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ENTERIC PATHOGENS AND ANTIMICROBIAL RESISTANCE IN TURKEY VULTURES (*CATHARTES AURA*) FEEDING AT THE WILDLIFE–LIVESTOCK INTERFACE

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Abstract: Free-flying turkey vultures (*Cathartes aura*) were sampled in California to investigate the fecal shedding prevalence and antimicrobial susceptibility of *Salmonella enterica*, *Campylobacter* spp., and *Escherichia coli*. Nine different serotypes of *Salmonella enterica* were detected in cloacal swabs from turkey vultures, and 6% of vultures were shedding *Campylobacter* spp.. Turkey vultures sampled at a location with range sheep were more likely to shed tetracycline-resistant *E. coli*, suggesting that proximity to livestock facilities could facilitate acquisition of drug-resistant bacteria in avian scavengers. These findings illustrate the importance of assessing drug-resistant pathogen transfer at the livestock–wildlife interface.

Key words: Antimicrobial, *Cathartes aura*, drug resistant, livestock, pathogen, turkey vulture.

BRIEF COMMUNICATION

Global trends in conversion of wild lands to agriculture have been associated with shifts in food resources for avian scavengers, with an increased dependence of some populations on domestic animal carrion and farm refuse.[9] Regular consumption of livestock remains places scavenging birds at heightened risk of exposure to veterinary drug residues, as evidenced by vulture die-offs from diclofenac toxicity on the Indian subcontinent.[9] A Canadian study found that wildlife residing near livestock operations exhibited greater antimicrobial resistance relative to other habitats.[2] Given these findings, and the problem of drug resistance in the agricultural industry,[8] closer monitoring of antimicrobial resistance in wildlife populations at the livestock–wildlife interface is warranted.

To assess antimicrobial resistance associated with the livestock–wildlife interface in an abundant wide-ranging avian scavenger species, free-flying turkey vultures (*Cathartes aura*) were sampled at two sites in California, where birds scavenge opportunistically on wild animal carrion and have variable access to nearby livestock remains. Bacteria targeted for culture included *Salmonella enterica*, *Campylobacter* spp., and *Escherichia coli*. These microbes were selected because of their wide host range, zoonotic potential, and documented association with antimicrobial resistance.[8,15]

Cloacal swab samples were obtained from 18 turkey vultures captured at University of California Landels-Hill Big Creek Reserve (LHBCR) (36°03′51′′N 121°34′28′′W) near Big Sur, California, and from 38 free-ranging turkey vultures captured at the Hopland Research and Extension Center (HREC) (38°59′39′′N 123°04′02′′W) near Hopland, California, in spring 2009. At the time of capture, all birds appeared to be healthy and in good body condition. The HREC maintains a flock of approximately 1,600 sheep that graze the rangeland at this location.

Turkey vultures were captured outside of their migratory period so that samples would reflect local subpopulation dietary preferences, although it is recognized that pathogen shedding and antimicrobial resistance may persist in a long-term carrier state and therefore could be influenced by premigration exposure to pathogens. Vulture age class and sex were determined according to standard guidelines[7] (Sex Made Easy™, Zoogen DNA Services, Zoogen Inc., Davis, California 95617, USA). Cloacal specimens were collected from birds on transport swabs, refrigerated, and cultured within 48 hr at University of California–Davis. Fecal samples were also obtained opportunistically from 26 domestic sheep at HREC.

Salmonella enterica, *Campylobacter* spp., and *E. coli* were isolated using selective media and standard protocols. Cultures for the target bacteria were performed using selective enrichment

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Table 1. Prevalence (%) of turkey vultures (*C. aura*) shedding *E. coli*, *Salmonella enterica*, or *Campylobacter* spp. (alone or in combination) captured at Landels-Hill Big Creek Reserve (LHBCR) and Hopland Research and Extension Center (HREC).

	% prevalence (animals positive/animals sampled)		
Bacterial species	LHBCR	HREC	Total
E. coli	83 (15/18)	92 (34/37)	89 (49/55)
Salmonella enterica	17 (3/18)	22 (8/37)	20 (11/55)
Campylobacter spp.	11 (2/18)	3 (1/37)	6 (3/55)

and media,[14] with *Salmonella* serotyping performed at the National Veterinary Services Laboratory (National Veterinary Services Laboratory, USDA–APHIS–VS–NVSL, Ames, Iowa 50010, USA). Antimicrobial susceptibilities for *E. coli* and *Salmonella enterica* isolates were determined using broth microdilution[14] with standard panels (COMEQ3F and COMPAN1F Veterinary Plates, Trek Diagnostic Systems, Inc., Cleveland, Ohio 44131, USA). Prevalence of antimicrobial resistance and multidrug resistance patterns were determined for vultures and sheep sampled for this study. Pearson's chi-square test of independence was performed to assess whether age class, sex, or capture location of turkey vulture was associated with the type of isolate or antimicrobial resistance.

Shedding prevalences of *Salmonella enterica* and *Campylobacter* spp. were similar in the HREC and LHBCR turkey vulture populations (Table 1). In total, 20% (11/55) of turkey vultures were positive for *Salmonella enterica*, constituting nine serotypes: *Salmonella* Anatum, *Salmonella* Typhimurium, and *Salmonella* Newport cultured from LHBCR vultures; and *Salmonella* Give, *Salmonella* Montevideo, *Salmonella* Enteritidis, *Salmonella* Arizonae Type Rough O:i:z, *Salmonella* Arizonae Type III_18:z4:z23, and *Salmonella* Arizonae Type III_50:T:z cultured from HREC vultures. The prevalence of *Salmonella enterica* shedding was 17% (3/18) in LHBCR birds and 22% (8/37) in HREC birds. Only 5.5% (3/55) of all turkey vultures sampled were shedding *Campylobacter* spp., comprising 11% (2/18) of the LHBCR sample population and 3% (1/37) of the HREC population. The single *Campylobacter* spp. isolate cultured from the HREC vulture was identified as *Campylobacter jejuni*; species identification could not be established for the two LHBCR vulture *Campylobacter* spp. isolates. The most common bacterial species isolated from turkey vulture cloacal specimens in both locations was nonhemolytic *E. coli*. There were no significant associations between the prevalence of microbe isolated in relation to sex or age class of turkey vulture. Nonhemolytic *E. coli* was the only target bacteria isolated from HREC sheep (26/26).

Overall, 20% of turkey vultures were shedding antimicrobial-resistant *E. coli*. Excluding resistance to clindamycin, erythromycin, oxacillin, and penicillin because *E. coli* spp. exhibit universal resistance to these antimicrobials,[11,12] the prevalence of single drug resistance in *E. coli* isolates was 7% in LHBCR vultures and 26% in HREC vultures ($P = 0.11$). Tetracycline resistance in *E. coli* isolates was detected in HREC vultures only, with a prevalence of 24% compared to 0% in LHBCR vultures ($P = 0.04$; Table 2). Among HREC sheep, 12% (3/26) were positive for tetracycline-resistant *E. coli* isolates, including one individual that also exhibited resistance to several other antimicrobials (Table 2). With the exception of universal resistance of *E. coli* to clindamycin, erythromycin, oxacillin, and penicillin, multidrug resistance was absent in vultures and rare in HREC sheep. Antimicrobial resistance was not observed in any *Salmonella enterica* isolates.

Although there are no reports of *Salmonella enterica* and *Campylobacter* spp. causing clinical illness in turkey vultures, salmonellosis has been implicated in mass mortality events in other wild bird populations[6] and has caused significant production losses in the livestock industry.[13] Domestic fowl, in particular, are known to experience high morbidity due to a variety of *Salmonella enterica* serotypes, including some of those identified in turkey vultures sampled for this study (i.e., *Salmonella* Enteritidis, *Salmonella* Typhimurium, *Salmonella* Anatum, and *Salmonella* Arizonae Type III_18:z4:z23).[5,10] Unlike *Salmonella enterica*, the health impacts of *Campylobacter* spp. in wild and domestic animals are less known. Severe clinical manifestations of campylobacteriosis appear to be rare in most domestic species,[4] and *Campylobacter* spp. seems to exist mainly in a carrier state in birds.[1]

From a public health standpoint, both *Campylobacter* spp. and *Salmonella enterica* are important zoonoses, and many of the isolates identified in this study (i.e., *C. jejuni*, *Salmonella* Enteritidis, *Salmonella* Typhimurium, and *Salmonella* Newport) are commonly linked to food-borne outbreaks worldwide.[15] Although livestock reservoirs are the primary source of *Campylobacter* spp. and *Salmonella enterica* infections in humans,[15] wild birds can serve as vectors or carriers of disease.[1]

Table 2. Prevalence of antimicrobial resistance in *E. coli*[a] shed by domestic sheep and turkey vultures (*C. aura*) at two study sites in California.

	% prevalence (animals positive/animals sampled)[c]		
Resistance pattern[b]	HREC sheep	LHBCR turkey vultures	HREC turkey vultures
AMK, AMP, CEPH, CHL, ENR, GEN, MAR, TET, TIC, TMS	4 (1/26)	—	—
AMP	—	—	3 (1/34)
GEN	—	7 (1/15)	—
TET	8 (2/26)	—	24 (8/34)

[a] All *E. coli* isolates were resistant to clindamycin, erythromycin, oxacillin, and penicillin regardless of the animal from which the isolate was cultured.

[b] AMK, amikacin; AMP, ampicillin; CEPH, cephalothin; CHL, chloramphenicol; ENR, enrofloxacin; GEN, gentamicin; MAR, marbofloxacin; TET, tetracycline; TIC, ticarcillin; TMS, trimethoprim-sulfamethoxazole.

[c] HREC, Hopland Research and Extension Center; LHBCR, Landels-Hill Big Creek Reserve.

Frequent use of antibiotics in agriculture, and in the health industry as a whole, has been linked to the emergence of multidrug-resistant strains of *Salmonella enterica* and *Campylobacter* spp..[8] Investigations of disease transmission at the livestock–wildlife interface are therefore critical to understanding selective pressure for drug resistance in multiple species as well as for determining the extent to which wildlife populations contribute to the maintenance and spread of these microbes.

The source of tetracycline-resistant *E. coli* detected in HREC vultures sampled in this study cannot be definitively ascertained; however, research has established that tetracycline is commonly used in farming operations.[8] One study reported the prevalence of tetracycline-resistant *E. coli* among sheep in the United States to be 33%.[3] Our finding, which revealed that several range sheep sampled at HREC were shedding tetracycline-resistant *E. coli*, is consistent with these observed patterns. Given that vultures in this area share habitat with these sheep and have been observed scavenging on sheep carcasses on facility grounds, it is plausible that there may be spillover of tetracycline-resistant *E. coli* from domestic sheep to vultures on this rangeland. In this preliminary investigation, small sample sizes likely impeded our ability to detect less common patterns in drug resistance among turkey vultures, including multidrug resistance, which constituted a rare finding in sheep sampled at HREC. In spite of these limitations, our data suggest that tetracycline resistance could be common in enteric bacteria shed by scavenging birds that forage on livestock. Dispersal of antimicrobial resistance by scavenging birds could have significant economic and public health implications. Avian scavengers, in particular, may be highly effective at disseminating antimicrobial-resistant pathogens because of their wide ranging and migratory behavior.[1] More in-depth investigations of antimicrobial resistance in scavengers are therefore warranted to improve our understanding of cross-species transfer of antibiotic resistance to wild animals.

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