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Source: Avian Diseases, 52(4):558-566. 2008.
Published By: American Association of Avian Pathologists
DOI: http://dx.doi.org/10.1637/8298-032808-Reg.1
URL: http://www.bioone.org/doi/full/10.1637/8298-032808-Reg.1

AVIAN DISEASES 52:558–566, 2008

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Received 31 March 2008; Accepted and published ahead of print 4 June 2008

SUMMARY. Pathogens of free-ranging chickens create a risk of disease for wild birds, some of which migrate to the United States, as well as potential economic losses for resource-poor farmers. Free-roaming backyard chickens are commonly kept in shade-grown coffee plantations, habitats that attract large numbers of wild birds. The husbandry and pathogen prevalence of backyard chicken flocks in San Luis, Costa Rica, were investigated. Based on serologic evidence, Newcastle disease virus, infectious laryngotracheitis virus, infectious bronchitis virus, chicken anemia virus, and infectious bursal disease virus, as well as both *Mycoplasma gallisepticum* and *Mycoplasma synoviae*, appear to be significant diseases of this population, and thus, we consider these backyard chickens potential reservoirs for these diseases. There was no evidence of avian influenza. Interviews, clinical examinations, and microscopic examination of tissues led us to believe that poxvirus is also a significant cause of morbidity and mortality in these chickens. We found that *Escherichia coli* isolates were resistant against tilmicosin, tetracycline, ampicillin, amoxicillin with clavulanic acid, ticarcillin, and cephalothin, and contained genes considered responsible for conferring tetracycline resistance. Additionally, although production was not measured, we suspect that husbandry and lack of preventative medicine are directly related to the diseases reported, all of which negatively affect production.

RESUMEN. Las parvadas de aves de traspatio representan un riesgo de enfermedad para las aves tropicales en Costa Rica.

Los patógenos de las aves de traspatio generan un riesgo de enfermedad para las aves silvestres, algunas de las cuales migran a los Estados Unidos, generando a su vez potenciales pérdidas económicas para granjeros de escasos recursos. Las aves de traspatio criadas a la intemperie son comúnmente mantenidas en plantaciones de café con abundante sombra, un habitat que atrae un gran número de aves silvestres. En San Luis, Costa Rica, se investigó el manejo y la prevalencia de patógenos en aves de traspatio. Basado en evidencia serológica, los virus de la enfermedad de Newcastle, laringotraqueitis infecciosa, bronquitis infecciosa, anemia infecciosa aviar y enfermedad infecciosa de la bolsa, así como el *Mycoplasma gallisepticum* y el *Mycoplasma synoviae*, son agentes causantes de enfermedades en esta población y en consecuencia se consideran a estas aves de traspatio como reservorios potenciales de estas enfermedades. No se encontró evidencia de influenza aviar. Entrevistas, exámenes clínicos y evaluaciones microscópicas de tejidos nos llevan a creer que el virus de la viruela aviar es también una causa significativa de morbilidad y mortalidad en estas aves. Se demostró que los aislamientos de *Escherichia coli* eran resistentes a la tilmicosina, tetraciclina, ampicilina, amoxiciclina y ácido clavulánico, ticarciclina, cefalocina y contenían genes considerados responsables de conferir la resistencia a la tetraciciclina. Adicionalmente, aunque no se midió la producción, se sospecha que las prácticas de manejo y la falta de medicina preventiva están directamente relacionadas con las enfermedades reportadas, todas capaces de afectar negativamente la producción.

Key words: husbandry, free-ranging, backyard chickens, wild birds, pathogens, antimicrobial resistance, Costa Rica

Abbreviations: AE = avian encephalomyelitis virus; AI = avian influenza; AMRP = antimicrobial resistance profile; APV = avian pneumovirus; CAV = chicken anemia virus; ELISA = enzyme-linked immunosorbent assay; IBD = infectious bursal disease; IBV = avian infectious bronchitis; ILT = infectious laryngotracheitis; MAG = Costa Rican Ministry of Agriculture; MIC = minimum inhibitory concentrations; MG = *Mycoplasma gallisepticum*; MS = *Mycoplasma synoviae*; NDV = Newcastle disease virus; PDRC = Population Diagnostic Research Center; PPMV = pigeon paramyxovirus; RT-PCR = real-time polymerase chain reaction; S/N ratio = signal-to-noise ratio; USDA = U.S. Department of Agriculture

In 2001, Friend (23) detailed examples where recent disease emergence has had significant effects on wild bird populations and made a plea to avian and conservation communities to support more proactive, comprehensive examinations of risk factors that could affect further emergence of diseases. In response to his recommendations, we aim to understand how specific human-altered systems and introduced avifauna can affect disease dynamics for wild birds. Costa Rica is one of the hot spots of the world's biodiversity (54). The highlands of the Republic of Costa Rica harbor the greatest avian species richness in Central American montane forests and one of the

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highest levels of avian endemism in the world (35). The Monteverde region, in the northwestern part of the country, is the second top ecotourist destination, attracting visitors specifically seeking its natural beauty and rich avian biodiversity (10). However, outside of protected reserves, the landscape continues to be deforested for agricultural use, an activity that threatens the status of neotropic migrants and resident avifauna in a variety of ways. Preservation of avian biodiversity in this region should, therefore, be a priority, both from an economic and conservation standpoint. One conservation incentive heavily promoted is the creation of shade-coffee parcels, considered forest-surrogate habitat, as they provide floristically and structurally diverse habitat, positively affecting avian biodiversity. Nevertheless, they might also pose disease risks by artificially concentrating and aggregating birds to areas rich in food resources and exposing them to invasive species—such as the domestic chicken. We hypothesized that free-ranging backyard chickens could serve as disease reservoirs for susceptible wild bird populations.

Backyard chicken flocks have begun to receive attention because of their role in the epidemiology of avian influenza in Asian countries and are currently being closely scrutinized in many countries (6,8,70,71,75). Additionally, game chicken flocks have been involved in outbreaks of economically significant diseases, such as Newcastle disease, leading to the slaughter of many birds and to expensive biosecurity efforts (11,27). Although the export of poultry products by Central American countries is small, and mostly confined to trade within the region, Costa Rica is Central America's principal poultry exporter and is currently marketing to the United States, explaining Costa Rica's efforts to be declared Newcastle disease–free (73). Backyard flocks can act as potential reservoirs for diseases that can affect commercial poultry operations, especially of diseases that have become rare in these operations (40,75). A recent review by the U.S. Department of Agriculture (USDA) has recommended examining backyard chicken flocks near commercial operations more closely (52). Despite disease concerns and typical maintenance of backyard chicken flocks similar to those in this study across the developing world, there is a paucity of published information regarding the pathogen prevalence and diversity of backyard chickens, particularly in Latin America (31,33,36,42,60,62). According to the Costa Rican Poultry Association (Musmanni, pers. comm.), large commercial poultry operations are well established in Costa Rica, but backyard flocks are still very common, with a majority of families in rural areas primarily dependent on poultry for their sustenance. Poor preventative medicine negatively affects production, and information on the health and disease status of backyard chicken flocks is needed to generate recommendations that benefit rural communities.

Here, we present the results of a disease survey conducted with the objective of determining whether free-ranging backyard chickens inhabiting shade-grown coffee parcels pose a source of pathogens for wild birds that share these habitats. In addition, we aimed at determining the baseline antimicrobial resistance pattern of fecal bacterial isolates as a model for studying microbial distribution patterns and transmission in this habitat. During the course of the study, it became obvious that the health and disease prevalence of backyard chickens is also important to people participating in sustainable agroforestry incentives and to nearby commercial poultry operations.

MATERIALS AND METHODS

Study area. The study took place in the town of San Luis (10°16′57.117″N, 84°47′53.747″W), 7 km southwest of the well-known Monteverde region in northwestern Costa Rica, which housed approximately 60 rural families. The landscape of this region comprises a small residential area, a cooperative farm that contains shade-grown coffee plantations, pasture, and a mixture of primary and secondary tropical, premontane forest fragments. The flocks selected for this study were either located within shade-grown coffee plantations or were immediately adjacent to such plantations (Fig. 1).

Study subjects, interviews, and examinations. The 151 chickens (*Gallus domesticus*) from 13 flocks were randomly captured for physical examination and biologic sample collection during three separate time periods in July 2005, November 2005, and February 2007. All chicken owners were interviewed in Spanish during the sampling procedure by the one of us (S. M. H.-D.).

A physical exam was performed by palpation of the pectoral musculature to gauge body condition (scored on a scale of 1 to 5: 1 = emaciated, 2 = thin, 3 = ideal, 4 = overweight, 5 = obese), the presence and degree of mite infestation was subjectively scored (as low = <50 mites/wing, moderate = 50–150 mites/wing, or severe = >200 mites/wing), and any visible abnormalities were noted. If animals had a body condition of 2/5 or less, showed severe levels of mites, or had any other physical abnormalities or clinical signs, they were considered *abnormal*.

Biologic sample collection and disease surveillance. Blood was collected from 151 chickens. A thin blood smear was made immediately, dried, stained with Wright's stain, and examined for the presence or absence of hemoparasites (69). At least five fresh fecal samples were collected from each flock, preserved in a 2.5% potassium dichromate solution, and examined microscopically, first directly, and subsequently, by standard flotation technique with Sheather sugar solution (69). Ectoparasites were collected and stored in 70% ethanol for later identification using morphologic characteristics. Serum was collected and maintained at −80 C until processing. Serum samples were tested using a commercial enzyme-linked immunosorbent assay (ELISA; IDEXX Inc., Westbrook, ME) for avian pneumovirus (APV), infectious laryngotracheitis (ILT), infectious bursal disease (IBD), avian encephalomyelitis virus (AE), chicken anemia virus (CAV), Newcastle disease virus (NDV), avian influenza (AI), infectious bronchitis virus (IBV), *Mycoplasma gallisepticum*, (MG), and *Mycoplasma synoviae* (MS) at the School of Veterinary Medicine, Universidad Nacional de Costa Rica (Heredia, Costa Rica), or at the University of Georgia, Poultry Diagnostic and Research Center (PDRC; Athens, GA).

Choanal swabs were collected from 21 birds for MG and/or MS nucleic acid detection. DNA was extracted using a commercial available QIAamp Mini Kit (Qiagen, Valencia, CA), following the manufacturer's recommendations. The DNA was frozen at −70 C until processing. MG DNA was detected with a Real-Time TaqMan® polymerase chain reaction (RT-PCR), and MS DNA was detected with a PCR assay described by Lauerman *et al.* (7,46)

Cloacal swabs from nine birds from three flocks were collected and prepared for virus isolation by inoculating chickens embryos. The allantoic fluid was tested for hemagglutinating activity, and the samples were placed on FTA® classic cards (Whatman International Ltd, Kent, U.K.) for the molecular detection of Newcastle disease virus, as previously described (58).

Fresh fecal samples were collected, and fecal bacteria was propagated on MacConkey media plates and incubated at 37 C for 12–18 hr. Individual colonies were introduced into stable storage media (0.2% tryptone, 0.02% yeast extract, and 0.5% agar in distilled water) and maintained at 4 C until export. Once in the United States, bacteria was streaked for reisolation onto both blood and MacConkey agar plates and incubated at 35 ± 2 C for 12–18 hr to confirm purity. Bacterial identification was done through standard biochemical reactions (triple sugar iron, motility, indole, ornithine, oxidase, and citrate) or with commercially available *Enterobacteriaceae* identification strips (API 20E; bioMérieux USA, Durham, NC). Minimum inhibitory concentrations (MIC) were determined using TREK diagnostic system plates (Trek Diagnostics, Cleveland, OH), following the manufacturer's instructions (9), for MIC plates containing a series of titrations of 13 different antibiotics. Resistance breakpoints were determined based on previously published data (55). Whole-cell templates were made of pure culture stock of lactose fermenters (64). PCR was used to identify the samples that contained drug-resistant genes, such as the class I integron (*int1*)

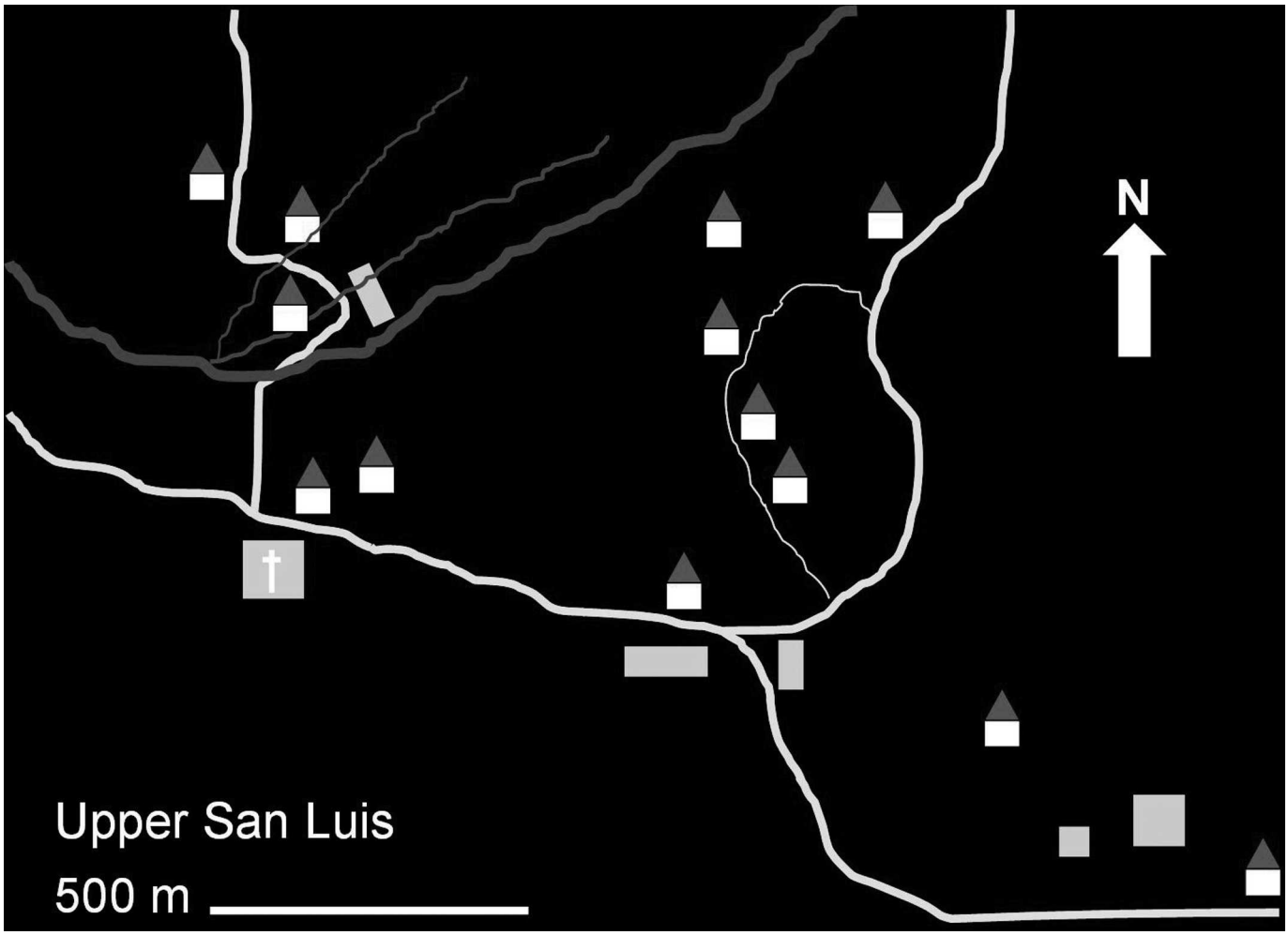

Fig. 1. A survey of the diseases of backyard flocks in the town of San Luis, Costa Rica was conducted. The relative location of the flocks sampled is represented by the houses of the parcels inhabited by the flocks. For orientation, the town's roads (white) and rivers (grey), as well as the cemetery (cross), are depicted.

and *tet*(A) and *tet*(B), as previously described (3,29,56). A list of the primers used is summarized in Table 1. Fourteen chickens, which belonged to 12 owners, were examined and humanely euthanatized. A gross necropsy was performed immediately after euthanasia. For each animal, a representative sample of all organs was collected and preserved in 10% buffered formalin until examination.

All biologic samples were prepared for importation following USDA guidelines for pathogen inactivation and were imported into the United States under a USDA import permit and a Ministry of Agriculture of Costa Rica (MAG) export permit. All of the work was approved by the University of Georgia's Institutional Animal Care and Use Committee.

RESULTS

Interviews and examinations. Chickens were the responsibility of the women in the household, and they were maintained primarily for personal use of the meat and eggs and for breeding. Five flock owners indicated they ''sometimes'' obtained some chickens from the MAG. Chickens distributed by MAG are vaccinated against NDV, IBD, IBV, and pox virus. Chickens foraged within the boundaries of the owner's properties but frequently entered nearby farms or forest fragments throughout the day and were penned in rustic coops at night. The chickens primarily foraged for food but were also provided with kitchen scraps, crops (i.e., bananas), cracked corn, and commercial poultry rations, which did not contain antimicrobials or coccidiostats. None of the owners routinely vaccinated their chickens.

Owners reported an average 5%–20% mortality rate of chicks during the first 4 wk, primarily from diarrhea and respiratory signs, and an annual mortality rate of 0%–17% in their adult birds. The disease syndromes owners perceived as the most important and that caused mortality in the flocks were a Newcastle disease–like

Table 1. Genotypic antimicrobial resistance for *Enterobacteriaceae* isolated from fecal samples was determined from backyard chickens in Costa Rica. Genotypic antimicrobial resistance was determined by testing for *int1*, *tet*(A) and *tet*(B).[A] The primer sequences used are listed herein.

Gene	Forward primer	Reverse primer	Reference
Int1	5′-CCT CCC GCA CGA TGA-3′	5′-TCC ACG CAT CGT CAG GC-3′	3, 29
tet(A)	5′-GCT ACA TCC TGC TTG CCT TC-3′	5′-CAT AGA TCG CCG TGA AGA GC-3′	5, 56
tet(B)	5′-TTG GTT AGG GGC AAG TTT TG-3′	5′-GTA ATG GGC CAA TAA CAC CG-3′	56

[A]From Integrated DNA Technologies, Coralville, IA.

Table 2. ELISA antibody titers for NDV, ILT, IBV, and APV from backyard chickens in Costa Rica.

Virus	No. of samples with titer				Total no. tested
	<1000	1000–1999	2000–3999	>4000	
NDV	99	19	19	14	151
IBV	95	11	13	9	128
ILT	25	38	15	12	90
APV	39	44	6	1	90

syndrome, fowl pox, respiratory disease, and an undetermined acute, anemia syndrome. Upon examination, the percentage of abnormal physical findings in the chickens from each flock varied from 43% to 99%. Abnormalities found were severe mite infestations (42%; range, 28%–100%); thin body conditions (67%; range, 14%–100%); respiratory signs (13%; range, 0%–25%); evidence of current or previous pox lesions on head, face, or legs (1%); loss of normal leg scales (100% in animals >2 yr of age).

Disease surveillance. The results of tests for NDV, ILT, IBV, and APV and for MG, and MS are presented in Tables 2, 3, respectively. Of the 128 chickens tested, 72 had a signal-to-noise (S/N) ratio of 0.029–0.700 and were considered positive for CAV, whereas 56 had an S/N ratio between 0.700 and 0.921 and were considered negative. Of the 151 ELISA extended-range titers acquired for IBD, 33 were <1000, 17 were between 1000 and 1999, 41 fell between 2000 and 3999, and 60 were >4000. Interpretation of antibody titers depends on many factors, such as type of vaccine (e.g., live *vs.* inactivated), level of challenge in the field, and host immune status. Based on previous experience with the clinical significance of antibody titers in poultry, and the test manufacturer's recommendations, we grouped the titer results into categories such that <1000 was considered negative or very low, 1000–2000 was low to moderate, 2000–4000 was considered high, and >4000 was very high. Additionally, 38 birds were tested against AE, of which, 13 showed antibodies above the manufacturer's threshold against AE (34%); however, only two individuals (5%) from two separate flocks had titers >1500. Of the 118 birds tested for the presence of antibodies against AI, 12 (10%) showed the presence of antibody ranges above the manufacturer-recommended threshold for seropositivity (range, 26–1495). None of these were confirmed positive by agar gel immunodiffusion. The hemagglutination test performed with the allantoic fluid obtained from embryos after three passages was negative for NDV. Additionally, paramyxovirus nucleic acid was not detected *via* PCR in any of the samples tested. Twenty-one birds from six flocks were tested via RT-PCR for MG, which was detected in six samples (29%), and of the 21 birds tested by regular PCR for MS, it was detected in 14 (67%) of the samples.

No hemoparasites were found in the 96 blood smears examined. Direct and flotation techniques on 65 fecal samples from 13 flocks yielded 1) *Eimeria* oocysts (*Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunette*, and *Eimeria necatrix*) in 46% of samples; 2) nematode ova, identified as capillarids (54%), *Dispharynx* sp. (8%), and *Ascaridia galli* (20%); and 3) cestode eggs consistent with *Raillietina* sp. (42%). More than one species of parasite was present in 86% of the samples examined. Two species of mites collected were identified as a wing mite, *Pterolichus obtusus*, (Robin; family Pterolichidae), and a body mite, *Megninia cubitalis* (Megnin; family Analgidae). The lice collected were the shaft louse, *Menopon gallinae* (Linnaeus; family Menoponidae); a wing louse, *Lipeurus caponis* (Linnaeus; family Philopteridae); and *Oxylipeurus dentatus* (Sugimoto; family Philopteridae).

Table 3. ELISA antibody titers for MG and MS from backyard chickens in Costa Rica.

Mycoplasma sp.	No. of samples with titer				Total no. tested
	<1000	1000–1999	2000–3999	>4000	
MG	95	9	14	29	147
MS	48	8	14	58	128

Of the fecal samples collected, only those that yielded bacterial isolates that were lactose fermenters were analyzed; therefore, the results discussed are from 48 isolates. The majority of lactose fermenter isolates were identified as *Escherichia coli* (91.6%), and the remaining isolates were other genera in the group *Enterobacteriaceae* (8.4%). The antibiotic-resistance profile and the prevalence of gene presence are represented on Tables 4, 5. Specifically, the presence of resistance-conferring genes in the 18 samples that displayed phenotypic resistance against tetracycline, are represented on Table 5. Also of significance, 14/44 (32%) *E. coli* isolates displayed intermediate resistance to cephalothin (16 μg/ml), 27/44 (61%) to florfenicol (4 μg/ml), 4/44 (9%) to difloxacin, 2/44 (5%) to orbifloxacin (2–4 μg/ml), 41/44 (93%) displayed the lowest susceptibility for ceftiofur (≤2 μg/ml), 26/44 (59%) to amoxicillin with clavulanic acid (≤4:2 μg/ml), and 15/44 (34%) to cephalothin (≤8 μg/ml).

Fourteen chickens from 12 owners were submitted to complete pathology examinations. In general, all animals were found to have moderate-to-severe ectoparasite infestations (mites, lice), evidence of mild-to-moderate endoparasitism, and poor body condition, and all birds older than 1 yr of age were found to have moderate-to-severe dermatitis of the lower legs from *Knemidocoptes* sp. infestations. Parasites found during gross necropsy and microscopic examinations were consistent with *Capillaria* spp., *Ascaridia* spp., *Heterakis* spp., protozoal organisms consistent with *Eimeria necatrix*, and flagellates consistent with *Tetratrichomonas gallinarum*. Significant gross and microscopic findings included 1) subcutaneous heterophilic granulomas containing branching hyphae, of which, pure, heavy growth of *Aspergillus clavatus*, *Aspergillus flavus*, and *Aspergillus fumigatus* was cultured ($n = 2$); 2) hyperplastic epithelium with ballooning degeneration and large eosinophilic intracytoplasmic inclusions consistent with poxvirus ($n = 2$); 3) lymphocytic sinusitis highly suggestive of MG ($n = 1$); 4) splenic lymphoid atrophy, suggestive of immunosuppressive agents ($n = 2$); 5) ovarian adenocarcinoma ($n = 1$); 6) anthracosis ($n = 10$); and 7) lymphocytic myocarditis and epicarditis, suggestive of reovirus ($n = 2$). Minor findings included 1) protozoal typhlitis ($n = 4$), and 2) mild-to-moderate parasitic enteritis ($n = 8$).

DISCUSSION

Free-roaming chickens are at a disadvantage compared with commercial poultry for maintaining their health because they do not receive vaccinations nor are they afforded treatments typically applied to commercial poultry. These chickens are on a poor plane of nutrition and run in flocks of mixed ages, placing susceptible younger chicks in contact with adults that are potential reservoirs of disease. Additionally, most commercial poultry breeder flocks are maintained free of certain infectious diseases that can be transmitted from the hen to progeny. It is likely that the diseases to which these chickens are exposed, both singly and in combination, are responsible for the high mortality among the young and the potentially decreased reproductive success. On the other hand, their

Table 4. The antibiotic susceptibility pattern, as determined by MIC, of 48 isolates of commensal fecal *Enterobacteriaceae* isolates from 13 flocks of backyard chickens in Costa Rica.

Organism	No. of resistant strains (% strains resistant)[A]						
	TIL	TET	AMP	A/C	TIC	CELOT	GENT
E. coli ($n = 44$)	35 (80)	18 (41)	13 (30)	11 (25)	7 (16)	12 (27)	0
Other *Enterobacteriaceae* ($n = 4$)	1 (25)	0	2 (50)	0	1 (25)	0	0

[A]Abbreviations: TIL = tilmicosin; TET = tetracycline; AMP = ampicillin; A/C = amoxicillin with clavulanic acid; TIC = ticarcillin; CELOT = cephalothin; GENT = gentamicin. All isolates were susceptible to florfenicol, difloxacin, ceftiofur, enrofloxacin, and orbifloxacin, and thus, they are not represented on this table. Break points for resistance were determined as per CLSI, 2006 (9).

relative low densities, hybrid vigor, and capability to free-roam away from excrement may prevent them from suffering from more substantial clinical disease. Lack of education, geographic isolation, and cost of veterinary services preclude the owners in this community from applying standard preventative-medicine protocols. Morbidity and mortalities reported are directly linked to the lack of preventative medicine and lack of shelter and to keeping chickens of different ages in the same group and allowing the introduction of new individuals into the flock (4) Owners reported sporadic deaths from a ''chicken plague,'' which, based on the history and clinical signs, the high prevalence of NDV antibodies among the population, and the elevated antibody titers against NDV, led us to conclude was most consistent with outbreaks of Newcastle disease.

At least one report indicated that poxvirus is not uncommon in backyard chicken flocks in the United States (32) Given the lack of vaccination, the presence of insect vectors, and the clinical and microscopic examination findings in this study, we suspect that poxvirus will continue to be an important cause of decreased production in these flocks. Although pox viruses are typically host-specific, there is a possibility of recombination of chicken and passerine poxvirus strains, which could affect virulence.

Given our serology results and interviews, it is likely that the respiratory syndrome described by owners is caused by NDV, IBV, mycoplasmosis, or ILT. Tumors caused by Marek's disease or avian leukosis or other conditions causing severe anemia might explain the other syndrome owners reported as anemia, weight loss, and death, because at least one of the birds necropsied exhibited splenic atrophy, often associated with immunosuppressive agents like Marek's, chicken infectious anemia, or IBD.

Given the significant number of birds with titers >4000, we assume that backyard chickens in this region have been in contact with a pathogenic strain of NDV, even after taking into consideration that some birds might have received live vaccines. The USDA and the Office International des Epizooties consider Costa Rica a country free of exotic Newcastle disease, but the status of backyard chickens is unknown (73). Worldwide, NDV has the potential to cause morbidity and mortality and has been detected in a variety of wild birds (27,30,41,43,48). Of those relevant to this region, high level of susceptibility to NDV is reported in Galliformes, Psittaciformes, and Columbiformes. Lesser susceptibility occurs in Falconiformes, Accipitriformes, and Passeriformes. Gilchrist (25) provides a recent comprehensive review of wild bird susceptibility to NDV. Of particular concern would be other members of the Galliformes, of which, certain species are frequently observed in shade-grown coffee plantations or in immediately adjacent forest fragments, such as three members in the family Cracidae, and one in the family Odontophoridae. In this region, there are six Columbiformes inhabiting shade-grown coffee parcels, one of which is considered rare. Five species of psittacines, highly coveted by ecotourists, make use of coffee parcels. Because of the feeding ecology of members of the Galliformes and Columbiformes, contact with chickens or their excrement is considered likely. For example, one of the authors (S. M. H.-D.) has observed Inca doves (*Columbina inca*) and white-tipped doves (*Leptotila verreauxi*) feeding with chickens (68). Pigeon paramyxovirus (PPMV-1) antibodies were detected from a variety of wild birds, with the highest frequency during a regional outbreak of PPMV-1 in white-collared doves (*Streptopelia decaocto*) in Florida, suggesting that this virus had spread to other wild birds. Both that study (72) and the Gohm *et al.* (27) serosurvey of wild birds during an NDV epizootic illustrate the subtlety with which this virus circulates in natural populations.

Close inspection of the birds with titers >4000 for IBV indicated that they did not originate from flocks where owners reported acquiring birds from the MAG, and we would consider those titers significant. Seropositivity against IBV has been reported in wild birds, such as pigeons (2). Jimenez *et. al.* (38) reported on the widespread distribution of IBV in Costa Rica, a similar prevalence (42%) in the backyard poultry they examined, and the prevalence of IBV antibodies in free-ranging Columbiformes, including species that routinely inhabit shade-grown coffee plantations. Again, the aforementioned wild birds in the orders Galliforme and Columbiforme would be considered at highest risk.

Because these backyard chickens were not vaccinated against ILT, the 65 birds with titers >1000 were likely previously infected with virus. Currently, Costa Rica is experiencing sporadic outbreaks of ILT in its commercial operations, in which more than 50% of the animals are seropositive (Jimenez, pers comm.). Therefore, it appears that ILT could also be a significant disease for this population of backyard chickens. ILT has been reported in members of Phasanidae and Numididae, but the susceptibility of their New World counterparts in the Cracidae and Odontophoridae families is unknown (39,74)

Table 5. Presence of class 1 integrase and genes associated with tetracycline resistance in 48 isolates from fecal samples of backyard chickens in Costa Rica.

Organism	No. of strains positive (% strains positive)			
	int1	*tet*(A)	*tet*(B)	*tet*(A) and *tet*(B)
All *E. coli* ($n = 44$)	9 (20)[A]	21 (48)	4 (9)	3 (7)
E. coli resistant to tetracycline ($n = 18$)	4 (22)[A]	12 (67)	5 (28)	3 (17)
Other *Enterobacteriaceae* ($n = 4$)	2 (50)[A]	0	0	0

[A]Polymerase chain reaction of the 5′–3′ region failed to show the presence of integrated resistance genes.

Based on serology and PCR, these chickens were also infected with MG and MS. It appears that *Mycoplasma* sp. diseases are common in backyard chicken flocks (36). The DNA presence of Mycoplasma mimicked the antibody seroprevalence. All of the birds in which MG nucleic acid was detected were less than 1 yr of age, and although the sample size in this study precluded us from definitively investigating the relationship between age and antigen detection, we speculated that juveniles harbored more MG organisms. At least one study (24) states that lower quantities of MG DNA are found in older birds. MG causes respiratory disease in a variety of wild birds (22,50,51), and a variant associated with poultry and turkeys has caused population declines from conjunctivitis and mortality of house finches and other members of Fringillidae (13,18). There are 134 species of Passeriformes that inhabit this region, of which, at least 74 regularly inhabit shade coffee plantations, and of those, 18 species forage primarily on the ground and could be considered at risk (Hernandez-Divers, unpubl. data). Twenty-five of the common Passeriformes in shade-grown coffee parcels are North American migrants that rely heavily on surrogate habitat.

Because 56% and 40% of chickens had titers consistent with infection with CAV and IBD, respectively, it appears these diseases are also common in this population of chickens. Antibodies against IBD have been reported in a variety of wild birds (37,57). Of relevance to our study are birds in the genera *Corvus* and *Columba*, although antibodies have been found in other members of the Passeriformes (25). As this virus causes immunosuppression, clinical disease might be expressed in terms of secondary infections, leading to indirect causes of mortality (e.g., predation).

Multiple infections with a variety of parasites are common in free-range chickens (17,33,36,40,59,62,66). Losses in weight, egg production, and longevity of free-roaming chickens from parasitic disease might not be as apparent, when compared with viral or bacterial disease, but can be far more significant (34,59,66). Coinfections with three species of *Eimeria* were noted in high numbers on fecal exams and were associated with clinical disease in pathologic examinations. Coccidiosis can be a major cause of mortality among chicks and a cause of morbidity and loss of condition among adult chickens (53). Except for *Knemidocoptes* sp., which caused visible irritation and dermatitis, the other mites and lice recovered are host-specific and not considered particularly pathogenic. A subtle, yet important, factor affecting production is the interrelationship of parasitic infections and other diseases, and currently, there is interest in understanding this relationship (12,16). Although parasitic infections are generally host-specific, some important exceptions exist. For example, in addition to members of the Galliformes, both *Dispharynx* sp. and *Capillaria* sp. are nematodes that have the capability of infecting a variety of Passerine hosts (20,61). Although nematodes found in chickens have always been considered host-specific, *Syngamus trachea*, for example, has been reported in a variety of wild birds (44). In fact, preliminary data have demonstrated *Syngamus* sp. ova in wild passerines inhabiting these plantations (Hernandez-Divers, unpubl. data). *Dispharynx* sp. has been reported to cause clinical disease in wild birds and might be an important pathogen for nestlings (61). At least one study suggests that *Ascaridia galli* infections in nonchicken hosts had been acquired from chickens (19). Areas with large concentrations of fecal material, such as chicken-feeding stations and corrals, are typical in shade-grown coffee parcels and can provide a focus of fecal contamination for the environment and for intermediate hosts (26).

The diseases for which these animals were tested also have significant economic importance for the poultry industry, a rising industry in Costa Rica (63). There is one large commercial poultry operation approximately 20 km from San Luis. Although biosecurity is stringent in these operations, it is important for the Costa Rican authorities to be aware of the diseases free-roaming chickens harbor, in case of a biosecurity breach that might lead to an epizootic.

The addition of chickens to the Monteverde landscape is likely to have an effect on environmental bacterial populations. In particular, we were concerned that antibiotic-resistant plasmids carried by chicken phenotypes would be available for horizontal transfer, making shade-coffee plantations foci for exchange of bacterial genetic material. The poultry literature reports antimicrobial resistance patterns for commercial operations, and within the context of antimicrobial use (1,5,14,67). With the exception of sporadic, individual-animal use of oxytetracycline by three owners, the chickens in San Luis are not routinely exposed to antimicrobials, and we did not find a significant difference in the prevalence of tetracycline resistance among those flocks that had been exposed to oxytetracycline and those that were not. Even though this study supports the premise that isolates from free-range chickens display lower tetracycline resistance than found commercial operations, the resistance is still significant (1,12,67). This is supported by the presence of resistance genes *tet*(A) and *tet*(B). A variety of genes have been found to mediate resistance to tetracycline; however, *tet*(A) to *tet*(E) are the most prevalent elements found in tetracycline-resistant *E. coli* isolates, and within that group, the majority of resistance appears to be derived from *tet*(A) and *tet*(B) (56). Current reports in the literature describe higher prevalence of antimicrobial resistance in isolates from wild birds associated with human-disturbed habitats than from birds that are not exposed to human-associated activities. For example, *E. coli* isolates from black-headed gulls (*Larus ridibundus*), nesting in agricultural regions of the Czech republic, displayed a 19% resistance to tetracycline, whereas only 7.6% of *E. coli* isolates from rooks (*Corvus frugilegus*), nesting in remote regions, were resistant to tetracycline (15,49). A recent study (65) of the antimicrobial resistance of *E. coli* isolated from wild birds in the Arctic reported low prevalence (8%) but proposed that migratory birds were the vehicles for transport of resistance genes. Class I integrons, contained within mobile DNA elements, have been shown to be of importance in the transmission of antibiotic resistance in chickens and a useful tool for studying antimicrobial-resistance transmission (29,47). The prevalence of *intI* in our isolates was much lower than previously reported in chickens (29). In addition, no resistance gene cassettes were found, by PCR, integrated in the few integrase positive isolates. Thus class 1 integron does not appear to play a significant role in the resistance we observed but remains a potential vehicle of antimicrobial-resistance transmission. In our study, either *tet*(A) or *tet*(B), but not both, was likely responsible for the recorded tetracycline resistance in the *E. coli* isolates. The results we obtained do not support the theory that antimicrobial use is the primary selection mechanisms responsible for resistance but do seem to demonstrate that there is a pool of resistance genes in this population, not necessarily selected through direct antimicrobial use either therapeutically or subtherapeutically in the feed or water. This pool of resistance genes not only poses a threat to the wild bird population but also can be used as a model of microbial transmission within habitats, as has been done in previous reports (28)

Forest-surrogate environments, such as shade-grown coffee plantations, provide suitable habitats, maintaining wild bird species richness and abundance. However, they are human-altered systems, which may pose a potential risk to wild birds through exposure to

highly mobile backyard chickens and their pathogens. In accord with Friend *et al.* (23), we recognize the need, from an ecologic perspective, to focus more attention on disease issues as direct and indirect causes of declining avian populations. Unfortunately, disease investigations in wild birds are often undertaken only following a massive mortality event or on highly endangered species, and they typically focus on mortality alone, ignoring subtle, sublethal or indirect effects caused by one or a combination of diseases. The prevalence and diversity of pathogens of the wild birds sympatric with backyard chickens is currently being investigated (Hernandez-Divers, unpubl. data). Although, to our knowledge, no visible mortality events of wild birds have occurred as a result of the introduction of backyard chickens in forest-surrogate habitats, we suggest that if wild birds become infected with the aforementioned diseases, there are likely fitness trade-offs to individuals that are associated with infection and immune defense against viruses and parasite loads, which may translate to effects on population dynamics through indirect and sublethal effects (45,46). Additionally, free-range chickens can serve as reservoirs for antimicrobial-resistance bacteria, which could be disseminated to birds using shade coffee plantations. Recognizing the need for creating more available habitat for avian conservation, further sustainable agroforestry incentives, such as shade-grown cacao, are the wave of the future (21,26). Thus, studies understanding the disease dynamics of wild birds inhabiting these forest-surrogate habitats to determine their significance as foci of risk for diseases will motivate policy changes for conservation organizations.

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ACKNOWLEDGMENTS

We would particularly like to acknowledge the chicken owners and the San Luis community. Without their trust and enthusiasm, this project could not have been realized. Specifically, we would like to thank Adan Fuentes, our field assistant and community liaison. We would like to thank the UGA San Luis Research Station for providing lodging and logistic support. This project was funded by a National Science Foundation Graduate Student Fellowship. Harmony Seahorn (Poultry Diagnostic Research Center, UGA), Nate Chenoweth (College of Veterinary Medicine, GA), Natasha James (UGA), and Rocio Cortes and Jorge Prendas (Universidad Nacional, CR) provided instrumental technical assistance.