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Source: Journal of Wildlife Diseases, 54(2) : 414-418

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/2017-08-191

DOI: 10.7589/2017-08-191

Journal of Wildlife Diseases, 54(2), 2018, pp. 414–418

Serologic Surveillance of Wild and Pen-reared Ring-necked Pheasants (*Phasianus colchicus*) as a Method of Understanding Disease Reservoirs

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ABSTRACT: We investigated exposure to infectious diseases in wild (n=33) and pen-reared (n=12) Ring-necked Pheasants (*Phasianus colchicus*) in the Central Valley of California, US during 2014 and 2015. Serologic tests were positive for antibodies against hemorrhagic enteritis, infectious bursal disease, and Newcastle disease viruses in both wild and pen-reared pheasants.

The practice of pen rearing and releasing Ring-necked Pheasants (*Phasianus colchicus*) on public and private wildlands coupled with the widespread distribution of hunting areas (CDFW 2017; USFWS 2017) within California, US (Fig. 1) may provide a mechanism by which disease can potentially spread to wildlife and commercial poultry. Although wild birds are known reservoirs of pathogens that affect domesticated and nondomesticated birds (Cooper 1993; Hanson et al. 2005), released pen-reared pheasants are a potential reservoir of disease for avian (Tompkins et al. 2000) and terrestrial wildlife (Anderson et al. 2006). Furthermore, most release locations for pheasants are associated with wetlands or rice agriculture along major waterfowl flyways, which often provide favorable conditions for pathogen survival and transmission among waterfowl (Hanson et al. 2005; Hoye et al. 2011). Therefore, investigating the prevalence of certain infectious diseases in pheasants could be an important aspect of disease surveillance for understanding overall disease transmission risk to other wildlife and to commercial poultry.

We sampled pheasants across four study sites in the Central Valley of California: Yolo Bypass Wildlife Area, Gray Lodge Wildlife Area (GLWA), Roosevelt Ranch Duck Club, and Mandeville Island Duck Club (Fig. 1). Using night spotlighting techniques adapted from Wakkinen et al. (1992), we captured and collected antemortem blood from wild and pen-reared pheasants at our study sites, not including breeding farms. We classified individuals that were not reared in captivity as "wild," and individuals reared in captivity as "pen-reared" pheasants. Wild pheasants sampled at Yolo Bypass Wildlife Area, GLWA, and Mandeville Island Duck Club spatially overlapped with pen-reared pheasants released at these sites during both years of the study. We sampled pen-reared pheasants from two breeding farms licensed by the California Department of Fish and Wildlife (CDFW 2015). Seven pen-reared pheasants from a farm in Butte County, California, were sampled at GLWA prior to release, and five were sampled at a pheasant breeding farm in Glenn County, California. Neither of these farms vaccinated pheasants produced on site, but the farm in Glenn County vaccinated their breeding stock for *Mycoplasma gallisepticum* and *Salmonella enterica* serovar Pullorum (SP). None of the pen-reared pheasants sampled at these farms tested positive for titers against these diseases.

We conducted enzyme-linked immunosorbent assay (ELISA) analyses to test for titers specific to avian influenza (AI), Newcastle disease (ND), infectious bursal disease (IBD), infectious bronchitis (IB), hemorrhagic enteritis (HE), infectious laryngotracheitis (ILT), and *Pasteurella multocida* (PM). Additionally, we used microagglutination techniques to test for SP. Sera samples were transferred to the California Animal Health and Food Safety

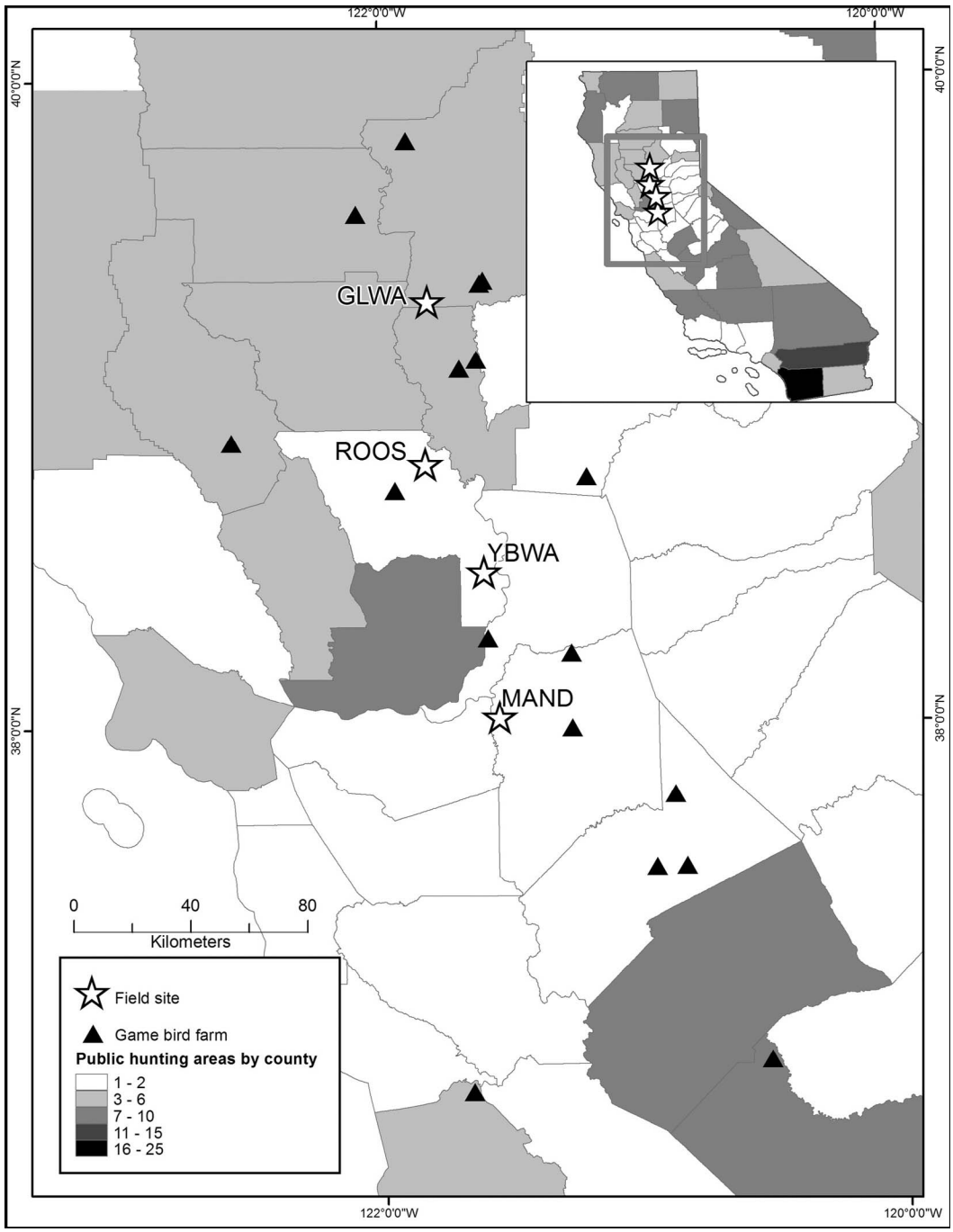

FIGURE 1. Locations of collection of serum samples from Ring-necked Pheasants (*Phasianus colchicus*) used for testing by enzyme-linked immunosorbent assay and microagglutination assay for infectious diseases in 2014 and 2015. Study sites (stars) and game bird farms (triangles) are shown in relation to the number of public hunting areas by county in California, USA. GLWA=Gray Lodge Wildlife Area; ROOS=Roosevelt Ranch Duck Club; YWBA=Yolo Bypass Wildlife Area; MAND=Mandeville Island Duck Club.

diagnostic laboratory (Turlock, California). Separate ELISA kits were used for AI, ND, IBD, IB, and PM (IDEXX Laboratories Inc., Westbrook, Maine, USA) and for HE and ILT (Synbiotics®, Zoetis Inc., Parsippany, New Jersey, USA). These ELISA tests have only been validated in chickens and turkeys, and the diagnostic sensitivity and specificity for each test is above 95% for validated species. Hence, there may be greater potential for false positives when used on pheasants. However, there are common antigenic sites across turkey, pheasant, and chicken immunoglobulins (Narat et al. 2004) that can cross react with the immunoglobulin conjugate used in the ELISA kits. Results from the ELISA tests suggest exposure to screened diseases and are primarily qualitative evidence supporting further investigation of diseases affecting wild and pen-reared pheasants. The following titer group cutoffs were used: "seronegative" when ELISA <1 and "seropositive" when ELISA >1.

Serologic tests completed in 2015 from two locations (n=12) showed positive serology for HE, IBD, and ND viruses in pen-reared pheasants sampled from game bird farms in the Central Valley. Of the 12 pen-reared pheasants sampled in 2015, seven (58%) tested seropositive for HE, 10 (83%) for IBD, and six for ND (50%). During 2014 and 2015, wild pheasants sampled (2014, n=14; 2015, n=19, total n=33) in the same geographic area were shown to be seropositive for HE (15%), IBD (69%), and ND (18%). Additionally, we found positive serology for antibodies against IB (6%), ILT (3%), and PM (9%) in wild pheasants across both years (Table 1). None of the sampled pen-reared or wild birds showed positive serology for AI or SP; some of the sampled birds were seropositive for more than one agent.

The unique role of pheasant farms in supplying game to hunting clubs and refuges may increase the probability of spreading disease to wildlife at release sites. Furthermore, pen-reared Galliformes introduced to public and private lands may not only cointroduce novel pathogens (Viggers et al. 1993) but may also decrease the breeding success of wild birds that pair with pen-reared birds (Rands and Hayward 1987), increase predator abundance (Robertson 1988), and increase the occurrence of parasite transmission to other birds (Tompkins et al. 2000). Outbreaks of disease have contributed to the decline of several endangered species when coupled with other environmental pressures such as habitat loss and hunting (Viggers et al. 1993). For example, the introduction of the Domestic Turkey (*Meleagris gallopavo*) onto an island refuge with a remnant Heath Hen (*Tympanuchus cupido*) population led to the introduction of *Histomonas meleagridis* (Simberloff 1986). Coates et al. (2017) found that wild pheasant populations in California were affected by similar environmental and anthropogenic factors leading to habitat loss resultant from changes in farming practices. Although a culmination of factors is likely influencing the decline of wild pheasants in California, the introduction of pathogens from released pen-reared pheasants may exacerbate the effects of these other factors.

Regardless of whether the pheasants were wild or pen-reared, these data suggest past exposure to disease. Therefore, further investigation of the potential for pheasants to be reservoirs of avian diseases may be warranted. An important caveat is that the presence of antibodies shows past exposure to antigens and does not necessarily imply the development of clinical symptoms (Cooper 1993). To our knowledge, this is the first evaluation of diseases in relation to both pen-reared and wild pheasants that occupy the same geographic areas. A more in-depth study that uses isolation techniques to detect pathogens and that investigates disease prevalence associated with release sites before and after the release of captive birds would likely improve our ability to estimate the occurrence of disease transmission.

This research was funded through Pheasants Forever, the California Department of Fish and Wildlife Upland Game Bird Stamp Program, and the Center for Food Animal Health. We thank J. Kohl, S. Haynes, J. Juanitas, and R. Buer for their diligence in collecting data in the field. We also thank D.

TABLE 1. Prevalence of antibody detection to infectious diseases as determined from enzyme-linked immunosorbent assay and microagglutination analyses on serum samples collected from wild and pen-reared Ring-necked Pheasants (*Phasianus colchicus*) during 2014–15 in the Central Valley, California, USA. No samples from any location were positive for antibodies to avian influenza or to *Salmonella enterica* serovar Pullorum.

Source[a]	Year	*n*	% Positive					
			Newcastle disease	Infectious bursal disease	Infectious bronchitis	Hemorrhagic enteritis	Infectious laryngotracheitis	*Pasteurella multocida*
Wild								
YBWA	2014	7	28	85	0	0	0	0
YBWA	2015	7	0	28	0	0	0	0
GLWA	2014	4	25	100	25	50	25	25
GLWA	2015	4	0	50	25	50	0	25
Mandeville Island Duck Club (San Joaquin County)	2014	3	0	66	0	66	0	0
Roosevelt Ranch Duck Club (Yolo County)	2015	8	37	87	0	37	0	12
Pen reared								
GLWA	2014	7	85	100	0	57	0	0
Game bird farm (Glenn County)	2015	5	0	60	0	60	0	0
Totals								
Wild		33	18	69	6	15	3	9
Pen reared		12	50	83	0	58	0	0

[a] YBWA = Yolo Bypass Wildlife Area (Yolo County); GLWA = Gray Lodge Wildlife Area (Butte County).

Connelly with Pheasants Forever and S. Gardner, M. Meshriy, B. Burkholder, J. Stoddard, A. Atkinson, and D. Van Baren with the California Department of Fish and Wildlife for logistic support. We are grateful to Mandeville Island and Roosevelt Ranch for providing access onto their private lands. The use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the US Government.

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Submitted for publication 10 August 2017.
Accepted 6 October 2017.