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Health surveillance of a potential bridge host: Pathogen exposure risks posed to avian populations augmented with captive-bred pheasants

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Abstract

Augmentation of wild populations with captive-bred individuals presents an inherent risk of co-introducing novel pathogens to naïve species, but it can be an important tool for supplementing small or declining populations. Game species used for human enterprise and recreation such as the ring-necked pheasant (*Phasianus colchicus*) are commonly raised in captivity and released onto public and private wildlands as a method of augmenting naturalized pheasant populations. This study presents findings on pathogen exposure from three sources of serological data collected in California during 2014–2017 including (a) 71 pen-reared pheasants sampled across seven game bird breeding farms, (b) six previously released pen-reared pheasants captured at two study sites where wild pheasants occurred and (c) 79 wild pheasants captured across six study sites. In both pen-reared and wild pheasants, antibodies were detected against haemorrhagic enteritis virus (HEV), infectious laryngotracheitis (ILT), infectious bursal disease virus (IBDV), paramyxovirus type 1 (PMV-1) and *Pasteurella multocida* (PM). Previously released pen-reared pheasants were seropositive for HEV, ILT, and PM. Generalized linear mixed models accounting for intraclass correlation within groups indicated that pen-reared pheasants were more than twice as likely to test positive for HEV antibodies. Necropsy and ancillary diagnostics were performed in addition to serological testing on 40 pen-reared pheasants sampled from five of the seven farms. Pheasants from three of these farms tested positive by PCR for Siadenovirus, the causative agent of both haemorrhagic enteritis in turkeys and marble spleen disease of pheasants, which are serologically indistinguishable. Following necropsy, owners from the five farms were surveyed regarding husbandry and biosecurity practices. Farms ranged in size from 10,000 to more than 100,000 birds, two farms raised other game bird species on premises, and two farms used some form of vaccination. Biosecurity practices varied by farm, but the largest farm implemented the strictest practices.

KEYWORDS

California, game birds, pen-reared, poultry diseases, ring-necked pheasant, surveillance

[Correction added on 4 Decmber 2021, after first online publication: The copyright line was changed.]

1 | INTRODUCTION

Human-mediated introduction of pathogens through the release of captively reared animals presents a risk to susceptible wildlife populations and is an important consideration for wildlife introduction programs. High host density in captive-rearing conditions can facilitate pathogen transmission as well as indirectly increase disease susceptibility of individuals when captive conditions are stressful (Scott, 1988). Captive-rearing conditions are often studied from the perspective of protecting domesticated species from wildlife, but the impacts of disease on wildlife populations are not generally considered in the context of releasing animals reared in captivity. Health surveillance of individuals prior to release can reduce the potential of disease spreading to naïve wildlife, as well as increase the effectiveness of target population augmentation (Mathews et al., 2006; Viggers et al., 1993).

Pathogen transmission risk to wildlife from game birds reared in captivity and then released into novel wildlife habitats is not well known (Garber et al., 2007; Miller et al., 2013), but pathogen transmission between wild birds, backyard poultry and commercial poultry is relatively well-documented (Kinde et al., 2003; Soberano et al., 2009; Woolcock et al., 2003). In California, recent outbreaks of virulent Newcastle disease (vND) in backyard and commercial poultry flocks (CDFA, 2019) as well as previous outbreaks that have spread to commercial poultry (Burridge et al., 1975; Kinde et al., 2005; Soberano et al., 2009) exemplify the potential for disease in small backyard flocks to spill over to the commercial poultry industry. Similarly, detection of avian influenza A virus (AIV) in waterfowl along the Pacific Flyway (Bevins et al., 2016; Hill et al., 2010; Siembieda et al., 2010) as well as in commercial poultry in California (Kinde et al., 2003; Stoute et al., 2016; Woolcock et al., 2003) demonstrates how pathogens can spread between wildlife and larger-scale commercial poultry. However, game bird farms are unique in that the majority of birds produced are released into the wild. Although most previous outbreaks associated with farm-raised pheasants and other game birds have been documented in Europe (Alexander et al., 1997; Höfle et al., 2004; Vitula et al., 2011), these outbreaks demonstrated the vulnerability of avian wildlife to infectious diseases carried by released domestic birds. Therefore, the release of game birds reared in captivity such as ring-necked pheasants (*Phasianus colchicus*; hereafter pheasants) may facilitate conditions by which disease agents can be released into the environment (Viggers et al., 1993).

Pheasants have a long history of facilitated introductions to novel environments across Europe and North America (Hart, 1990; Poole, 2010). Statewide hunter harvest of wild pheasants in California was more than one million birds per year in the 1960s (Hart, 1990) but is currently less than one hundred thousand (CDFW, 2019). Concomitant stressors including habitat loss, habitat degradation and increased pesticide application attributed to agricultural intensification across the state have led to precipitous declines in wild pheasant populations (Coates et al., 2017). Hence, wildlife managers tend to purchase and release captive-bred pheasants with three immediate goals in mind: (a) provide hunters with more opportunities to harvest pheasants by releasing them just before the hunting season, (b) supplement hunted populations by releasing pen-reared pheasants after the hunting season and (c) establish new populations as necessary (Sokos et al., 2008). To this end, tens of thousands of pen-reared ring-necked pheasants are released onto private hunting clubs and public wildlife areas in the Central Valley of California annually (Fleskes, Skalos, Kohl, & Loughman, U.S. Geological Survey, unpublished data summary).

Captive-bred game birds including pheasants are commonly raised outdoors in wire pens, which creates the potential for birds raised in captivity to come in contact with wildlife and potentially increase the wildlife-livestock disease interface (Miller et al., 2013). For example, vegetation is often allowed to grow in the pens for cover, creating perching substrate for small wild birds. Additionally, shared parasites between captive-bred and wild game birds such as *Heterakis gallinarum* have been previously reported to negatively influence host body condition (Tompkins, Draycott, & Hudson, 2000), which can exacerbate other challenges to the survival of released game birds such as increased predator abundance (Robertson, 1988) and lower disease resistance (Sokos et al., 2008).

Monitoring of pathogen prevalence in game bird breeding operations can help inform game bird breeders of the health of their flocks while elucidating potential reservoirs of disease. Avian diseases that have been reported in wild and commercially raised pheasants are described in Appendix S1. This study measured potential for pathogen exposure using serological surveillance from sampled pheasants among three different sources including (a) pen-reared pheasants within game bird breeding farms, (b) pen-reared pheasants that were previously released onto wildlands in which wild pheasant populations occurred and (c) wild pheasants on public and private hunting areas within northern California. The main objective was to compare differences in pathogen exposure between groups of pheasants based on evidence of antibody response from serological tests. In wild and pen-reared pheasant populations, we carried out surveillance for bacterial and viral pathogens that were previously reported in wild and commercially raised pheasants (Appendix S1).

2 | MATERIAL AND METHODS

2.1 | Pen-reared pheasant serologic surveillance

Pen-reared pheasants were sampled at seven game bird breeding farms located across five counties within the Sacramento and San Joaquin Valley (Figure 1) during 2015–2017. A pilot effort was carried out during the first two years of the study (2014–2015) to test sampling methodologies. Findings from the pilot study were published in Dwight et al., (2018). Farms were named based on the California county in which the premises were located. Birds were purchased from or donated by game bird breeders for diagnostic testing, and up to ten pheasants from each farm were tested in a given year. Approximately 1–2 ml of blood was taken from the cutaneous ulnar vein using a 3 ml syringe. All blood samples were stored

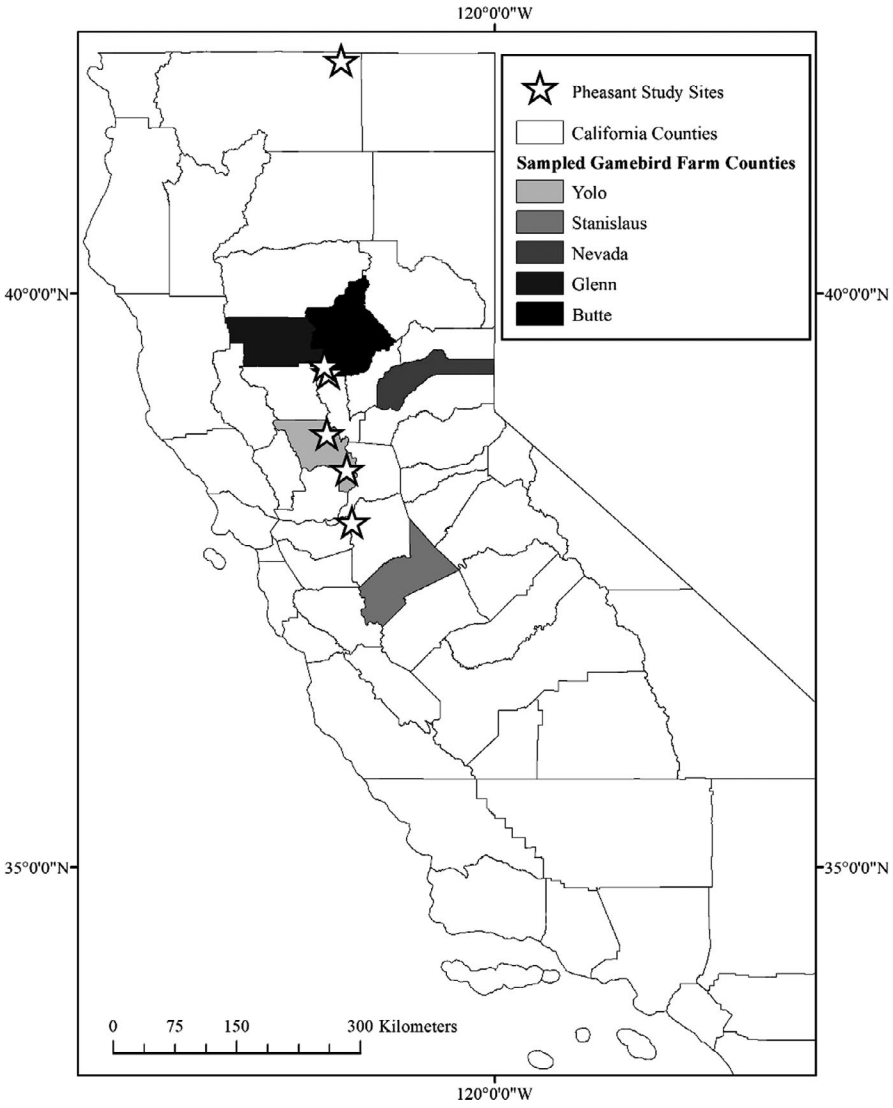

FIGURE 1 Map showing field sites where wild ring-necked pheasants (*Phasianus colchicus*) were captured and counties where pen-reared ring-necked pheasant were sampled in the northern California, USA, 2014–2017

upright in serum separator tubes (BD Vacutainer®, Franklin Lakes, NJ) in a cooler or refrigerator prior to being spun-down in a centrifuge within 48 hr of collection, and serum taken from these samples was kept in 1 ml Nalgene™ long-term storage cryogenic tubes (Thermo Fisher Scientific, Waltham, MA) at −80°C prior to performing diagnostic tests.

All tests were performed at the California Animal Health and Food Safety (CAHFS) diagnostic laboratory in Turlock, California. Enzyme-linked immunosorbent assays (ELISA) were used to test for antibody titres against AIV, paramyxovirus type 1 (PMV-1), infectious bursal disease virus (IBDV), *Pasteurella multocida* (PM) (IDEXX Laboratories Inc., Westbrook, ME), haemorrhagic enteritis virus (HEV) and infectious laryngotracheitis (ILT) virus (Synbiotics®, Zoetis Inc., Parsippany, NJ). The microagglutination test (MAT) was used to detect antibodies specific to *Salmonella enterica* serovar Pullorum (SP) (Charles River Laboratories, Wilmington, MA). Titre-group cutoffs for a positive or negative result were based on test manufacturer specifications. The AIV, PMV-1, IBDV and ILT ELISA tests were validated for chicken species, while the HEV and PM kits were validated for turkey species. The sensitivity and specificity of these tests in closely related avian species such as pheasants is unknown.

2.2 | Necropsy surveillance

Necropsy diagnostic surveillance was performed on clinically normal, pen-reared pheasants from five game bird farms in the Sacramento and San Joaquin Valley during November and December 2017. Submissions of eight live pheasants from each of 5 farm premises were submitted to CAHFS Turlock diagnostic laboratory over the two-month period. All submitted pheasants were hatched during 2017 and were approximately 24–32 weeks old. At necropsy, all pheasants were humanely euthanized with CO_2 gas, and approximately 3 ml of blood was collected from the femoral vein from each bird for serological testing. Tracheal swabs were collected for ILT PCR (Callison et al., 2007) and *Mycoplasma gallisepticum* (MG)/*Mycoplasma synoviae* (MS) multiplex PCR (Pang et al., 2002) using a commercial IDEXX kit (IDEXX Laboratories, Westbrook, ME). Bursa of Fabricius sections was collected for IBDV RT-PCR

(Jackwood & Sommer, 2005), and spleen sections were collected for HEV PCR (Hess et al., 1999). Histopathology was conducted on three sections of bursa, trachea, lung, spleen, intestines and liver. *Salmonella* cultures were performed on the caecal intestinal pool. Aerobic cultures were done for macroscopic lesions that were identified. Separate but similar serological tests were completed in conjunction with necropsy diagnostic surveillance. ELISA testing done in conjunction with necropsy submissions was performed against HEV (Synbiotics®, Zoetis Inc., Parsippany, NJ), IBDV and PM (IDEXX Laboratories, Westbrook, ME) using the same tests as previously stated. MAT was also used to detect antibodies specific to SP (Charles River Laboratories, Wilmington, MA). The plate agglutination test was performed against MG and MS, and haemagglutination inhibition (HI) testing was performed for PMV-1 antibody.

2.3 | On-farm surveys

Game bird farm owners were asked to participate in a voluntary survey in conjunction with necropsy submissions. A standard questionnaire was developed and submitted to the Institutional Review Board at the University of California Davis, from which an exemption was received, prior to carrying out the survey. Each farm owner was asked a set of 42 questions related to husbandry and biosecurity practices on the farm. General categories of questions asked during the survey included the following: sources for eggs and chicks, vaccination, number and types of species produced, general biosecurity practices and pathogen monitoring of the flock. Names and exact locations were redacted, and farms were identified by county only.

2.4 | Wild pheasant serologic surveillance

Wild pheasants were sampled across six study sites in northern California during 2015–2017: Yolo Bypass Wildlife Area (Yolo Bypass), Gray Lodge Wildlife Area (Gray Lodge), Upper Butte Basin Wildlife Area (Upper Butte), Lower Klamath National Wildlife Refuge (Lower Klamath), Roosevelt Ranch Duck Club (Roosevelt Ranch) and Mandeville Island Duck Club (Mandeville Island; Figure 1). Yolo Bypass, Gray Lodge and Upper Butte were state-managed wildlife areas that released pen-reared pheasants in differing quantities (e.g. 50–200 birds) during each year that we sampled wild pheasants at these sites. Lower Klamath was a federally managed wildlife refuge that did not release pheasants during the years in which wild pheasants were sampled. The two privately owned hunting clubs, Roosevelt Ranch and Mandeville Island, were both primarily managed for waterfowl hunting but offered pheasant hunting opportunities as well. Roosevelt Ranch did not release pheasants during the study period, while Mandeville Island released several thousand pen-reared pheasants each year of the study in an effort to augment the population post-harvest. Roosevelt Ranch was assumed to have an entirely wild population, and the pheasant population at Mandeville Island was assumed to be mostly pen-reared and released birds. However, birds sampled at Mandeville Island that hatched from nests initiated by previously released birds were considered naturalized and categorized as wild.

Pheasants sampled from our study sites included both wild pheasants and previously released pen-reared pheasants. Blood collection methods were the same for wild as for pen-reared pheasants sampled at game farms. Blood samples collected in the field were stored upright in serum separator tubes in a cooler with ice or refrigerator prior to being spun-down in a centrifuge. Previously released pen-reared pheasants sampled at wild pheasant study sites could not be linked back to the farm of origin, but physically distinguishable characteristics such as a clipped toe or leg band allowed us to differentiate previously released pen-reared birds from wild birds. Pheasants were captured at our study sites using night-spotlighting live-capture techniques adapted from Wakkinen et al., (1992), and blood samples were collected at the study sites during the fall (September–October) as well as during the winter and early spring (January–April) to minimize capturing pheasants that could be nesting. All capture, handling, blood extraction and necropsy procedures for pen-reared and wild pheasants were approved by U.S. Geological Survey Institutional Animal Care and Use Committee (IACUC; WERC-2016-01) and University of California Davis IACUC Protocol (#20785).

2.5 | Model development

Serology data collected from study sites and game bird breeding farms during 2014–2017 were combined and used in statistical analyses to take advantage of the longitudinal nature of the study and make the most of limited sample sizes from individual sampling locations. Only samples from wild pheasants captured at our study sites and pen-reared pheasants sampled directly from breeding farms were included in the models. Pen-reared birds captured in the wild were removed as a group because too few samples were collected ($n = 6$) to make statistical comparisons.

Generalized linear mixed models within a Bayesian framework were used to evaluate the probability of testing positive for selected pathogens as a pen-reared or wild pheasant. Separate models were evaluated for each selected pathogen such that the response variable represented the positive or negative outcome for a single ELISA test on a serum sample. Each model took the form:

$$Y = \beta_0 + X\beta_1 + \gamma + \delta$$

where β_0 is the fixed effect intercept, $X\beta_1$ represents the slope coefficient β_1 of the categorical predictor X, with $x = 1$ indicating a pen-reared bird and $x = 0$ indicating a wild bird, γ is a random effect for year, and δ is a random effect for site. The random effects of site and year were nested and represented variation attributed to sampled farms or field sites given the inherent differences in spatial clustering of pheasants at farms and field sites in different years. The response observations Y followed a Bernoulli distribution using a logit link function, with $y = 1$ indicating

positive ELISA test and y = 0 indicating a negative test. Uninformative priors for β_0 and β_1 were normally distributed with a mean of 0.

Models were developed with package *rjags* (Plummer, 2016) using R statistical software version 3.5.1 (R Core Team, 2018), which implements JAGS 4.3.0. Markov Chain Monte Carlo methods were used with 3 chains of 100,000 iterations, a burn-in of 10,000 iterations and retaining every 10th sample. We checked chain convergence visually and with Gelman-Rubin statistic ($\hat{r}$ <1.05). Estimated coefficients for each group (i.e. pen-reared or wild) were transformed and reported as probabilities with 95% credible intervals (CRI). For all coefficients, median values of the posterior distribution and 95% CRI are reported unless otherwise stated. The odds ratios estimated from the models were also reported to quantify the relative risk of having a positive result for a specific antibody titre given that a bird was pen-reared or wild.

3 | RESULTS

3.1 | Pen-reared pheasant serologic surveillance

Serological data were collected from a total of 71 pen-reared pheasants, including necropsied birds, sampled across seven game bird farms during 2015–2017. The proportion of positive ELISA results for selected pathogens at each game bird breeding farm are illustrated in Figure S1a of Appendix S2. Positive serology was detected for antibodies against HEV in 47 birds (66%), ILT in 9 birds (13%), IBDV in 17 birds (24%), PMV-1 in 7 birds (10%) and PM in 6 birds (8%). Exposure to HEV was identified in all but one flock-year combination across pheasant breeding farms during the study (Table 1). All samples were seronegative for antibodies against AIV and SP.

3.2 | Necropsy surveillance

Of the 40 pen-reared pheasants examined by necropsy, 39 were tested via ELISA for antibody titres due to the death of one bird prior to reaching the diagnostic laboratory, which precluded blood sampling. Mild to severe lymphofollicular splenitis and congestion were observed in all eight pheasants from three of five submissions (Table 2). Spleen tissue samples from all eight pheasants in those submissions were positive for HEV by qPCR. Likewise, ELISA results were positive for all but one sample from those three submissions. Indeterminate results for HEV by qPCR were found in a fourth submission. No pathogens were detected by PCR in the fifth submission, but two birds were positive for HEV by ELISA. All other PCR tests completed for the other submissions were negative. Samples from the second submission were positive for MG by MAT, and samples from the fifth submission were positive for both MG and MS by MAT. However, all samples were negative by the HI test, which is the confirmatory test. All samples were negative for SP by MAT and negative for AIV by ELISA. Bacteriology for *Salmonella* species by aerobic culture were also negative for all submissions. Mild to moderate roundworm (*Heterakis gallinarium*) infections of the caeca were found in at least one bird from all five submissions (Table 2).

3.3 | On-farm surveys

Owners from the five game breeding farms were interviewed following the submission of pheasants to the CAHFS diagnostic laboratory. All five farms participated in the National Poultry Improvement Plan (NPIP) administered by the California Poultry Health Board. Three of the five farms raised only pheasants, one farm-raised pheasants and chukar (*Alectoris chukar*), and one farm-raised pheasants, chukar, wild turkey (*Meleagris gallopavo*) and rock pigeon (raised as squab; *Columba livia domestica*; Table 3). All farms housed pheasants in outdoor pens as well as indoor barn enclosures. Chicks were housed in brooders within the indoor barn enclosures. Each farm-raised differing numbers of pheasants ranging from approximately 15,000 to 60,000 birds each year (Table 3). Chukar partridges were housed in battery-style cages off the ground and kept separately from pheasants. Two of the five farms vaccinated birds raised on premises. One

TABLE 1 Serology results from pen-reared ring-necked pheasant (*Phasianus colchicus*) flocks sampled at each farm by year in the Sacramento and San Joaquin Valley, California, 2015–2017

			Farm-year serology result				
Source	Year	*n*	HEV	IBDV	ILT	PM	PMV–1
Butte County 1	2015	7	+	+	–	–	+
Butte County 2	2016	10	+	–	+	–	+
Butte County 3	2017	8	+	+	–	+	–
Glenn County	2015	5	+	+	–	–	–
Glenn County	2017	8	+	+	–	+	–
Nevada County	2017	8	+	+	–	–	–
Stanislaus County	2017	7	–	–	–	–	–
Yolo County	2016	10	+	–	+	+	–
Yolo County	2017	8	+	–	+	–	–

Note: Abbreviations: HEV, haemorrhagic enteritis virus; IBDV, infectious bursal disease virus; ILT; infectious laryngotracheitis; *n*, sample of individuals per flock; PM, *Pasteurella multocida*; PMV-1, Paramyxovirus type 1.

TABLE 2 Description of necropsy findings by farm for each diagnostic technique for pheasants submitted November and December 2017 to the California Animal Health and Food Safety Laboratory Turlock, CA, USA

Diagnostic technique	Butte County 3	Glenn County	Nevada County	Stanislaus County	Yolo County
Histopathology	Mild to severe splenitis and congestion observed in 8/8 birds	Mild to severe splenitis and congestion observed in 8/8 birds	Mild to severe splenitis and congestion observed in 8/8 birds	No abnormalities detected	Moderate splenitis and congestion in 3/8 birds
Molecular diagnostics	Positive for HEV by qPCR in 8/8 spleen sections. Negative for all other pathogens	Positive for HEV by qPCR in 8/8 spleen sections. Negative for all other pathogens	Positive for HEV by qPCR in 8/8 spleen sections. Negative for all other pathogens	Indeterminate results for HEV by PCR. Negative for all other pathogens	No pathogens detected
Bacteriology	Negative for SP by aerobic culture	Negative for SP by aerobic culture	Negative for SP by aerobic culture	Negative for SP by aerobic culture	Negative for SP by aerobic culture
Immunology	Positive by ELISA for HEV 8/8, IBDV 3/8, PM 4/8	Positive by ELISA for HEV 7/8, IBDV 2/8, PM 1/8	Positive by ELISA for HEV 8/8, IBDV 2/8, Positive by agglutination for MG 8/8 but negative by HI test	Negative by ELISA	Positive by ELISA for HEV 8/8, IBDV 2/8. Positive by agglutination for MG 8/8, MS 8/8 but negative by HI test
Parasitology	Small number of *Heterakis* roundworms detected in 4/8 birds	Mild to moderate *Heterakis* roundworm infections in caeca sections in 6/8 birds	Small number of *Heterakis* roundworms detected in caeca of 3/8 birds	Small number of *Heterakis* roundworms detected in caeca of 3/8 birds	Moderate number of *Heterakis* roundworms detected in caeca of 1 bird

Note: Abbreviations: HEV, haemorrhagic enteritis virus; IBDV, infectious bursal disease virus; MG, Mycoplasma gallisepticum; MS, Mycoplasma synoviae; PM, Pasteurella multocida; PMV-1, Paramyxovirus type 1; SP, Salmonella enterica serovar Pullorum.

TABLE 3 Summary table of findings from the survey of game farms after necropsy submissions completed in the Sacramento and San Joaquin Valley, California, 2016–2017

Characteristic	Butte County 3	Glenn County	Nevada County	Stanislaus County	Yolo County
Species raised	Pheasants only	Pheasants, chukar	Pheasants only	Pheasants, chukar, wild turkey, squab	Pheasants only
Number of pheasants raised	10,000–25,000	30,000–35,000	10,000–25,000	40,000	50,000–60,000
Total number of birds raised	10,000–25,000	40,000–50,000	10,000–25,000	100,000+	50,000–60,000
Vaccinations	None	Pheasants for MG and PM	None	Squab for PMV1	None
Health problems reported at time of survey	None	None	Feed refusal, decreased production	None	None
Monitor for disease (blood sampling)	Once per 3 years	Once per 3 years	Once per 3 years	Every 6 months	Every 6 months
Consult a veterinarian	At least once in last 3 years	At least once in last 3 years	Not in last 3 years	More than once per year	Not in last 3 years
Biosecurity signage	Signage at the entrance of property	Signage at the entrance of property	No signage	Signage at the entrance of property	No signage
Perimeter security	Fence and gate	Fence and gate	Fence and gate	Not secured	Not secured
Vehicle or boot wash station	Boot wash station	None	None	Boot wash station	Vehicle wash station
Footbaths	No	No	No	Yes	Yes
Litter disposal	NS	Composted on site	On-farm trench	Composted and sold	On-farm incinerator
Mortality disposal	NS	Composted on site	On-farm landfill	On-farm incinerator	On-farm incinerator
Wild birds observed on the farm or in the pens	Small passerines, raptors	Waterfowl, small passerines, raptors	Waterfowl, small passerines,	Small passerines	Waterfowl, gulls, small passerines, raptors

Note: Abbreviations: MG, Mycoplasma gallisepticum; NS, Not specified; PM, Pasteurella multocida; PMV-1, Paramyxovirus type 1.

of the farms vaccinated pheasant breeding stock for MG and PM, and the other vaccinated squab for PMV-1. Serological testing for AIV, MG, MS and Pullorum-typhoid were done on all farms based on NPIP blood testing procedures at least once every 3 years. Two of the five farms had birds tested at least twice per year. Only one farm consulted a veterinarian more than once per year, and all but two farms had consulted a veterinarian within the last 3 years (Table 3).

Biosecurity signage was placed near the entrances of three of five farms, and a perimeter fence with a gate was observed at all but one farm (Table 3). However, none of the premises were open to the public, and all those purchasing birds from the farms were required to give notice before entering the property. Two of five farms had a wash station for personnel (boots and clothing), and foot baths were used at three farms. None of the game farms had a wash station specifically for vehicle tires and undercarriages. The largest farm had biosecurity assessments conducted once a year, and the other four farms once every 3 years or more. One game farm owner stated that he did not remember the last time a biosecurity assessment was carried out. At all farms, vegetation was allowed to grow in the outdoor pens for cover and small songbirds were often observed perching inside or near the pens. Waterfowl and birds of prey were also commonly observed flying over, and a pond was observed on or within a kilometre of all five farms. However, it was not common for terrestrial mammals or larger birds to be observed in the pens, and some form of animal control was used on all farms to prevent small mammals, including rodents, from entering the pens. Lastly, two farms disposed of mortalities using an on-farm incinerator, one used an on-farm compost pile, one an on-farm landfill, and one did not specify (Table 3).

3.4 | Wild pheasant serologic surveillance

Wild pheasants were sampled from February 2014 to April 2017 across the six study sites displayed in Figure 1. The proportion of positive ELISA results for selected pathogens at each wild pheasant study site are illustrated in Figure S1b of Appendix S2. However, not all study sites were sampled during every year of the study (Table 4). Of the 79 total wild pheasants sampled, antibodies were detected against HEV in 23 birds (29%), ILT in 1 bird (1%), IBDV in 27 birds

(34%), PMV-1 in 6 birds (8%) and PM in 24 birds (30%). All samples were seronegative for AIV and SP. The number of samples by site and year is listed in Table 4. Six previously released pen-reared pheasants were also captured at Gray Lodge ($n = 3$) and Mandeville Island ($n = 3$) in 2016, from which we found positive serology for antibodies against HEV in 2 birds, ILT in 3 birds and PM in all 6 birds (Table 4). We did not find positive antibody titre results for AIV or SP in any sampled group.

3.5 | Model interpretation

Bayesian CRIs for the posterior probability distribution of pen-reared and wild groups testing positive for HEV, ILT, IBDV, PMV1 and PM are described in Table S1 of Appendix S2. However, previously released pen-reared birds were not used in statistical analyses models due to low sample sizes that precluded inference. Similarly, ILT and PMV1 were not included in the interpretations because few birds across groups tested positive for these pathogens. Lastly, models for MG and MS were not developed because results positive by ELISA were negative by the HI test.

Based on model inference, the median probability of a pen-reared pheasant testing positive for HEV was 71.5% (95% CRI; 13.2%–98%) while the probability for a wild pheasant testing positive was 27.6% (95% CRI; 2.5%–84.2%; Figure 2). The estimated odds ratio indicated that the probability of a positive HEV titre result given that pheasant was pen-reared was 2.5 (95% CRI; 0.2–48.2) times higher than for a wild pheasant. The probability of a pen-reared pheasant being positive for IBDV was 37.1% (95% CRI; 0.001%–99.9%), and the probability for a wild pheasant was 21.3% (95% CRI; 0.001%–99.9%; Figure 2). By contrast, wild pheasants had an estimated probability of 31.6% (95% CRI; 1.0%–91.2%) and pen-reared pheasants 5.7% (95% CRI; 0%–37.1%) for detecting antibody titres against PM (Figure 2).

TABLE 4 Number of positive serology samples from individual wild and previously released pen-reared ring-necked pheasants (*Phasianus colchicus*) captured at each field site in the Sacramento and San Joaquin Valley and Klamath Basin, California, 2014–2017

			Number of seropositive samples				
Source[a]	Year	*n*	HEV	ILT	IBDV	PMV–1	PM
Wild							
Gray Lodge	2014	4	2	1	4	1	1
	2015	5	2	0	2	0	2
	2016	2	0	0	0	0	1
Lower Klamath	2016	10	1	0	1	0	5
	2017	6	4	0	0	0	2
Mandeville Island	2014	3	2	0	2	0	0
	2016	1	0	0	0	0	0
Roosevelt Ranch	2015	10	4	0	7	3	1
	2016	2	2	0	0	0	2
	2017	4	3	0	2	0	2
Upper Butte	2015	6	0	0	1	0	2
	2016	3	1	0	0	0	2
Yolo Bypass	2014	6	0	0	6	2	0
	2015	15	1	0	2	0	3
	2016	2	1	0	0	0	1
Released							
Gray Lodge	2016	3	2	2	0	0	3
Mandeville Island	2016	3	0	1	0	0	3
Totals							
Wild	2014–2017	79	23	1	27	6	24
Released	2014–2017	6	2	3	0	0	6

Note: Abbreviations for pathogens tested: HEV, haemorrhagic enteritis virus; IBDV, infectious bursal disease virus; ILT; infectious laryngotracheitis; *n*, sample size; PM, *Pasteurella multocida*; PMV-1, Paramyxovirus type 1.

[a]Yolo Bypass = Yolo Bypass Wildlife Area (Yolo County); Gray Lodge = Gray Lodge Wildlife Area (Butte County); Mandeville Island = Mandeville Island Duck Club (San Joaquin County); Roosevelt Ranch = Roosevelt Ranch Duck Club (Yolo County); Lower Klamath = Lower Klamath National Wildlife Refuge (Siskiyou County); Upper Butte = Upper Butte Basin Wildlife Area (Butte County).

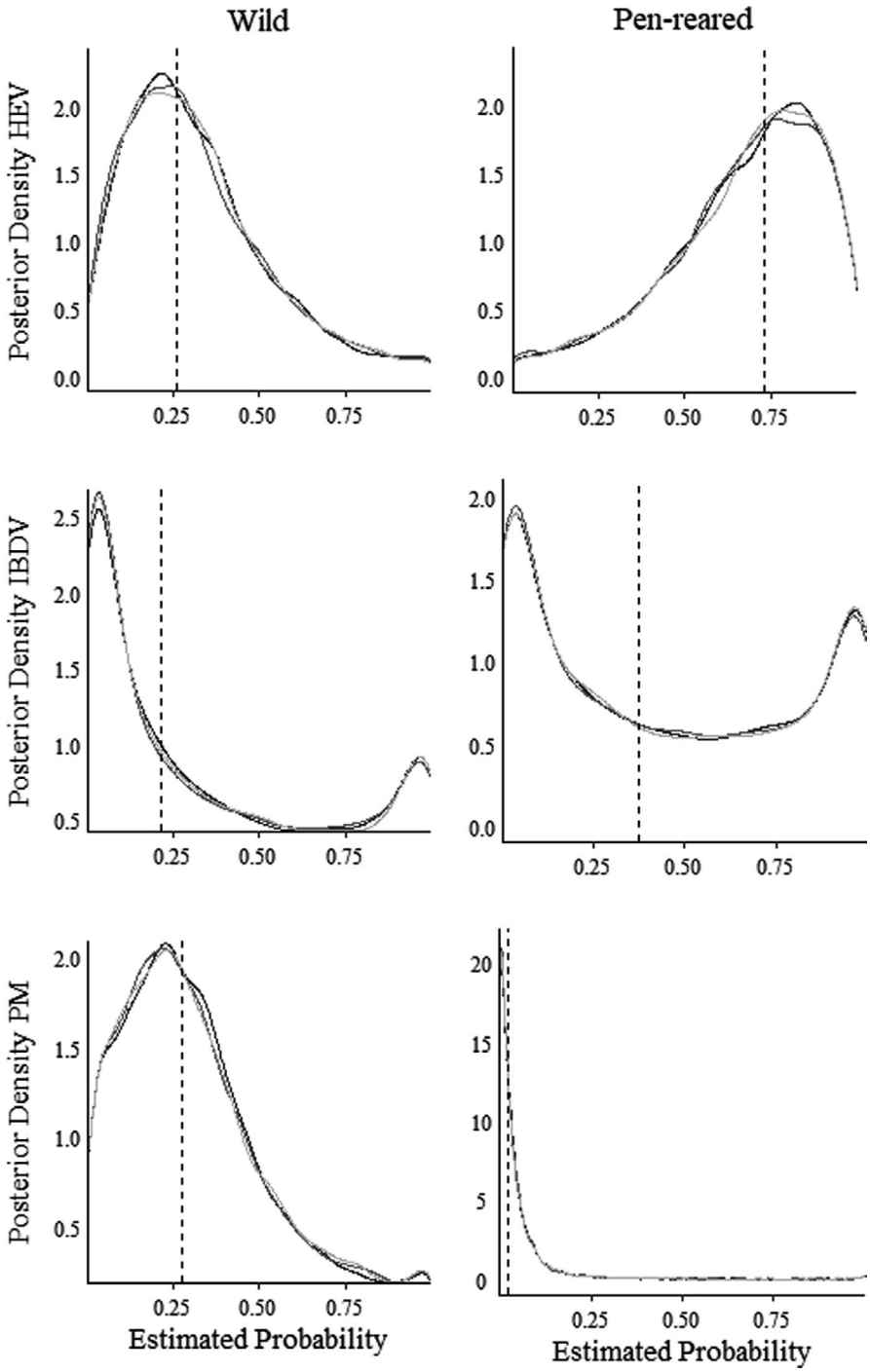

FIGURE 2 Posterior densities for the probability of pen-reared pheasants sampled from game bird farms and wild pheasant sampled at study sites in northern California, USA, testing positive for haemorrhagic enteritis virus (HEV), infectious bursal disease virus (IBDV) and *Pasteurella multocida* (PM). The dashed line represents the median value for the estimated probability of testing positive for a tested pathogen

4 | DISCUSSION

The practice of releasing captive-reared animals into wildlife habitat carries inherent risk, yet health surveillance is often overlooked in wildlife reintroduction programs (Matthews et al., 2006). Based on findings from this study, risk is apparent in the context of releasing captive-bred game birds for the purpose of population augmentation or increasing recreational opportunity. During this study, pheasants tested positive for antibodies specific to HEV at six of seven farms sampled during the study (Table 1), and virus was detected by PCR in pheasants at three farms (Table 4). Importantly, the marble spleen disease (MSD) virus in pheasants is almost serologically indistinguishable from HEV of turkeys and avian adenovirus splenomegaly of chickens (Pierson & Fitzgerald, 2008). Therefore, birds positive for HEV by PCR and ELISA may have actually been infected with the MSD virus. Both antigens can cause splenomegaly in pheasants (Fitzgerald & Reed, 1989), but MSD has been documented many more times in commercial pheasant production systems (Fitzgerald & Reed, 1989) and has been shown to be immunosuppressive in pheasants, chickens, and turkeys (Domermuth et al., 1979; Sharma, 1994). Spleens from necropsied birds that tested positive for HEV were enlarged, and the survey findings indicated that sampled game bird

breeders did not vaccinate for MSD virus using an HEV-derived vaccine (Sharma, 1994). Hence, HEV-positive pheasants most likely had ongoing MSD virus infections. In previous literature, MSD was considered one of the most important diseases impacting captive-reared pheasant production (Fitzgerald & Reed, 1989), but the availability of vaccines in more recent times has decreased its impact (Sharma, 1994). Therefore, if farms are not testing or vaccinating for MSD, it may be endemic on some premises without producers knowing it.

The necropsy findings provided important validation of ELISA test results, in that birds testing positive for HEV by ELISA were also positive for the infectious agent by PCR and showed clinical signs associated with a HEV or MSD virus infection (Table 2). Further, the ELISA results for HEV antigen detection were consistent across years, and at least one bird in both wild and pen-reared groups tested positive for HEV across almost all sampling locations (Tables 1 and 4). Although sample sizes from farms were small (5–10 birds per flock-year), individual pheasants sampled at the same farm were likely exposed to the same pathogens, especially if housed in the same enclosure within the farm. As a caveat, it is important to recognize that HEV of turkeys is not necessarily the same pathogen as MSD virus of pheasants, and therefore, results should be interpreted with caution. Whole genome sequencing of viral amplicons isolated from the spleens of sampled pheasants would better discern the exact aetiologic agent.

The implications of releasing birds infected with MSD virus rather than HEV are different with respect to commercial poultry and wildlife at release sites. The host-specific properties of MSD virus and HEV confer different clinical responses in different target species, and avirulent strains of both viruses have been used effectively to vaccinate pheasants and domestic turkey (Sharma, 1994). Although both viruses are transmissible to pheasants, turkeys, chickens and other game bird species (Pierson & Fitzgerald, 2008), HEV would be of greater concern if spread to the turkey industry as MSD virus is less pathogenic in non-target hosts (Sharma, 1994). Likewise, MSD virus is of greater concern to wild pheasants at release sites, and co-introducing this pathogen into wildlife habitat may decrease the efficacy of population augmentation. Game bird breeders did not vaccinate for HEV or MSD at the farms sampled in this study. One farmer reported noticeable depression, a decrease in food intake and a decline in egg-laying from his pen-reared birds. Hence, the virulence of the viral strain infecting the Siadenovirus positive pheasant flocks may have been variable across farms, and the viral load present at each seropositive farm may also have played a role in the severity of symptoms. Splenomegaly in the necropsied birds suggested immunodepression was likely within the infected flocks, but no outward clinical signs were observed prior to euthanasia.

Antibodies for ILT were not tested by ELISA in birds submitted for necropsy; only ILT PCR tests were conducted for those birds, which limited the sample size and precluded inference of past exposure to ILT in necropsied birds. Few wild or pen-reared pheasants tested positive for ILT or PMV-1, which also precluded model inference. Model estimates indicated that pheasants sampled in both pen-reared and wild groups appeared to be exposed to IBDV at similar rates (Figure 2). Lastly, pheasants captured at our field sites were often associated with seasonal wetlands, which may explain why PM was detected more often in wild pheasants than in pen-reared birds. Probability estimates for testing positive for a pathogen indicated large variation in positive samples possibly attributed to variation explained by sampling year and location. Based on model estimates, the difference in probability of antibody detection between pen-reared and wild groups was greatest for HEV, but this difference did not demonstrate a causal relationship that suggested HEV titres detected in wild birds originated from pen-reared birds.

Difficulties in acquiring enough independent samples also precluded the ability to reliably estimate prevalence across all groups as well as compare individual antibody titres of previously released pen-reared pheasants to the other groups (i.e. pen-reared and wild). Further, although we attempted to capture pen-reared pheasants at our field sites, too few were successfully captured and sampled to provide a good estimation of prevalence within this group. Trapping efforts on the hunting areas could only take place after the hunting season when most of the released pheasants had likely died or dispersed. In addition, pen-reared pheasants often have low rates of survival post-release (Musil & Connelly, 2009) and were hunted prior to trapping efforts. Hence, without a random block design in which wild and pen-reared pheasants are sampled in replicate across both environments (i.e. farms and wild pheasant sites), it is difficult to provide quantitative evidence that adequately demonstrates a difference in Siadenovirus prevalence between farms and wild pheasant study sites.

The farms chosen for the necropsy study were selected based on their proximity to public hunting areas, raising of game birds for release onto hunting areas, and their participation in NPIP. All five game breeders surveyed after necropsy were part of the NPIP, and 36 game breeders were listed on the California public game breeder list (CDFW, 2017). However, game breeders in California can elect not to have their name and contact information listed publicly, and not all farms enrolled in NPIP were listed on the public game breeder list. A total of six farms were enrolled in NPIP at the time of the study, which is less than 10% of licensed game bird farms in California (California Poultry Federation, personal communication). Larger farms may be more likely to list contact information publicly and participate in programs like the NPIP. Therefore, it is difficult to estimate the total number of game birds sold and released in California based on publicly available information.

Farmers primarily sought NPIP certification to legally transport birds across state lines, gain access to educational resources and maintain healthy flocks through preventive action. However, game bird producers that transported birds across state lines were required to be certified by NPIP as U.S. Pullorum-Typhoid Clean and U.S. MG clean. The farmers interviewed in the study participated in the certification programs based on where they transported birds. Specifically, all five interviewed farmers belonged to the North American Gamebird Association (NAGA) in addition to being NPIP

participants. At least two of the farm owners stated that they regularly attended annual conferences sponsored by NAGA. Game bird farms in this study were often multi-generational or had been established for at least 20 years. Farm owners that participated in the NPIP reported that they often shared information with other game bird breeders but were reluctant to consult with a veterinarian due to the added expense. Four of the five farmers that were interviewed did not regularly consult a veterinarian, but said they were willing to submit sick birds to the CAHFS diagnostic laboratory on an as-needed basis.

Survey questions were meant to gather information on production practices that are relevant to disease transmission risk and to gain some understanding of the level of biosecurity on the premises. Maintaining adequate biosecurity on game bird breeding farms presents inherent challenges given that an all-in-all-out production system is usually not feasible. New birds were introduced to the flocks throughout the year as hatch-outs occurred at regular intervals during the breeding season, essentially creating mixed-age flocks. Young birds were moved from indoor brooders to outdoor enclosures where they were held until they reached maturity and could be sold. The outdoor wire-mesh enclosures used for pheasants had vegetation growing inside, which was important for perching and for cover from the heat. However, this practice increased the likelihood of physical interaction between the pheasants, other wildlife and the outside environment. Game bird farm owners that were surveyed stated that small songbirds were also able to utilize the vegetation in the enclosures for perching and cover. Farmers reported that small mammals such as mice entered the enclosures in search of food and that predatory birds would sometimes attempt to capture pheasants from the top of the mesh enclosure. Furthermore, maintaining vegetation in the outdoor pens that house birds throughout the breeding season and longer precluded the ability to fully clean and sanitize the pens mid-season. Farmers waited until the end of the breeding season when birds have been sold before removing vegetation and fully cleaning the pens. Therefore, removing sick or dead birds from the pens likely did not prevent secondary exposure from contaminated litter or soil, which is the primary mode of transmission for MSD virus (Pierson & Fitzgerald, 2008).

The farm in Stanislaus County was the only game bird producer near (i.e. within 5 km) commercial poultry producers. This farm was also the largest with approximately 40,000 pheasants and 60,000 chukar raised annually. Likely due to the size of this farm, they employed a greater level of biosecurity relative to the other farms that participated in the survey. The Stanislaus farm had biosecurity signage at the entrance to the property, as well as foot baths at the entrances to every brooder house. They employed a variety of wildlife control measures, including traps and rodent bait stations, to minimize the interaction of wildlife with pheasants or other game birds raised on the property.

Although farms did not have a vehicle wash station, they did not allow people to come on the property without prior authorization. Only two of five farms used a wash station of any kind that was separate from foot baths, and two farms required vehicles to remain outside of the farm perimeter when clients or vendors visited the property (Table 4). Although game breeders interviewed during the study did not always adhere to biosecurity guidelines recommended by NPIP, in general, they understood the importance of minimizing points of contact that could lead to pathogen transmission on the farm. They did not share equipment such as crates, trailers or other farming equipment with other breeders. They also stated that they used their own vehicles and personnel to transport birds to release sites or to clients purchasing birds across state lines. Game breeders sought to balance biosecurity on the farm with the size of their flocks, and implementation of biosecurity guidelines was not necessarily equivalent to the game breeders' understanding of biosecurity. Rather, farmers likely weighed the risk of not following certain biosecurity principles with the cost of implementing that principle. However, adequate surveillance and preventive action is still likely the best means of minimizing the potential for disease to be released into wildlife environments or otherwise spill over into backyard flocks or commercial poultry.

5 | CONCLUSIONS

An apparent exposure of pen-reared pheasants to pathogens based on findings from our health surveillance, coupled with the common management practice of releasing these pheasants onto public and private lands where they likely interact with wild pheasants and other wildlife, substantiates the need for further investigation of disease prevalence in captive breeding and release systems. To our knowledge, our study is the first to quantify differences in apparent pathogen exposure and infection between pen-reared and wild pheasants, as well as characterize production practices for commercial game bird farms in California. Captive-rearing conditions, whether pheasants are housed in wire pens outdoors or in brood houses, inherently allow more contact between individuals than if pheasants are spread out across a wild population. These conditions and resulting release may facilitate pen-reared pheasants acting as a bridge host (Caron et al., 2015) in which pathogens such as MSD virus spread among a maintenance population of pheasants (i.e. on the farm), and upon release into wildlife habitat, those birds come into contact with a target population of pheasants as well as other wildlife. Studies that test for a wider range of pathogens (e.g. MSD virus) in pen-reared pheasants could prove very beneficial at informing farming practices that improve flock health for pen-reared and wild pheasants. The risk of pen-reared birds spreading disease among wild bird populations may be greater in other species reared for release, such as chukar, that have relatively larger home ranges than those of pheasants. Additional research that utilizes whole genome sequencing techniques can potentially link pathogens found on farms to those detected in birds from release sites, which allows for tracing of a pathogen from farm to the environment. Further, investigations of social networks formed by game bird farms, suppliers and customers could elucidate points of contact that may lead to pathogen transmission.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The US National Research Council's guidelines for the Care and Use of Laboratory Animals were followed.

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CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship, or publication of this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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