 day-old broiler chicks were imported onto the island of Santa Cruz to support the local broiler industry (Servicio Ecuatoriano de Sanidad Agropecuaria [SESA]-Galápagos, unpubl. data). This increased to 126,200 chicks in 2004 and 143,000 in 2005. Poultry farms exist on the human-populated islands of Santa Cruz, Isabela, San Cristobal, and Floreana and include small- to medium-scale broiler operations (300–4,000 birds), small- to medium-scale egg-layer farms (1,500–5,200 birds), and backyard chicken flocks (1–100 birds) (Gottdenker et al. 2005, SESA-Galápagos unpubl. data). On the island of Santa Cruz, there are currently 25–30 broiler farms registered with SESA-Galápagos. Day-old broiler chicks are imported to Galápagos from mainland Ecuador and reared within enclosed barns until they are slaughtered at seven or eight weeks of age. Broiler barns typically have metal roofs, concrete floors, and walls constructed from concrete, wood, wire mesh, chain-link fencing, or tarps. By contrast, most backyard chickens are allowed to range freely, do not have a set lifespan, and may travel hundreds of meters from the farm site to forage, returning regularly for supplemental feed provided by the farmer. It is unknown how many backyard chicken flocks currently exist on the four inhabited islands.

Expansion of the poultry–wildlife interface increases the potential for transfer of pathogens from chickens to immunologically naive resident wildlife. Introduction of poultry diseases into endemic Galápagos bird species has the potential to drive small, susceptible populations to extinction (Williams et al. 1988, Gerber et al. 2005). A recent serosurvey of poultry farms on Santa Cruz and San Cristobal, conducted in 2001–2003, identified evidence of exposure to a number of pathogens, including avian paramyxovirus-1 (Newcastle disease virus) and *Mycoplasma gallisepticum*, which are pathogens of major concern to wildlife (Gottdenker et al. 2005). These pathogens are known to cause mortality and population declines in wild avian species in other parts of the world (Kuiken 1999, Schelling et al. 1999, Hochachka and Dhondt 2000, Bengis et al. 2002) and, thus, may have significant effects on multiple avian species in the Galápagos archipelago. The objectives of the present study were (1) to begin to characterize the threats that different types of poultry farming may represent to native fauna of the Galápagos Islands by comparing health status and serological responses to selected pathogens between broiler and backyard chickens and (2) to begin evaluating the health status of wild birds found in association with farms.

Methods

Farm and control site selection.—In June 2005, on Isla Santa Cruz, 25 broiler farms registered with SESA-Galápagos were visited, and farmers were interviewed to evaluate their willingness to participate in this investigation. Number and age of chickens in operation and GPS points for each farm were recorded. Eleven backyard chicken flocks with ≥50 chickens were visited, interviews were conducted, and data were recorded in the same manner as for broiler farms. All broiler farms and backyard flocks were mapped in ARCVIEW GIS, version 3.1 (Environmental Systems Research Institute, Redlands, California), overlaying topographical maps of Santa Cruz. Three broiler farms and four backyard chicken flocks located throughout the agricultural zone were selected for the present study (Fig. 1). We also located three control sites ≥2 km away from selected poultry farms (Fig. 1). Broiler farms, backyard flocks, and control sites at similar altitudes were selected to minimize differences in potential confounding factors, such as avian species composition and insect-vector diversity and abundance. Consent from farmers to participate in the study was voluntary, and all sampling of chickens and wild birds was conducted between 7 and 30 July 2005.

Sampling of chickens.—Thirty chickens were sampled at each broiler farm and backyard flock. Each chicken was examined, and any lesions or abnormalities were recorded. Blood samples were collected by ulnar or jugular venipuncture, and blood smears were immediately prepared and were later stained for future evaluation for hemoparasites. A small amount of blood was stored in a lysis buffer preservative solution (Longmire et al. 1988) for future genetic analyses (e.g., hemoparasite identification, molecular sexing). Remaining blood was placed in serum collection tubes and stored on ice packs in coolers until processed later on the same day. Tubes were centrifuged for 20–30 min until serum was separated, and serum samples were subsequently frozen in cryogenic vials at –80°C. Ectoparasites were opportunistically collected and preserved in 95% ethanol for identification. Feces and two swabs each of conjunctiva, choana, and cloaca were collected and stored in microcentrifuge tubes at –80°C for future analyses. Swabs of abnormal mucus or exudate from eyes, nares, and choana were also collected and frozen.

Samples from endemic birds.—Using mist nets and Potter traps, we attempted to trap a minimum of 30 resident passerine birds on farms and control sites. Mist nets and Potter traps were set up immediately adjacent to chicken barns or enclosures, or within barns or enclosures when feasible. Physical examinations and sample collection were conducted as described above for chickens (except that swabs collected for future analysis were not taken in duplicate), and mass and wing chord length were recorded. All birds were marked with unique color-coded and numbered leg

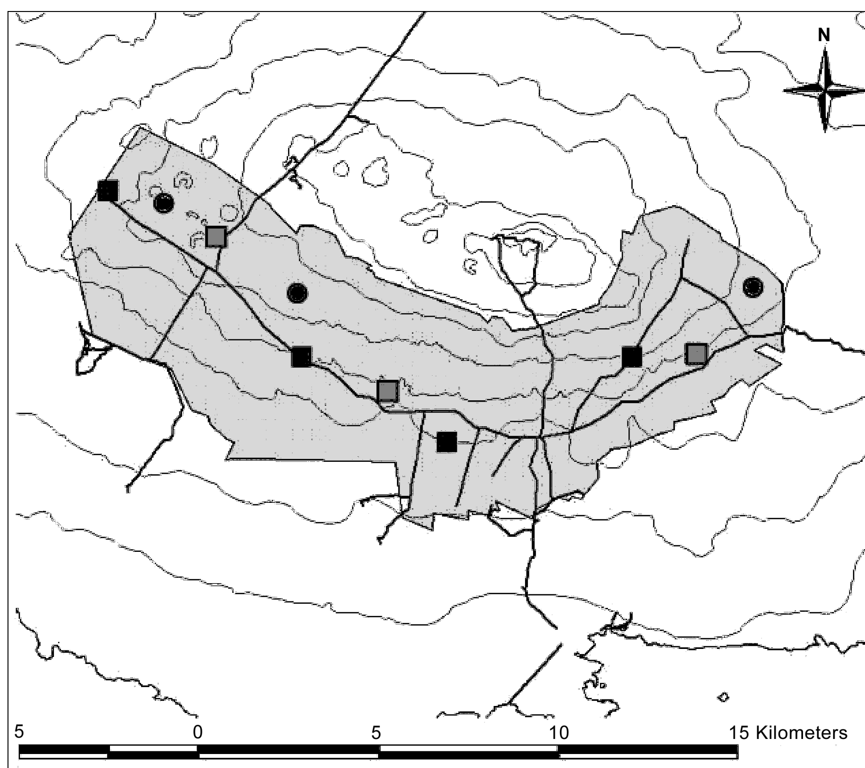

Fig. 1. Map of the agricultural zone (light gray area) on Isla Santa Cruz illustrating general location of farm and control sites selected for the present study. Filled squares are backyard flocks, dark gray squares are broiler farms, and filled circles are control sites. Each contour line represents a 100-m elevation (southernmost contour line is shoreline at 0 m). Solid black lines indicate road system.

bands. Because of the limited volume of blood that could safely be collected from the very small species (≤100 µL per 10 g body mass), analyses of sera from wild passerines were prioritized on the basis of initial screening of poultry serology.

Serological analyses.—Antibody titers to avian paramyxovirus serotype 1 (PMV-1, Newcastle disease virus), *Mycoplasma gallisepticum* (MG), infectious bursal disease virus (IBD), avian encephalomyelitis virus (AEV), avian reovirus, and infectious laryngotracheitis virus (ILT) were determined using enzyme-linked immunosorbent assays (ELISA). Given that ELISAs are specific for chicken sera, wild bird serum samples were tested for PMV-1 and MG using hemagglutination inhibition (HI) tests and for IBD using virus neutralization (VN) tests. Hemagglutination inhibition tests were employed to evaluate titers to infectious bronchitis virus, both Connecticut (IBV-Conn) and Massachussetts (IBV-Mass) strains. Exposure to avian influenza type A virus, group I avian adenovirus, and Marek's disease virus (MDV) were determined using agar gel precipitin tests (AGP), which yield positive or negative test results. Tube agglutination (TA) tests were used to evaluate exposure to *Salmonella typhimurium* and *S. pullorum.* Serological testing was done at the Poultry Diagnostic Research Center in Athens, Georgia.

Statistical analyses.—Multilevel modeling was used to account for the hierarchical structure of our data, with individual birds (level-1) clustered within farm sites (level-2), using MLWIN, version 1.10.0006 (Multilevel Models Project, Institute of Education, London; Rasbash et al. 2000). We used multilevel binomial logistic models to determine whether broiler and backyard chickens differed in their likelihood of (1) showing clinical signs of disease and (2) being seropositive to pathogens. Two-level random intercept models were employed to compare log-transformed antibody titers between broiler and backyard chickens. Similar binomial and continuous multilevel analyses were used to determine whether there were effects of sex and age on clinical disease and serological responses of backyard chickens only. For wild bird species with adequate sample sizes, two-level random intercept models were used to compare mass or body-condition index (residuals of mass over wing chord) among birds captured at broiler farms, backyard flocks, and control sites.

For binomial logistic models, the effects of explanatory variables were expressed as odds ratios (with 95% confidence limits), which were obtained by taking the natural antilog of the regression coefficients. Explanatory variables were tested for significance using the Wald test ($P < 0.05$). Multilevel modeling also allows one to estimate the contribution of each level of organization to the total variance of the explanatory variable of interest (Dohoo et al. 2001). For continuous two-level models, the variance partition coefficient (VPC) is level-2 variance divided by the total variance (Rasbash et al. 2000) and refers to the proportion of the total residual variation that is attributable to differences among farm sites. This

value is also interpreted as the intraclass correlation coefficient (ICC), which measures the degree of similarity among individuals within the same group (i.e., farm site) for the outcome of interest (Snijders and Bosker 1999, Rasbash et al. 2000). A high ICC (>0.20) is indicative of significant clustering among farms (Snijders and Bosker 1999). For binomial logistic models, the latent variable approach was used to estimate ICCs (Dohoo et al. 2001, 2003).

RESULTS

Chickens

Chickens sampled.—We sampled 210 chickens from three broiler and four backyard flocks. Chickens from broiler farms were four, seven, and eight weeks old. Exact ages of backyard chickens were unknown and were categorized as juvenile or adult. Of the 120 backyard chickens sampled, 89 (74.2%) were adult and 31 (25.8%) were juvenile. Furthermore, 87 (72.5%) of the backyard chickens were female and 33 (27.5%) were male.

Prevalence of clinical signs of disease.—Overall prevalence of clinical signs of disease in chickens was 19.0% (40/210). Of the clinical signs observed, respiratory signs such as nasal and choanal discharge, swollen sinuses, and open-mouthed breathing were most common (11.9%, 25/210). Ocular lesions (6.2%, 13/210) and cutaneous masses or tumors (2.9%, 6/210) were also observed. Clinical signs were more prevalent in backyard chickens (30.8%; $n = 120$) than in broiler chickens (3.3%; $n = 90$). Farm prevalence of clinical signs ranged from 13.3 to 40.0% in backyard chicken flocks and from 0 to 6.7% in broiler farms. Backyard chickens were 13.6× more likely than broilers to exhibit clinical signs (95% confidence limits [CL]: 3.3–56.6, $P = 0.0003$, $n = 210$). For this model, farm type explained 33.5% of the total variance of the null model and reduced ICC of the null model from 0.372 to 0.055. Hence, farm type accounted for most of the variation in clinical signs observed at the farm level. Of the backyard chickens, juveniles and subadults were 3.3× more likely than adults to show clinical signs (95% CL: 1.3–8.3, $\chi^2 = 6.56$, $P = 0.01$, $n = 120$) and 12.4× more likely to show respiratory signs (95% CL: 2.0–77.7, $\chi^2 = 7.189$, $P = 0.007$, $n = 120$). Male and female backyard chickens did not differ in prevalence of clinical signs ($\chi^2 = 0.828$, $P = 0.36$, $n = 120$). Ectoparasitism, ranging from mild to marked infestations of biting lice, was common in backyard chickens (72.5%, 87/120) but not observed in broilers (0/90). In backyard chickens, there was no effect of age ($\chi^2 = 0.922$, $P = 0.337$) or sex ($\chi^2 = 2.711$, $P = 0.10$) on likelihood of ectoparasitism. Ocular nematodes (*Oxyspirura* sp., likely *O. mansoni*) were identified in 2 of 120 (1.6%) backyard chickens but not in broilers.

Overall seroprevalence.—Overall seroprevalence data for the 13 pathogens tested are displayed in Table 1. All chickens were seronegative for avian influenza virus, *S. typhimurium,* and *S. pullorum.* Seroprevalence for all other pathogens ranged from 23.2 to 81.6% (Table 1).

Comparison of seroprevalence between backyard and broiler chickens.—There was no difference in seroprevalence between backyard and broiler chickens for PMV-1, IBD, AEV, and avian adenovirus group I (Table 2). Seroprevalence for IBD was 81.6%

TABLE 1. Seroprevalence of selected poultry pathogens in backyard flocks and broiler farms on Isla Santa Cruz, Galápagos, in July 2005.

	Backyard flocks					Broiler farms				
	1 ($n = 30$)	2 ($n = 30$)	3 ($n = 30$)	4 ($n = 29$)	Overall for backyard chickens ($n = 119$)	5 ($n = 29$)	6 ($n = 30$)	7 ($n = 29$)	Overall for broilers ($n = 88$)	Overall ($n = 207$)
Avian influenza virus	0	0	0	0	0	0	0	0	0	0
Mycoplasma gallisepticum	0.067	0.900	0.733	0.138	0.462	0	0.067	0.034	0.034	0.280
Avian paramyxovirus-I	0.433	0.967	0.067	0.310	0.445	0	0.600	0.483	0.364	0.411
Marek's disease virus	0.433	0.500	0.467	0.207	0.403	0	0	0	0	0.232
Infectious laryngotracheitis virus	0.300	0.600	0.600	0.448	0.487	0	0.033	0.241	0.091	0.319
Infectious bronchitis virus (Massachusetts)	0.933	0.967	0.690[a]	0.586	0.797[b]	0.241	0	0.069	0.102	0.500[c]
Infectious bronchitis virus (Connecticut)	0.933	0.933	0.700	0.759	0.832	0.069	0.033	0.034	0.045	0.498
Infectious bursal disease virus	0.933	1.000	0.767	0.828	0.882	0.207	0.967	1.000	0.727	0.816
Reovirus	0.433	0.833	0.867	0.828	0.739	0.034	0.367	0.138	0.182	0.502
Avian encephalomyelitis virus	0.467	0.900	0.133	0.241	0.437	0	0.133	0.793	0.307	0.382
Adenovirus	0.533	0.600	0.600	0.724	0.613	0.241	0.833	0.414	0.500	0.565
Salmonella typhimurium	0	0	0	0	0	0	0	0	0	0
S. pullorum	0	0	0	0	0	0	0	0	0	0

[a] $n = 29$.
[b] $n = 118$.
[c] $n = 206$.

Table 2. Summary of binomial multilevel models comparing likelihood of seroprevalence of pathogens between broiler and backyard chickens. For all models, reference category is broiler chickens.

	n	B	SE	Odds ratio[a]	Odds ratio 95% confidence limits: Lower	Odds ratio 95% confidence limits: Upper	P	Between-farm variance	Within-farm variance ($\pi^2/3$)[b]	Total variance of model	Model ICC/VPC	Total variance of null model	Null ICC/VPC	Percentage of total null variance explained by farm type
MG	207	3.302	1.621	27.2	1.1	651.4	0.042	3.544	3.29	6.834	0.519	8.804	0.626	22.4
PMV-1	207	0.482	2.135	1.6	0.0	106.3	0.821	5.993	3.29	9.283	0.646	8.313	0.604	0
ILT	207	2.716	0.868	15.1	2.8	82.9	0.0018	0.796	3.29	4.086	0.195	6.141	0.464	33.5
IBV-Mass	206	4.816	1.354	123.5	8.7	1754.3	0.0004	2.085	3.29	5.375	0.388	10.804	0.695	50.3
IBV-Conn	207	4.940	0.795	139.8	29.4	663.9	<0.0001	0.417	3.29	3.707	0.112	13.812	0.762	73.2
IBD	207	0.400	1.911	1.5	0.0	63.2	0.834	5.333	3.29	8.623	0.618	7.566	0.565	0
Reovirus	207	3.157	0.963	23.5	3.6	155.2	0.001	1.216	3.29	4.506	0.270	7.484	0.560	39.8
AEV	207	0.962	2.935	2.6	0.0	824.4	0.742	7.890	3.29	11.180	0.706	8.489	0.612	0
Adenovirus	207	0.500	0.723	1.6	0.4	6.8	0.489	0.729	3.29	4.019	0.181	3.911	0.159	0

Abbreviations: MG = *Mycoplasma gallisepticum*, PMV-1 = paramyxovirus-1, ILT = infectious laryngotracheitis virus, IBV-Mass = infectious bronchitis virus (Massachusetts strain), IBV-Conn = infectious bronchitis virus (Connecticut strain), IBD = infectious bursal disease virus, AEV = avian encephalomyelitis virus, ICC = intraclass coefficient, and VPC = variance partition coefficient.

[a]Odds ratios in this table represent how much more likely (order of magnitude) backyard chickens were to be seropositive for a particular pathogen than were broilers, for models that were statistically significant ($P < 0.05$). For instance, backyard chickens were 27.2× more likely to be seropositive for MG than broiler chickens ($P = 0.042$).

[b]The latent-variable method was used to estimate total variance and ICC; hence, values for these are not accurate estimates but merely approximations.

overall and was relatively high within each farm (Table 1). Seroprevalence for PMV-1 varied remarkably among farms, ranging from 0 to 60% in broiler farms and from 6.7 to 96.7% in backyard flocks (Table 1 and Fig. 2). Seroprevalence for AEV also varied among farms, ranging from 0 to 79.3% in broiler farms and from 13.3 to 90.0% in backyard flocks (Table 1 and Fig. 2). Backyard chickens were significantly more likely than broiler chickens to be seropositive for MG, ILT, IBV-Mass, IBV-Conn, and avian reovirus (Table 2). Odds ratios comparing magnitude of the differences were high, ranging from 15.1 (for ILT) to 139.8 (for IBV-Conn; Table 2). In these models, farm type explained 22.4–73.2% of the total variance of their corresponding null models (Table 2). Although the addition of farm type to the models markedly decreased farm-level variance, ICCs remained relatively high (11.2–51.9%; Table 2), which indicates residual variation at the farm level (i.e., clustering) not explained by farm type. Seroprevalence for MDV also was high in backyard chickens (20.7–50.0%) compared with broiler chickens, which were all seronegative (Table 1). There were no pathogens for which broiler chickens were more likely to be seropositive than backyard chickens (Table 2).

Comparison of serum antibody titers between backyard and broiler chickens.—In general, results of multilevel models on log-transformed serum titers paralleled the results of binomial models for seroprevalence data (details of models not shown). Serum titers for MG ($\chi^2 = 6.63$, $P = 0.01$, $n = 207$), ILT ($\chi^2 = 18.64$, $P < 0.0001$, $n = 207$), IBV-Mass ($\chi^2 = 38.9$, $P < 0.0001$, $n = 206$), IBV-Conn ($\chi^2 = 131.6$, $P < 0.0001$, $n = 207$), and reovirus ($\chi^2 = 21.3$, $P < 0.0001$, $n = 207$) were significantly higher in backyard chickens than in broiler chickens, but there was no difference between backyard and broiler chickens in antibody titers to PMV-1 ($\chi^2 = 0.113$, $P = 0.73$, $n = 207$), IBD ($\chi^2 = 0.845$, $P = 0.36$, $n = 207$), and AEV ($\chi^2 = 2.07$, $P = 0.15$, $n = 207$).

Effects of age and sex on serological responses in backyard chickens.—Among the backyard chickens, adults were 5.8× more likely than juveniles or subadults to be seropositive for PMV-1 (95% CL: 1.0–33.9, $\chi^2 = 3.889$, $P = 0.049$, $n = 119$), 4.6× more likely to be seropositive for ILT (95% CL: 1.8–12.0, $\chi^2 = 9.667$, $P = 0.0012$, $n = 119$), and 9.5× more likely to be seropositive for IBV-Mass (95% CL: 2.5–36.9, $\chi^2 = 10.608$, $P = 0.0013$, $n = 118$). There also was a higher tendency (not statistically significant) for adult chickens to be seropositive for MDV (OR = 2.4, 95% CL: 0.9–6.2, $\chi^2 = 3.327$, $P = 0.068$, $n = 119$) and reovirus (OR = 2.4, 95% CL: 0.9–6.5, $\chi^2 = 2.909$, $P = 0.088$, $n = 119$) compared with younger chickens. Adults had significantly higher antibody titers to MG ($\chi^2 = 3.92$, $P = 0.48$, $n = 119$), ILT ($\chi^2 = 14.26$, $P = 0.0002$, $n = 119$), IBV-Mass ($\chi^2 = 22.66$, $P < 0.0001$, $n = 118$), and IBV-Conn ($\chi^2 = 8.27$, $P = 0.004$, $n = 119$), and nearly significantly higher titers to PMV-1 ($\chi^2 = 3.69$, $P = 0.055$, $n = 119$), than juvenile and subadult chickens. Age of backyard chickens had no effect on likelihood of seropositivity for MG ($\chi^2 = 1.931$, $P = 0.17$, $n = 119$), IBV-Conn ($\chi^2 = 2.268$, $P = 0.13$, $n = 119$), IBD ($\chi^2 = 0.347$, $P = 0.56$, $n = 119$), AEV ($\chi^2 = 0.699$, $P = 0.40$, $n = 119$), or adenovirus ($\chi^2 = 1.657$, $P = 0.20$, $n = 119$), and no effect on serum titers for IBD ($\chi^2 = 0.665$, $P = 0.42$, $n = 119$), reovirus ($\chi^2 = 2.15$, $P = 0.14$, $n = 119$), and AEV ($\chi^2 = 0.213$, $P = 0.64$, $n = 119$). There was no effect of sex on likelihood of seropositivity or titers to any of the pathogens tested in backyard chickens (data not shown).

Wild Birds

Wild birds sampled.—We captured and examined 338 wild birds on three broiler farms, four backyard flocks, and three control sites (Table 3). We obtained serum samples from 236 of these birds. Unlike chickens, none of the wild birds exhibited overt clinical signs of disease. Small Ground-Finches (*Geospiza fuliginosa*) captured

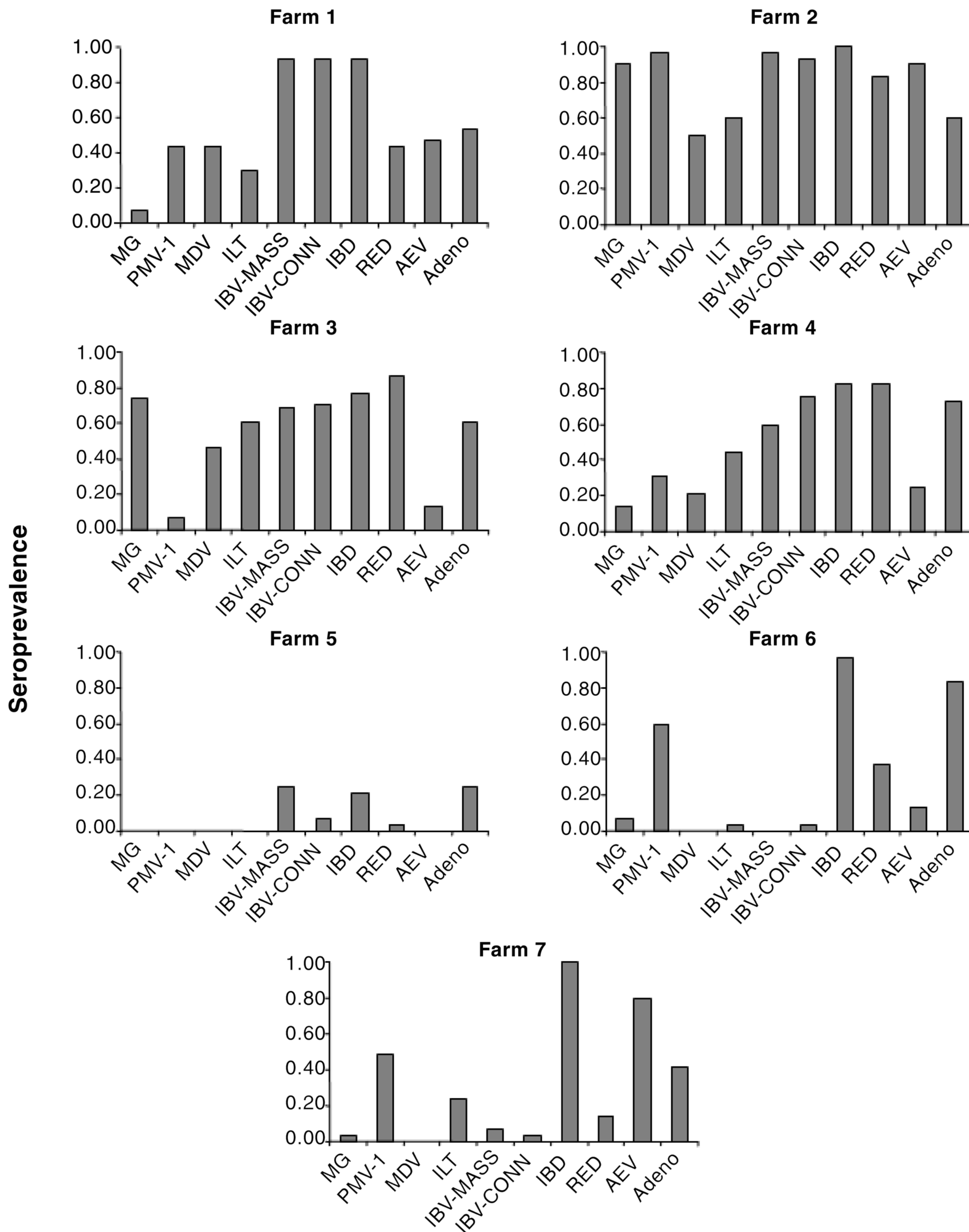

FIG. 2. Seroprevalence of pathogens displayed for each farm site. Farms 1 through 4 are backyard chicken flocks, and farms 5 through 7 are broiler farms. MG = Mycoplasma gallisepticum, PMV-1 = paramyxovirus-1, MDV = Marek's disease virus, ILT = infectious laryngotracheitis virus, IBV-Mass = infectious bronchitis virus (Massachussetts strain), IBV-Conn = infectious bronchitis virus (Connecticut strain), IBD = infectious bursal disease virus, REO = reovirus, AEV = avian encephalomyelitis virus, and Adeno = avian adenovirus.

Table 3. Numbers of individuals of wild bird species captured (with number sampled in parentheses) at control sites, broiler farms, and backyard flocks on Isla Santa Cruz, Galápagos, in July 2005.

Species	Control sites	Backyard flocks	Broiler farms	Total
Camarhynchus crassirostris	1 (1)	1 (1)	3 (3)	5 (5)
C. pallidus	0	2 (2)	1 (0)	3 (2)
C. parvulus	7 (5)	8 (6)	5 (3)	20 (14)
C. psittacula	0	1 (0)	0	1 (0)
Certhidea olivacea	0	0	2 (1)	2 (1)
Crotophaga ani	1 (1)	1 (1)	0	2 (2)
Dendroica petechia	8 (5)	16 (8)	14 (13)	38 (26)
Geospiza fortis	14 (11)	25 (22)	25 (23)	64 (56)
G. fuliginosa	64 (44)	69 (52)	61 (41)	194 (137)
Myiarchus magnirostris	2 (1)	0	0	2 (1)
Nesomimus parvulus	4 (4)	2 (2)	0	6 (6)
Zenaida galapagoensis	1 (0)	0	0	1 (0)
Total	102 (72)	125 (94)	111 (84)	338 (250)

near backyard flocks were significantly heavier (mean mass = 14.82 ± 1.12 [SD] g, $n = 69$; mean body-condition index = 0.32 ± 0.84, $n = 68$) and had significantly higher body-condition indices than those captured on broiler farms (mean mass = 14.06 ± 1.41 g, $n = 60$; mean body-condition index = –0.25 ± 1.08, $n = 60$) or control sites (mean mass = 14.26 ± 1.46 g, $n = 62$; mean body-condition index = –0.13 ± 1.0, $n = 60$), and there was no significant difference between Small Ground-Finches captured on broiler farms and those captured on control sites (multilevel model for mass: reference category = backyard farm finches, $b_{broiler} = -0.761 \pm 0.233$ [SE], $\chi^2 = 10.66$, $P = 0.001$; $b_{control} = -0.561 \pm 0.231$, $\chi^2 = 5.901$, $P = 0.015$, $n = 191$; multilevel model for condition index: $b_{broiler} = -0.572 \pm 0.173$, $\chi^2 = 10.956$, $P = 0.0009$; $b_{control} = -0.451 \pm 0.173$, $\chi^2 = 6.805$, $P = 0.009$, $n = 188$).

Seroprevalence.—Because of the tiny sample volumes obtained from small passerines, serological testing required pooling of samples. Sera from Small Ground-Finches were pooled by site for two broiler farms, two backyard flocks, and two control sites; samples collected from Small Ground-Finches at three remaining sites (including one control site, one broiler farm, and one backyard flock) were all pooled together. Samples from all other passerines were pooled together, which allowed us to test for a larger array of pathogens. Sera from Smooth-billed Anis (*Crotophaga ani*, an introduced species) were tested individually. Each serum pool of Small Ground-Finches was tested for PMV-1, MG, and IBD (except for the pool of combined sites, for which insufficient serum was available to test for PMV-1); there was sufficient serum from one backyard flock to additionally test for MDV, AIV, and adenovirus. The pool containing sera from all other passerines was tested for antibodies to PMV-1, MG, IBD, MDV, AIV, and adenovirus. Smooth-billed Anis were tested for exposure to all the above as well as *S. pullorum* and *S. typhimurium*. All serum samples from wild birds were seronegative for the pathogens tested.

Discussion

Investigation of poultry farms on Isla Santa Cruz revealed a high overall prevalence of clinical disease and evidence of exposure to numerous pathogens. Furthermore, compared with broiler chickens, backyard chickens were significantly more likely to show clinical signs of disease, be infected with ectoparasites, and be seropositive for most of the pathogens examined. This finding indicates that backyard chickens may pose a more significant and immediate threat of disease introduction to resident avifauna than broiler chickens.

Animals showing clinical signs of disease are more likely to be actively shedding the disease agent, for instance, through respiratory secretions or feces (Saif et al. 2003). Hence, wild birds are significantly more likely to come in contact with infectious material when foraging among or in the same area as backyard chickens than when foraging with broiler chickens.

Backyard and broiler chickens were seropositive for numerous pathogens, and overall pathogen seroprevalence was generally high. Serology results must be interpreted with caution, recognizing that a seropositive test reflects only that the animal had been exposed to the pathogen (or vaccine) at some point in its life and does not necessarily reflect current infection status (Saif et al. 2003, Wobeser 2006). Interpretation of serology results may be further complicated by the use of vaccines in chickens (Saif et al. 2003). Although vaccination of domestic animals is prohibited in the Galápagos Islands, day-old broiler chicks are commonly vaccinated for IBD, IBV, and MDV before being shipped to the Galápagos, and some hatcheries employ inactivated PMV-1 and IBV vaccines combined (M. Cisneros and F. Falconi, SESA, pers. comm.). Antibodies produced by day-old chicks in response to vaccines are generally short-lived, depending on the virus or vaccine (Saif et al. 2003), and are often undetectable by about four weeks of age (M. Cisneros pers. comm.); however, if live virus vaccines are employed, the vaccine virus may continue to circulate in the flock and give rise to persistent titers (Saif et al. 2003). Backyard chickens are unlikely to be vaccinated; hence, their antibody titers more likely reflect responses to field strains of viruses or bacteria circulating within the flock.

Preliminary serological results from wild birds sampled at broiler farms, backyard flocks, and control sites were negative. This could indicate that wild birds have not been exposed to poultry pathogens, which we consider unlikely given their proximity to backyard chickens and the high prevalences recorded in the chickens. It could also indicate that birds die when exposed to these pathogens and, therefore, were not available for capture during the investigation. It is more likely that prevalences in the wild bird population were too low for us to detect with the sample sizes we obtained. Other possible contributing factors include a dilution effect if pools contain few positive sera combined with many negative sera (Monzon et al. 1992, Maherchandani et al. 2004), as well as the possibility that the serological tests, which have been developed for use in chickens, are not valid in these wild bird species (Gardner et al. 1996, Wobeser 2006). Barring this last possibility, the absence of detectable antibodies in the wild birds suggests that wild birds are not reservoirs for these pathogens and are unlikely to be the main source of exposure for poultry.

Table 4. Routes of exposure and longevity for various poultry pathogens when outside the host.

Pathogen	Routes of exposure[a]	Survival outside host
Mycoplasma gallisepticum	R, M, V	Hours to days
Avian paramyxovirus-1 (Newcastle disease vrus)	R, I, M	Days to weeks
Marek's disease virus	R, C, MV	Months to years
Infectious laryngotracheitis virus	R, I, M	Days
Infectious bronchitis virus	R	Days (warm) to weeks (cold)
Infectious bursal disease	R, I, M, MV	Weeks to months (resistant to disinfectants and heat)
Reovirus	R, I	Stable; resistant to inactivation
Avian encephalomyelitis virus	I	Weeks; ≥4 weeks in feces
Adenovirus	R, I	Stable; resistant to inactivation and many disinfectants
Duck herpesvirus (duck plague)	I, M	Days
Haemophilus paragallinarum (infectious coryza)	R, M	Hours to days
Pasteurella multocida (fowl cholera)	R, M	Weeks
Avian influenza	R, I, M	Days (warm) to weeks (cold); 4 days in water at 22°C, >30 days at 4°C
Fowl poxvirus	R, M, C, MV	Months
Salmonella sp.	R, I, M	Weeks to months
Mycobacterium avium	R, I, M	Months to years
Coccidia sp.	I	Months

[a]Routes of exposure include inhalation–respiratory (R), ingestion (I), mucosal (M), cutaneous (C), vertical (V), and mechanical vector (MV; e.g., arthropod). Information was compiled from Charlton (2000), Saif et al.(2003), and websites of the Centre for Infectious Disease Research and Policy, University of Minnesota (www.cidrap.umn.edu/cidrap/content/influenza/avianflu/biofacts/avflu.html) and the University of California, Davis, Veterinary Medicine Teaching and Research Center (animalscience.ucdavis.edu/Avian/pfs26.htm).

We attribute the striking differences in clinical signs and seropositivity between backyard and broiler chickens to differences in management practices used. Broiler farms use an "all-in, all-out" process in which day-old chicks are imported from mainland Ecuador and reared together in the same barn or enclosure until they are slaughtered, usually around eight weeks of age. To break the cycle of infection and transmission, enclosures are then cleaned, disinfected, and left empty for ~15 days before new chicks are brought in for the next rearing cycle (D. Arana, SESA-Galápagos, pers. comm.). In backyard flocks, biosecurity measures are rarely employed, flocks are often composed of a mixture of ages, breeds, and species, and new individuals are continually added; thus, pathogens transmitted to backyard chickens are more likely to persist and circulate within the population and local environment (see Table 4).

The larger age range in backyard flocks than in broiler flocks (in which all birds are the same age) may also explain the higher seroprevalences and titers in backyard chickens. Because most of the backyard chickens we sampled were adults, they were older, on average, than the broiler chickens sampled. Older chickens from backyard flocks generally have higher seroprevalence and titers—and, hence, resistance to pathogens circulating within the flock—because of continued or multiple exposures to the same pathogens (Saif et al. 2003). Our results were consistent with this explanation: adult backyard chickens were more likely than subadult chickens to be seropositive and have higher titers to several pathogens, and less likely to show clinical signs.

For some of the serological tests, there was considerable residual variation among farms, even after farm type was accounted for. This was likely caused by the differences in management practices among farms as well as the general differences between broiler and backyard flocks, such as barn design, vaccination protocols, biosecurity practices, water availability, and previous history of disease. Environmental factors such as geographic area, altitude, climate, precipitation, vegetation, and arthropod or other vectors may also contribute to variation among farm sites.

Management practices that result in high disease prevalence and exposure in backyard chickens increase the risk of disease transmission between chickens and wild birds. This risk is amplified by the larger poultry–wildlife interface associated with backyard flocks, in which chickens are allowed to range freely in areas used by wild birds. Finches often feed alongside backyard chickens (Fig. 3A), which increases their risk of exposure to infectious material (e.g., respiratory secretions, saliva, feces, urates); this emphasizes the potential for "pathogen pollution" (Daszak et al. 2000) at backyard flocks. Consistent with this observation is our finding that Small Ground-Finches captured near backyard flocks were significantly heavier, and had higher condition indices, than those captured at broiler farms and control sites. This is likely attributable to increased foraging opportunities on feed provided outdoors to backyard chickens.

Because broiler chickens are housed indoors, the poultry–wildlife interface on broiler farms is not as large as it is for free-ranging chickens. However, it is common for wild birds, especially Small Ground-Finches and Yellow Warblers (*Dendroica petechia*), to enter enclosures through holes or through walls constructed with chain-link fences. We commonly observed wild finches and warblers at feeders and water troughs within broiler barns (Fig. 3B), and we trapped numerous wild birds within barns or enclosures during the study. Furthermore, many broiler farms have backyard chickens on the property, so bidirectional transmission between broiler and backyard chickens is possible. Poultry litter from broiler farms is used as fertilizer in agricultural fields and, if improperly composted, may be another source of infectious pathogens to wild birds foraging in these fields, because many pathogens can survive in the environment for days to years (Table 4). Thus,

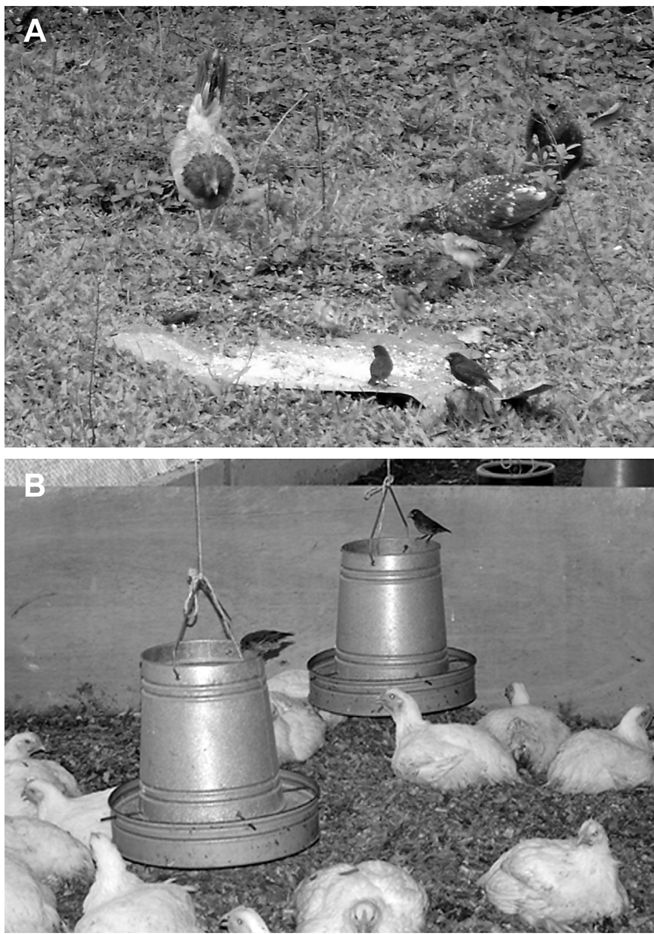

Fig. 3. Photographs illustrating the poultry–wildlife interface (A) near backyard flocks and (B) on broiler farms (photographs by C. Soos and M. Cruz).

although broiler chickens may be less likely to directly transmit infectious diseases to wild birds, several potential routes exist for indirect transmission (Table 4).

An additional risk is the potential for introduction of new diseases through the importation of day-old broiler chicks from the mainland. Currently, more than 140,000 day-old chicks are imported from the mainland each year, presumably from producers approved by SESA. Poultry operations authorized to export chickens to the Galápagos employ rigorous biosecurity procedures including shower-in, shower-out protocols. If the proper documentation from an authorized source is attached to crates containing chickens (often shipped through a third party; M. Cisneros pers. comm.), inspection of crates does not take place before shipment or upon arrival in the Galápagos (D. Arana, SESA-Galápagos, pers. comm.). Stressors associated with transport (e.g., inadequate temperatures; lack of food, water, and light; close confinement of chicks within crates) can cause immunosuppression and subsequent shedding of infectious pathogens, with rapid transmission among birds (Dohms and Metz 1991). Because imported crates are not routinely checked for sick or dead individuals and no quarantine or necropsy protocols are in place for imported chickens, new diseases may enter the Galápagos undetected through the importation of chickens from mainland Ecuador. The Asian strain of H5N1 avian influenza is but one example of an infectious disease that spilled over into wild bird populations from domestic poultry and was introduced into some countries because of inadequate quarantine procedures and poor veterinary infrastructure (Kilpatrick et al. 2006, Gauthier-Clerc et al. 2007, Karesh et al. 2007).

Consequences for disease emergence in wild populations: Pathogens of concern.—Implications of emergence of poultry pathogens into populations of wild birds in the Galápagos have been discussed in detail by Gottdenker et al. (2005). Some of these pathogens could strongly affect wild avian populations in the Galápagos, should spillover occur. Results from our preliminary serosurvey of apparently healthy wild passerines support previous studies that have demonstrated minimal to no prevalence of poultry pathogens in native Galápagos birds such as Galápagos Penguin (*Spheniscus mendiculus*), Flightless Cormorant (*Phalacrocorax harrisi*), Waved Albatross (*Phoebastria irrorata*), and Galápagos Dove (*Zenaida galapagoensis*) (Padilla et al. 2003, 2004; Travis et al. 2006a, 2006b). Furthermore, an ongoing dead-bird surveillance program, which was initiated in the Galápagos in 2003, has examined hundreds of wild bird carcasses (Gottdenker et al. 2008, St. Louis Zoo and University of Missouri–St. Louis Galápagos wildlife necropsy database) and has not detected the infectious pathogens investigated in the present study. The above studies indicate that Galápagos bird species are immunologically naive to poultry pathogens and, thus, may have a poor ability to cope with the introduction of these pathogens.

PMV-1 and MG are pathogens of immediate concern, and although they are highly prevalent in captive chicken populations, they have not been detected in any of the above-mentioned disease surveillance programs, which suggests that they are not endemic in wild bird populations and that, perhaps, they have not yet spilled over into wildlife. These pathogens are of particular concern because they have wide host ranges, with moderate to high epizootic potential based on the ability to spread rapidly, causing large-scale damage to wildlife in a short period, depending on strain and host susceptibility (Wobeser et al. 1993, Banerjee et al. 1994, Nolan et al. 1998, Kuiken 1999, Hochachka and Dhondt 2000, Hartup et al 2001, Bengis et al 2002). PMV-1 has caused high morbidity and mortality in populations of wild birds (e.g., Double-crested Cormorant (*Phalacrocorax auritus*); Bannerjee et al. 1994, Kuiken 1999) and has the potential to severely affect small populations of susceptible Galápagos species (e.g., Flightless Cormorant). The emergence of a novel strain of MG that causes chronic debilitating disease resulted in major population declines of House Finches (*Carpodacus mexicanus*) in North America (Nolan et al. 1998, Dhondt et al. 2005, Sydenstricker et al. 2006). Diseases with sublethal effects that depress reproductive output are capable of causing population limitation or regulation (Hudson et al. 1998) and may have serious consequences for small island populations in the long term.

Our results suggest that interactions at the poultry–wildlife interface may carry a risk to native and endemic birds in the Galápagos Islands and that backyard chickens have the potential to pose a more significant threat than broiler chickens. Further information on the degree of infectiousness of chickens (e.g., using

isolation or molecular techniques to identify the true prevalence of infectious pathogens) and the degree of contact between chickens and wild birds, or estimates of species composition and wild-bird densities around farms, would help us evaluate the relative risks of farm types more accurately. Furthermore, all samples were collected in July; there may be seasonal differences in clinical signs or seroprevalence that could affect the relationships observed here. Continued surveillance of avian diseases in both domestic and wild birds will allow us to identify patterns of disease emergence and to prioritize infectious-disease threats at the poultry–wildlife interface.

If introduced into wildlife populations, poultry pathogens will be difficult to control or eradicate because control measures for diseases in wild populations often entail culling and vaccination procedures (Wobeser 2002), which are likely to be considered socially, politically, and economically infeasible in the Galápagos Islands. Hence, it is urgent that proactive strategies be evaluated and implemented to prevent the spread of poultry diseases to wild birds. Primary objectives of a disease prevention plan must include minimizing the poultry–wildlife interface in the Galápagos Islands; reducing the prevalence of disease in poultry farms, with an emphasis on backyard flocks; and preventing introduction of poultry diseases into the Galápagos Islands. Improving farm biosecurity, eliminating or decreasing the numbers of free-ranging chickens, improving veterinary infrastructure, and modifying importation regulations and protocols must be carefully considered to achieve these goals. Development of management plans and regulations should involve farmers as well as SESA, Ministry of Agriculture and Livestock (Ministerio de Agricultura y Ganaderia), Ministry of Environment (Ministerio de Ambiente), Galápagos National Park, Charles Darwin Research Station, local veterinarians, poultry pathologists or specialists, wildlife disease specialists, and other local and international experts. It is important that a stronger, more integrated approach be taken toward importation and poultry practices in the Galápagos Islands. If the Galápagos Islands are to maintain their unique biodiversity of avian species, preventive measures must be taken to protect them from introduction of an increasing array of pathogens.

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