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Active and passive disease surveillance in wild turkeys (*Meleagris gallopavo*) from 2008 to 2018 in Pennsylvania, USA

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Abstract

There are increasing concerns about the effects of disease on wild turkeys (*Meleagris gallopavo*). Yet, many management agencies lack adequate data on wild turkey diseases and pathogens to address this concern. Toward that end, the Pennsylvania Game Commission increased surveillance efforts on wild turkeys beginning in 2013 (referred to hereafter as the enhanced surveillance period). From 2008–2018, 121 wild turkeys from Pennsylvania were submitted for necropsy, with 102/121 (84.3%) submitted during the enhanced surveillance period (2013–2018). We examined cases to determine causes of morbidity/mortality through gross and microscopic examinations and ancillary tests. The most common causes of morbidity/mortality in the examined wild turkeys were avian pox (66/121; 54.5%), chronic dermatitis (15/121; 12.4%), and trauma (10/121; 8.3%). We diagnosed additional diseases for the first time or more frequently during the enhanced surveillance period, including histomoniasis (7/121; 5.7%) and infectious sinusitis (1/121; 0.8%). Skin lesions were the most common cause of submission (94/121; 77.7%) and were most often attributed to avian pox (66/94, 70.2%), chronic dermatitis (15/94; 16.0%), or lymphoproliferative disease (3/94; 3.2%). During 2013–2018, tissues and sera were collected from any diagnostic cases and hunter-harvested turkeys to create a tissue repository. We used these samples to test for infection

or exposure to specific pathogens. We found that 75.3% (61/81) of wild turkeys were positive for lymphoproliferative disease virus, 61.9% (52/84) for *Heterakis gallinarum*, 28.6% (10/35) for *Toxoplasma gondii*, and 15.6% (15/32) for *Borrelia burgdorferi*. We detected antibodies (indicating exposure) to avian paramyxovirus-1 in 34.9% (22/63) of the wild turkeys and West Nile virus in 21% (13/62), but none were seropositive to influenza A viruses (0/62; 0%). The presence of diseases and pathogens in wild turkeys in Pennsylvania are being defined through active and passive surveillance approaches. Such data can begin to address the broader questions of disease impacts on wild turkey populations.

KEYWORDS

dermatitis, disease surveillance, lymphoproliferative disease virus, *Meleagris gallopavo*, poxvirus, West Nile virus, wild turkey

Perceived declines of wild turkey (*Meleagris gallopavo*) populations in the northeast are an increasing concern (Casalena et al. 2016). There are multiple potential contributors to these declines, including loss of habitat, weather, predation, and disease (Niedzielski and Bowman 2015, Casalena et al. 2016). Disease may affect wild turkeys at the individual or population level and through a variety of mechanisms, including direct morbidity/mortality, impaired growth rates, predisposal to other causes of mortality (e.g., predation or trauma), and decreased reproductive success (Ryser-Degiorgis 2013). Over the last 20 years, multiple pathogens have been identified in North America that could potentially impact the health of wild turkeys, including emerging (highly pathogenic avian influenza virus), re-emerging (*Histomonas meleagridis*), recently identified (lymphoproliferative disease virus, LPDV), or endemic (West Nile virus, WNV) pathogens. In addition to negatively affecting wild turkey health, some pathogens or contaminants harbored by wild turkeys may affect the health of humans, domestic animals, or other wildlife (e.g., *Salmonella typhimurium*, *Campylobacter jejuni*). However, many wildlife agencies lack adequate data on distribution of wild turkey diseases or pathogens to address broader questions relating to population impacts and the role that wild turkeys may play in the epidemiology of the diseases affecting them.

Disease surveillance in wildlife can focus on the detection of disease (i.e., deviation from normal function of any anatomic structure, organ, or system manifested in clinical signs or lesions) or the cause(s) of disease (e.g., pathogen or contaminants; referred hereafter as etiology). Disease data can be obtained through active or passive surveillance, which have their respective advantages and disadvantages. Passive surveillance involves the postmortem examination of sick and dead animals (or tissues) to determine the etiology of the observed morbidity/mortality (Stallknecht 2007). The primary advantage of passive surveillance is it provides data on occurrence of disease in a population. However, passive surveillance is inherently reliant on the detection of carcasses in the field and subsequent submission for postmortem examination. As such, passive surveillance is not ideal for accurate determination of prevalence or geographic distribution of a disease in a population. The quality of the data generated from passive surveillance is dependent on the condition of the carcasses (or tissues) examined. Consequently, there are numerous impediments and biases imposed on this surveillance approach in wildlife, including poor detection of carcasses in the field, scavenging, decomposition, availability of personnel and resources for appropriate diagnostic specimen handling prior to submission to a laboratory, and availability of diagnostic support for postmortem examinations. Finally, passive surveillance is reliant on postmortem examination of carcass.

As such, this is not a good approach for diseases that cause mild morbidity and no mortality or for pathogens that result in asymptomatic infections; instead, identification of these pathogens and diseases relies on active surveillance.

Active surveillance involves the targeted testing of a population or animal for a specific disease or etiology (Artois et al. 2009). A wide diversity of tissue samples can be collected from both live and dead animals depending on the pathogen or disease being targeted. A primary advantage of active surveillance is it can provide data on the distribution of pathogens and contaminants independent of disease or detection of disease. Many pathogens or contaminants that are harbored by wildlife result in subclinical or mild disease and, consequently, are unlikely to be detected through passive surveillance. Similarly, some diseases may selectively affect categories of animals (e.g., poults) that are unlikely to be detected through passive surveillance due to the aforementioned impediments. Thus, active surveillance can provide valuable data on the causes of disease even if morbidity/mortality does not result or is not detected. Where passive surveillance is dependent on opportunistic detection of sick or dead animals, active surveillance allows for a planned and systematic approach to disease/pathogen monitoring in a population, including selection of an appropriate study design for surveillance goals, determination of required sample size, definition of sample population, control of temporal and spatial variables for sample collection, and statistical analyses of data. Consequently, active surveillance can provide more accurate epidemiologic measures of a disease or etiology in a population, including prevalence, temporal patterns, and geographic distribution. One disadvantage of active surveillance is sampling can be expensive and time-consuming (e.g., live-animal trapping, management of check stations, road surveys for vehicle kills). Hence, active surveillance programs often focus on species and seasons when large numbers of samples can be obtained (e.g., hunting seasons). Active surveillance sampling can provide data on the distribution of pathogens or contaminants, even without clinical signs, but does not indicate anything about the disease itself, making it challenging to interpret the significance of the surveillance results to the individual animal or population.

When used in combination, active and passive surveillance provide complimentary data on occurrence of diseases in wildlife, as well as the distribution of pathogens and contaminants. For example, LPDV was first identified in a wild turkey with lymphoid tumors in 2009 via passive surveillance (Allison et al. 2014), which represented the first detection of this disease in North America as well as the first detection in wild turkeys. Prior to this detection, the virus had only been identified in domestic turkeys with lymphoid tumors in Europe and the Middle East (Ianconescu et al. 1983, Biggs 1997). Subsequent passive surveillance identified 5 additional wild turkeys with lymphoid tumors infected with LPDV (Allison et al. 2014). These detections led to concern among wildlife managers that LPDV was an emerging disease that could affect wild turkey populations in North America. To further investigate LPDV, multiple active surveillance projects were initiated throughout eastern North America (Allison et al. 2014, Alger et al. 2017). The results were consistent among studies, which indicated a high prevalence of LPDV infection among wild turkeys in North America, even in birds that were in apparent good health (Thomas et al. 2015, Alger et al. 2017, MacDonald et al. 2019*a*). Collectively, passive and active surveillance data defined the existing epidemiology of LPDV in wild turkeys; many turkeys are infected but only a small subset develops lymphoid tumors and associated clinical disease.

In response to increasing concerns of disease impacts on wild turkeys, the Pennsylvania Game Commission (PGC) initiated a multifaceted enhanced survey approach to generate data on diseases and pathogens in wild turkeys. Starting in 2013, the agency increased passive surveillance efforts by proactively soliciting the submission of diagnostic cases by agency personnel. Simultaneously, in 2013, a wild turkey tissue repository was created, both from diagnostic cases and hunter-harvested birds, that would enable active surveillance for specific pathogens or diseases, as warranted. Herein, we summarized data generated from the enhanced efforts and outline the value of this combined approach. We included data from 2008 to 2018 to highlight the increase in diagnostic submissions, and associated benefits, during the enhanced surveillance period that started in 2013.

METHODS

Passive surveillance

During 2008–2018, carcasses from wild turkeys that were found dead, euthanized due to severe clinical signs, or had outward signs of disease when harvested by hunters were submitted to the Pennsylvania Animal Diagnostic Laboratory System (PADLS), Southeastern Cooperative Wildlife Disease Study (SCWDS), or the PGC wildlife veterinarian for necropsy to determine cause of morbidity or mortality. Carcasses were collected and submitted for necropsy sporadically from 2008–2013. Beginning in 2013, efforts were initiated to increase wild turkey diagnostic case submissions via annual intra-agency training and requests to agency personnel for submission of all wild turkeys that were found dead without an obvious cause of mortality.

Data for each wild turkey case were collected on an agency diagnostic submission form that was submitted with carcasses to the laboratory, including age, sex, date when found (alive or deceased), location and environment found, and clinical signs (if observed). All carcasses or tissues were examined grossly for lesions at necropsy. Histologic examination of tissues and other ancillary diagnostic tests were performed as needed to determine the cause(s) of morbidity, mortality, or gross lesions. At necropsy, spleen, liver, heart, kidney, lung, bone marrow, and serum were collected from all birds, when available, and immediately stored in the PGC wild turkey tissue repository at −80°C. The intestinal tract from distal small intestine to vent (including ceca) was also collected and stored at −20°C to examine for the cecal nematode *Heterakis gallinarum* (see Active Surveillance below).

Historical and demographic data, diagnostic results, and primary diagnoses for each case were extracted from each wild turkey submission and its respective diagnostic report. Primary diagnoses were grouped into one of the following categories: avian pox, lymphoproliferative disease (lymphoid tumors), pasteurellosis (i.e., systemic bacterial infection with *Pasteurella multocida*), other systemic bacterial infection, chronic dermatitis (i.e., including both cases in which the causative agent was identified [bacterial and fungal], and those in which it was not determined), infectious sinusitis (*Mycoplasma* spp.), focal bacterial infection (i.e., abscesses), pneumonia, histomoniasis (*Histomonas meleagridis*), nematodiasis, trauma, hyperkeratosis, and undetermined.

We used Fisher's exact tests to examine associations in the proportions of wild turkeys testing positive for avian pox and/or chronic dermatitis and age and sex of the wild turkey, anatomical distribution of the disease, and season that the wild turkey was collected. We separated samples into 4 seasons for analysis of association between season and proportion testing positive: spring = March–May; summer = June–August; fall = September–November; and winter = December–February. We calculated odds ratios when Fisher's exact tests were statistically significant (Conover 1999). Threshold for statistical significance was $\alpha = 0.05$.

Active surveillance

As described above, spleen, liver, heart, lung, kidney, bone marrow, lower intestinal tract, and serum were collected from diagnostic cases for the PGC wild turkey sample repository beginning in 2013. Samples were also opportunistically collected from outwardly healthy hunter-harvested birds at check stations during organized hunts or from individual hunters that voluntarily collected tissues starting in 2015. Tissues were collected by a veterinarian or biologist, using a sterile scalpel blade, from all birds that delivered to the check station that were not field dressed. Tissue samples were placed in whirl-pak® bags and serum samples centrifuged, and both were then stored on ice packs in the field and placed in the sample repository at −80°C within 24 hours of collection. Banked samples were used, as warranted based on surveillance or research needs, to screen for diseases, pathogens, or exposure to pathogens that were of concern. All tests for specific pathogens were performed using published procedures (Table 3). None of the tissues that were collected from diagnostic cases were tested for pathogens that were the cause of morbidity or mortality in that host.

We tested serum samples in the repository for exposure (i.e., antibodies) to *Toxoplasma gondii* (Cerqueira-Cezar et al. 2019) and flaviviruses (e.g., West Nile virus and St. Louis Encephalitis Virus; Nemeth et al. 2021). We tested for avian paramyxovirus serotype-1 (APMV-1) using a commercially available ELISA kit (ProFLOK Newcastle Disease Virus (NDV) Antibody Test Kit; Zoetis, Parsippany, NJ, USA) and influenza A virus (IAV) by agar gel immunodiffusion (Thayer and Beard 2008). We molecularly tested bone marrow samples for proviral DNA of LPDV using PCR targeting a portion of the *gag* polyprotein (Thomas et al. 2015). None of the turkeys tested for LPDV had any evidence of lymphoid tumors. We molecularly tested spleen and bone marrow samples for infection with *Borrelia* spp. using a nested PCR targeting the flagellin gene that amplifies DNA from all *Borrelia* spp., including *B. burgdorferi* and *B. miyamotoi*, both of which have been reported in wild turkeys (Cleveland et al. 2020). The amplicons were sequenced from all positive samples to identify the species of *Borrelia*. We examined the ceca and large intestine samples for *Heterakis* spp. by emptying contents into a 1-mm sieve. We gently washed the content with water and examined the sieve for *Heterakis* spp. (Greenawalt et al. 2020). We counted all *Heterakis* spp. in a sample. All males were cleared (i.e., dehydrant was replaced with a fluid miscible with paraffin wax) using lactophenol and the species was identified based on morphologic characteristics. We confirmed female worms to be *Heterakis* spp., but the species cannot be identified based on morphology.

RESULTS

Passive surveillance

During 2008–2018, 121 wild turkeys were submitted for necropsy, 84.3% of which were submitted during the enhanced surveillance period (2013–2018; Figure 1). During 2008–2012, the mean annual wild turkey case load

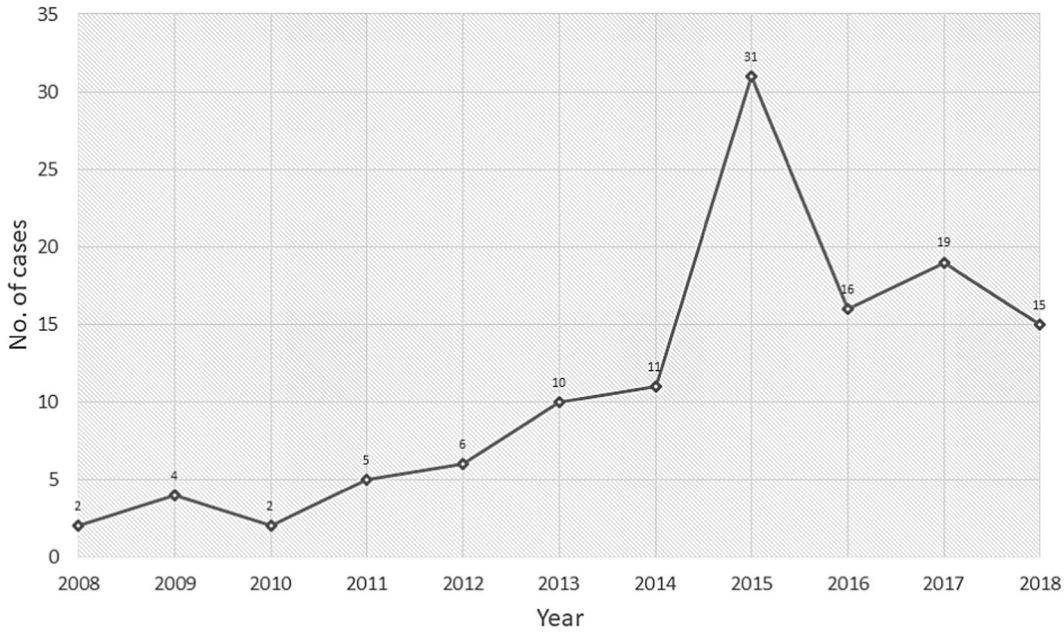

FIGURE 1 The number of wild turkey diagnostic cases submitted to the Pennsylvania Animal Diagnostic Laboratory System, Southeastern Cooperative Wildlife Disease Study, or Pennsylvania Game Commission, 2008–2018. Case submission is shown to increase during the enhanced surveillance period from 2013–2018.

was 3.8 (range = 2–6), whereas the mean annual case load once enhanced surveillance was initiated in 2013 was 17 (range = 10–31). Of the 121 wild turkeys necropsied, 90 (74.4%) were adults, 29 (24.0%) were juveniles, and 2 (1.7%) were of undetermined age. In addition, 72 (59.5%) were males, 47 (38.8%) were females, and sex was not determined in 2 (1.7%). By season, 33 (27.3%) were submitted during spring months, 15 (12.4%) in the summer, 53 (43.8%) in the fall, and 20 (16.5%) during the winter.

Avian pox was the primary cause of wild turkey morbidity/mortality during the entire study period (2008–2018) (Table 1). In addition to increasing number of annual cases, a greater diversity of diseases was identified in wild turkeys during the enhanced surveillance period. From 2008–2012, there were 19 primary diagnoses. Avian pox, chronic dermatitis, and trauma were the most common diagnoses (68.4%) during this period. During enhanced surveillance from 2013–2018, 102 primary diagnoses were made. Although avian pox, chronic dermatitis, and trauma remained the most common diagnoses (76.5%) during the enhanced surveillance period, we identified additional diseases of potential concern, including histomoniasis and infectious sinusitis.

Avian pox was the most common cause of morbidity/mortality (66/121; 54.5%), followed by chronic dermatitis (15/121; 12.4%) and trauma (10/121; 8.3%). Trauma was from a variety of sources, including penetrating wounds, blunt force trauma (e.g., collision with vehicles), fractures, and shotgun injuries. We observed skin lesions (Figure 2), largely affecting the unfeathered skin of the head, neck, and legs, in 77.7% of samples (94/121; Table 2). The most common cause of skin lesions was avian pox (66/94; 70.2%), followed by chronic dermatitis (15/94; 16.0%) and lymphoproliferative neoplasia (3/94; 3.2%). We identified a diversity of causes for skin lesions in the remaining 10.6% (10/94) of cases, including pasteurellosis, hyperkeratosis, and trauma.

For wild turkeys with avian pox, 98.5% (65/66) had proliferative skin lesions (i.e., dry pox) involving the head/neck, and a single turkey had wet pox lesions (in the oropharynx) without concurrent skin lesions. Of these cases

TABLE 1 Diagnostic category of mortality/morbidity cause, number diagnosed, and 95% confidence interval (CI) for 121 wild turkeys submitted to the Pennsylvania Animal Diagnostic Laboratory System, Southeastern Cooperative Wildlife Disease Study, and Pennsylvania Game Commission during passive surveillance, 2008–2018.

	Turkeys (n = 121)	
Category/Disease	**Number (%)**	**95% CI[a]**
Avian pox	66 (54.5)	45.7–63.1
Chronic dermatitis	15 (12.4)	7.7–19.4
Trauma	10 (8.3)	4.6–14.5
Undetermined	9 (7.4)	4.0–13.5
Histomoniasis	7 (5.7)	2.8–11.5
Lymphoproliferative disease	4 (3.3)	1.3–8.2
Pneumonia	2 (1.6)	0.5–5.8
Hyperkeratosis	2 (1.6)	0.5–5.8
Pasteurellosis	1 (0.8)	0.1–4.5
Infectious sinusitis	1 (0.8)	0.1–4.5
Chronic hepatitis	1 (0.8)	0.1–4.5
Systemic bacterial infection	1 (0.8)	0.1–4.5
Focal bacterial infection (abscesses)	1 (0.8)	0.1–4.5
Nematodes (*Ascarida* sp., *Heterakis* sp.)	1 (0.8)	0.1–4.5

[a]Wilson calculation method used to determine 95% confidence interval (CI).

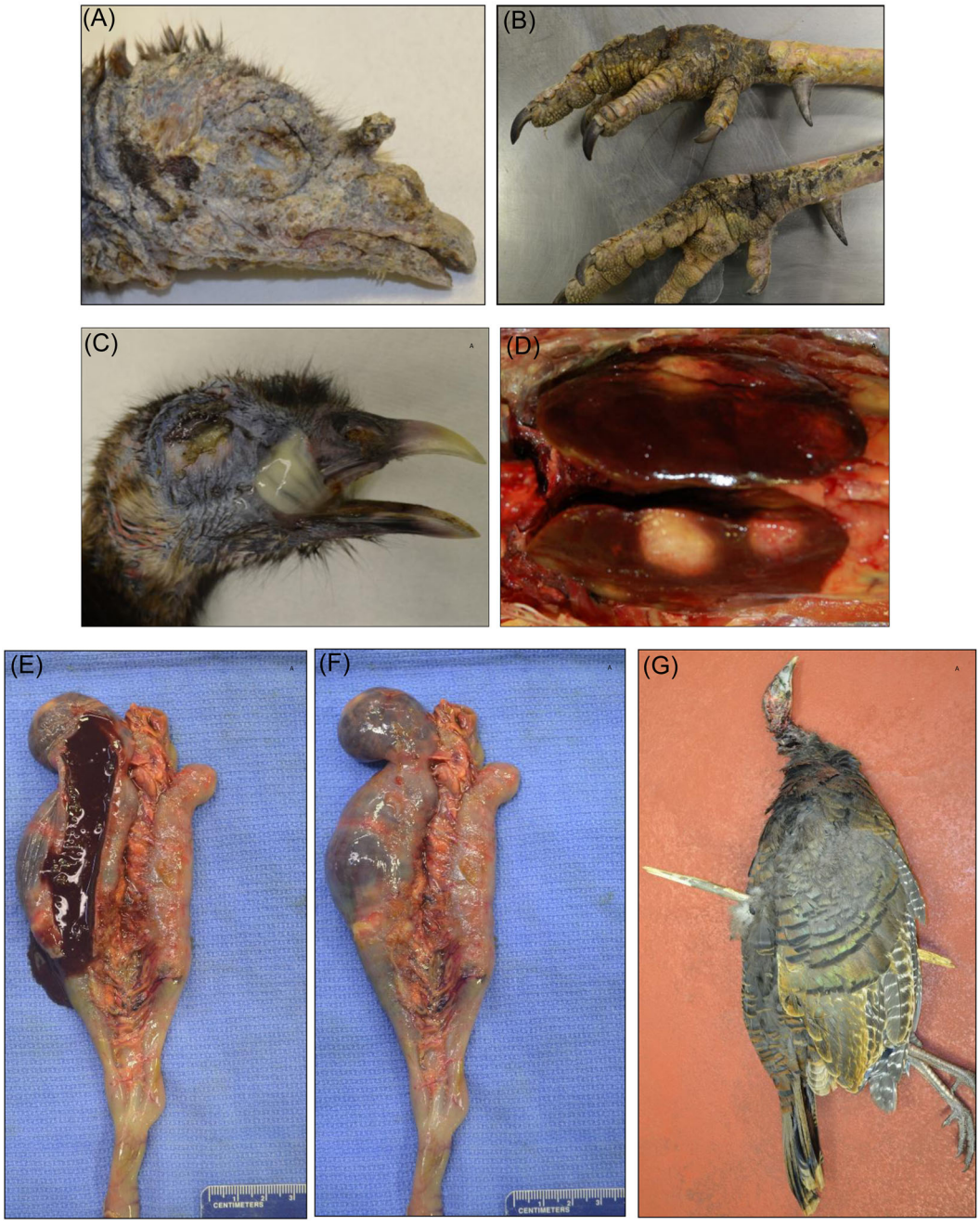

FIGURE 2 Gross lesions for various diseases diagnosed in wild turkeys from Pennsylvania through enhanced passive surveillance efforts from 2013 to 2018: chronic bacterial dermatitis (A, B), infectious sinusitis (C), blackhead (D, E, F), trauma (G), and avian pox (H, I).

with skin lesions on the head/neck, only 7.7% (5/65) also had proliferative skin lesions on the legs. No avian pox cases had proliferative skin lesions on the legs while being absent from the head/neck. Yellow/tan plaques on the mucosa of the oropharynx, esophagus, or trachea (i.e., wet pox) were observed in 72.7% (48/66) of pox cases. All but one of the turkeys with wet pox also had proliferative skin lesions on the head/neck consistent with dry pox.

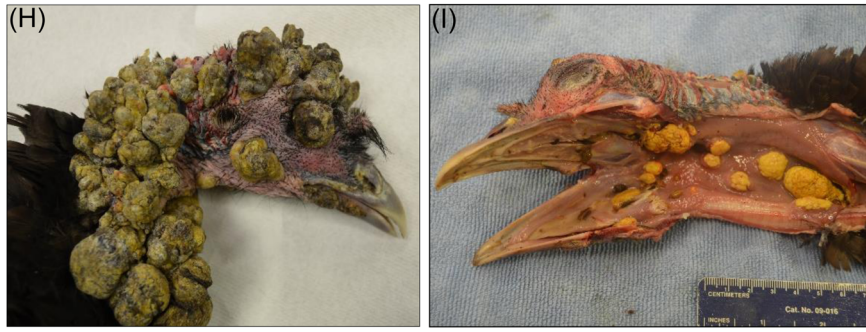

FIGURE 2 (Continued)

TABLE 2 Total number and anatomic distribution of skin lesions by disease category for 94 wild turkeys submitted to the Pennsylvania Animal Diagnostic Laboratory System, Southeastern Cooperative Wildlife Disease Study, and Pennsylvania Game Commission during passive surveillance, 2008–2018. Of the 121 wild turkeys examined during this period, 27 did not have skin lesions.

	Head and neck	Diphtheritic	Feathered skin	Legs	Feet
Avian pox	65	48	5	5	0
Chronic dermatitis	13	3	2	7	3
Lymphoproliferative disease	2	3	0	0	0
Other[a]	5	2	4	3	0

[a]'Other' includes pasteurellosis, hyperkeratosis, trauma, and undetermined.

There was no significant association for a diagnosis of avian pox and sex (males vs. females; Fisher's exact test $P = 0.57$), or age (adult vs. juveniles; Fisher's exact test $P = 0.14$). Turkeys with skin lesions on the head/neck were approximately 114 times more likely to test positive for avian pox (versus negative for pox; Fisher's exact test $P < 0.01$; odds ratio [OR] = 113.75; confidence interval [CI] = 14.64–883.57). Wild turkeys with yellow/tan plaques on the mucosa of the gastrointestinal or respiratory tract were approximately 16 times more likely to test positive for avian pox (versus negative for pox; Fisher's exact test $P < 0.01$; OR = 15.67, CI = 6.21–39.50). Skin lesions on the feathered skin of wild turkeys were not significantly associated with a diagnosis of avian pox (Fisher's exact test $P = 0.54$), nor were skin lesions on the legs and/or feet (Fisher's exact test $P = 0.10$). In addition to anatomic distribution of lesions, there were significant seasonal trends for avian pox cases in wild turkeys. Wild turkeys with skin lesions in fall or winter, when lesions were most often seen, were approximately 10 times more likely to test positive for avian pox than for chronic dermatitis (the second most common cause of skin lesions in wild turkeys) (Fisher's exact test $P < 0.01$; OR = 10.20; CI = 2.87–36.31).

Among the 15 wild turkeys diagnosed with chronic dermatitis, there was one (6.7%) with *Trichophyton* spp. identified, one (6.7%) with *Pasteurella multocida*, one (6.7%) with *Listeria monocytogenes*, and one (6.7%) with a mixed culture comprised mainly of *Staphylococcus aureus*. Also, one (6.7%) turkey had a mixed culture of *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*; one (6.7%) had *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus* sp., and 9 (60%) had no causative agent determined. Most of turkeys with chronic dermatitis (13/15; 86.7%) had lesions on the skin of the head and neck. Of the cases with head/neck skin lesions, 46.1% (6/13) also had lesions on the unfeathered skin of the legs. Two (13.3%) chronic dermatitis cases included lesions on the unfeathered skin of the legs, but not the head or neck. We observed diphtheritic lesions in 20.0%

TABLE 3 Summary of pathogen detection or pathogen exposure for wild turkey tissues obtained from the Pennsylvania Game Commission sample repository during active surveillance, 2013–2018.

Pathogen	Test	Sample	No. Pos./ Total (%)	95% CI[a]	Testing Facility[b]	Reference
Lymphoproliferative disease virus	PCR	Bone marrow	61/81 (75.3%)	0.6–0.8	SCWDS	Thomas et al. 2015
Heterakis gallinarum	Direct exam	Cecal content	52/84 (61.9%)	0.5–0.7	PGC Vet	Greenawalt et al. 2020
Avian paramyxovirus–1	ELISA (serology)	Serum	22/63 (34.9%)	0.2–0.5	PADLS	N/A
Toxoplasma gondii	MAT (serology); Bioassay	Serum, Heart	5/15 (33.3%); 5/20 (25.0%)	0.2–0.6; 0.1–0.5	USDA	Cerqueira-Cezar et al. 2019
Flaviviruses	PRNT (serology)	Serum	18/62 (29.0%)	0.2–0.4	SCWDS	Nemeth et al. In Press
Borrelia burgdorferi	PCR	Bone marrow &/or spleen	5/32 (15.6%)	0.1–0.3	SCWDS	Cleveland et al. 2020
Influenza A virus	AGID (serology)	Serum	0/62 (0.0%)	0.0–0.1	PADLS	Thayer and Beard 2008

[a]Wilson calculation method used to determine 95% confidence interval (CI).

[b]SCWDS = Southeastern Cooperative Wildlife Disease Study; PGC Vet = Pennsylvania Game Commission Wildlife Veterinarian; PADLS = Pennsylvania Animal Diagnostic Laboratory System; USDA = United States Department of Agriculture.

(3/15) of chronic dermatitis cases; all 3 of these turkeys also had lesions on the skin of the head and neck. Occurrence of chronic dermatitis was not different between males and females (Fisher's exact test $P = 0.76$), or between adult and juvenile birds (Fisher's exact test $P = 0.59$). There was no association between skin lesions on the head or neck and a diagnosis of chronic dermatitis (Fisher's exact test $P = 0.14$). When wild turkeys had lesions in the diphtheritic regions, they were about 77% less likely to test positive for chronic dermatitis than poxvirus (Fisher's exact test $P = 0.03$; OR = 0.23; CI = 0.06–0.84). There was no association between lesions on the feathered skin and chronic dermatitis (Fisher's exact test $P = 0.16$). When legs and feet were not affected by lesions, turkeys were 14 times more likely to not test positive for chronic dermatitis (Fisher's exact test $P < 0.01$; OR = 14.00; CI = 4.03–48.59).

Active surveillance

We detected lymphoproliferative disease virus proviral DNA in 75.3% (61/81) of the wild turkey bone marrow samples. *Heterakis* spp. were detected in the cecal contents of 61.9% (52/84) of the wild turkeys examined, and were identified as *Heterakis gallinarum* based on morphology. Infected wild turkeys generally harbored a moderately high nematode burden (mean = 16; range = 1–153) of *H. gallinarum* in their ceca. We detected *Borrelia* spp. in the spleen and/or bone marrow of 15.6% (5/32) turkeys. The *Borrelia* species detected was genetically identified as *B. burgdorferi*. Evidence of prior exposure to the following pathogens was detected in the serum of wild turkeys: *T. gondii* (10/35; 28.6%), WNV (13/62; 21.0%), and APMV-1 (22/63; 34.9%). None of the wild turkeys had detectable antibodies to IAV (0/62; 0%).

DISCUSSION

Efforts to enhance wild turkey diagnostic case submissions were successful, resulting in an approximately 5-fold increase in annual case submissions. The increased number of cases allowed for more robust data on both common (e.g., avian pox and chronic dermatitis) and less common diseases, as well as characterization of pathologic and seasonal trends. The increased number of cases also allowed for sample collection and creation of the PGC wild turkey tissue repository, which has been used to survey for multiple pathogens. Finally, the enhanced passive surveillance allowed for the identification of multiple diseases that had not been previously documented in wild turkeys in Pennsylvania, including histomoniasis and infectious sinusitis.

Consistent with previous studies, lesions on the unfeathered skin of the head, neck, and distal legs were the most common cause of morbidity/mortality diagnosed in wild turkeys (Elsmo et al. 2016). Lesions are highly visible and can result in debilitating disease; therefore, they are more likely to be noticed and submitted for necropsy by the general public (Davidson et al. 1985, Elsmo et al. 2016, MacDonald et al. 2016). Skin lesions in wild turkeys are diagnostically challenging because multiple diseases can produce grossly indistinguishable lesions and consequently, histology or ancillary tests are required for diagnosis. Consistent with previous studies, the most common cause of skin lesions in wild turkeys in this study was avian pox, followed by chronic dermatitis (Elsmo et al. 2016, Hydock et al. 2018, MacDonald et al. 2019*b*). Avian pox and chronic dermatitis cases in our study had seasonal and pathologic trends. Although histology and laboratory testing are necessary to confirm a diagnosis, the trends may provide some insights into the most likely causes of skin disease when carcasses or tissues are not available for examination (e.g., trail camera pictures or hunter harvested birds for which the carcass is no longer available). For wild turkeys with chronic skin lesions in the fall and winter, avian pox was the most likely cause. The seasonal occurrence of avian pox likely relates to increased viral transmission (i.e., via insect vectors) during summer and fall (van Riper et al. 2002). A greater prevalence of avian pox has been reported to correlate with higher vector abundance during the warmer, humid months (Akey et al. 1981, van Riper et al. 2002). If a wild turkey had skin lesions on the unfeathered skin of the head and neck it was more likely to be avian pox than other diagnoses. Similarly, if the wild turkey had yellow to tan plaques on mucosa of the upper gastrointestinal or respiratory tracts it was more likely to be avian pox.

We attributed most cases of chronic dermatitis to bacterial or fungal infections; however, in many cases the causative organism was not identified. The chronicity of lesions often presents a challenge in determining the etiology (i.e., the inciting pathogens have been cleared but the resulting lesions remain). For those chronic dermatitis cases in which bacteria were cultured, numerous species were isolated, including *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Listeria monocytogenes*, and *Bacillus* sp. Most of the species associated with chronic dermatitis are ubiquitous bacteria and/or normally occur on the skin of wild turkeys. Consistent with previous studies, there did not seem to be a single species of bacterium that was associated with these lesions; rather, multiple species commonly were cultured (Thogmartin et al. 1999, MacDonald et al. 2016). Most of the birds with chronic dermatitis were submitted during the summer and spring; however, sample size was insufficient to infer trends based on seasonal distribution. If skin lesions were observed on the legs, they were most likely to be caused by chronic dermatitis.

A high prevalence of infection with LPDV and *H. gallinarum* was detected in wild turkeys in Pennsylvania and is consistent with results from wild turkeys throughout the eastern United States and Canada (Allison et al. 2014, Alger et al. 2017, MacDonald et al. 2019*a*, *c*). As described above, our data provide perspective to the increasing, but relatively rare, reports of wild turkeys with lymphoproliferative disease. There are many unanswered questions relating to LPDV in wild turkeys, including the mode of transmission, impacts on poults, factors associated with development of disease, or whether other disease syndromes are associated with viral infection, especially because LPDV may result in immunosuppression (Niedringhaus et al. 2019). Additional research is needed to address these questions and more fully understand the effects of LPDV on wild turkeys. *Heterakis gallinarum* has frequently been reported in wild turkeys in the U.S. (Davidson and Wentworth 1992),

and although generally considered non-pathogenic in turkeys, it is the vector for *Histomonas meleagridis* (McJunkin et al. 2003). *Histomonas meleagridis* is the causative agent of histomoniasis, or blackhead disease, and can be an important factor in wild turkey mortality (Davidson et al. 1985). Although wild turkeys commonly harbor *H. gallinarum*, it currently is unknown how frequently these *Heterakis* nematodes are infected with *H. meleagridis*.

We detected a low prevalence of *T. gondii* and *B. burgdorferi* in wild turkeys in Pennsylvania. *Toxoplasma gondii* is a protozoan parasite that can cause toxoplasmosis in humans and animals and has been reported in numerous wildlife species, including wild turkeys (Quist et al. 1995). Although 25% of the wild turkeys in our study tested positive for *T. gondii*, related morbidity or mortality was not identified and appears to be uncommon. Toxoplasmosis is a potential cause of foodborne illness in humans; however, adequate cooking of meat will inactivate the parasite and prevent transmission of *T. gondii* from wild turkeys and other wildlife to humans. Recently, there was a report of *T. gondii* transmission from white-tailed deer (*Odocoileus viriginianus*) to hunters in Quebec, Canada who developed acute toxoplasmosis after consuming undercooked meat (Gaulin et al. 2020). In the eastern United States, *Ixodes* spp. ticks are the vectors for *B. burgdorfori*, the causative agent of Lyme disease (Scoles et al. 2001, Olsen 2007, Hamer et al. 2010). Previous studies conducted on wild turkeys in Tennessee and California also reported *B. burgdorfori*, with positive detections ranging from 1.1% (1/90) to 9.3% (21/226; Lane et al. 2006, Jordan et al. 2009). Data such as these are critical to fully understanding the epidemiology of Lyme disease, its vectors, and wild turkeys as potential reservoirs.

We documented evidence of previous exposure to a variety of viral pathogens in wild turkeys in Pennsylvania. Avian paramyxovirus-1 is a pathogen with potential human, domestic poultry, and wildlife health impacts, and is the causative agent of Newcastle disease. Antibodies to APMV-1 have previously been reported in wild turkeys in Arkansas and California, at similar and lower prevalence, respectively; however, related morbidity or mortality has not been reported (Hopkins et al. 1990, Charlton 2000). Future active surveillance studies are warranted to determine the prevalence, viral diversity, and epidemiology of APMV-1 infection in wild turkeys. West Nile virus has previously been implicated in the decline of ruffed grouse in Pennsylvania (Nemeth et al. 2017, Stauffer et al. 2018); however, the potential impacts of WNV on wild turkeys are not well-documented. Swayne et al. (2000) inoculated domestic turkey poults with WNV and results suggested that this virus is not a major disease concern for turkeys, although experimental infection on wild turkeys was not performed. Our results showed that nearly a quarter of wild turkeys tested in Pennsylvania had antibodies to WNV, indicating that they had been previously infected with the virus and survived. Additional research is needed to build upon and appropriately interpret these data. Specifically, it is unknown how susceptible wild turkeys are to WNV infection, and how many birds that are infected die. No antibodies to IAV were detected in wild turkeys from Pennsylvania., which is consistent with previous surveys for IAV in wild turkeys using serology or viral detection (molecular or virus isolation; Stallknecht et al. 2007, Jennelle et al. 2017, MacDonald et al. 2019*c*). Collectively, these results indicate wild turkeys have little role in the natural history or epidemiology of IAV. However, this role could change with future emergent IAV strains that may be highly virulent for gallinaceous species, and the role of wild turkeys may need to be reassessed.

CONCLUSIONS

The first step in assessing the potential effects of disease on wild turkeys is to define the pathogens and diseases that occur within populations. A combined approach of active and passive surveillance provides complementary data that begin to address this question. Over the last 20 years, we observed multiple pathogens emerge or re-emerge that could significantly impact the health of wild turkeys. The multifaceted surveillance approach described herein provides the reader with a process for collecting data on the diseases and pathogens that circulate

in wild turkeys. Such data are critical for interpreting emerging, re-emerging, or common diseases, communicating with the general public or hunters on diseases they observe, or collaborating with colleagues in other sectors on transboundary diseases (e.g., agriculture or public health). In addition, this approach provides a banked sample that can be tested for future pathogens that may arise.

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ETHICS STATEMENT

No ethical information provided.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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