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RISK FACTORS FOR AND SPATIAL DISTRIBUTION OF LYMPHOPROLIFERATIVE DISEASE VIRUS (LPDV) IN WILD TURKEYS (*MELEAGRIS GALLOPAVO*) IN NEW YORK STATE, USA

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ABSTRACT: Lymphoproliferative disease virus (LPDV) is an oncogenic avian retrovirus that was previously thought to exclusively infect domestic turkeys but was recently shown to be widespread in Wild Turkeys (*Meleagris gallopavo*) throughout most of the eastern US. In commercial flocks, the virus spreads between birds housed in close quarters, but there is little information about potential risk factors for infection in wild birds. Initial studies focused on distribution of LPDV nationally, but investigation of state-level data is necessary to assess potential predictors of infection and detect patterns in disease prevalence and distribution. We tested wild turkey bone marrow samples (n=2,538) obtained from hunter-harvested birds in New York State from 2012 to 2014 for LPDV infection. Statewide prevalence for those 3 yr was 55% with a 95% confidence interval (CI) of 53–57%. We evaluated a suite of demographic, anthropogenic, and land cover characteristics with logistic regression to identify potential predictors for infection based on odds ratio (OR). Age (OR=0.16, 95% CI=0.13–0.19) and sex (OR=1.3, 95% CI=1.03–1.24) were strong predictors of LPDV infection, with juveniles less likely to test positive than adults, and females more likely to test positive than males. The number of birds released during the state's 40-yr translocation program (OR=0.993, 95% CI=0.990–0.997) and the ratio of agriculture to forest cover (OR=1.13, 95% CI=1.03–1.19) were also predictive of LPDV infection. Prevalence distribution was analyzed using dual kernel density smoothing to produce a risk surface map, combined with Kulldorff's spatial scan statistic and the Anselin Local Moran's I to identify statistically significant geographic clusters of high or low prevalence. These methods revealed the prevalence of LPDV was high (>50%) throughout New York State, with regions of variation and several significant clusters. We revealed new information about the risk factors and distribution of LPDV in New York State, which may be beneficial to game bird managers and producers of organic or pasture-raised poultry.

Key words: Cluster detection, dual kernel density, LPDV, lymphoproliferative disease virus, *Meleagris gallopavo*, New York State, Wild Turkeys.

INTRODUCTION

Lymphoproliferative disease virus (LPDV) is one of three oncogenic avian retroviruses responsible for neoplastic disease in wild and domestic fowl (Payne and Venugopal 2000). Previous outbreaks occurred in the 1970s and were limited to commercial fattener flocks in Israel, Great Britain, and the Netherlands (Biggs et al. 1978). In these early cases, infected poults showed few clinical signs, and death occurred as quickly as 3–6 wk after infection (Biggs 1978; Gazit et al. 1979). This rapid clinical course, combined with subsequent flock mortality as high as 25%, initially led some researchers to believe the virus could be economically damaging (Schwarzbard et al. 1980). Chickens (*Gallus gallus domesticus*) were also found to be susceptible to LPDV—developing both viremia and disease in challenge studies—furthering concern for the agricultural industry (Ianconsecu et al. 1983). Ultimately, however, outbreaks were limited within only a handful of countries (Patel and Shilleto 1987; Yaniv et al. 1995) and have not reemerged in commercial poultry flocks on a substantial scale (Payne and Venugopal 2000).

In 2009, researchers detected LPDV in Wild Turkeys (*Meleagris gallopavo*) in the US (Allison et al. 2014). Follow-up surveillance detected LPDV throughout the eastern US and as far west as Colorado, with prevalence

estimates suggesting this virus is endemic in Wild Turkey populations (Thomas et al. 2015). In wild birds, the virus can induce internal or cutaneous tumor formation causing direct mortality or blindness and lack of mobility leading to starvation (Allison et al. 2014). Dead Wild Turkeys—particularly young birds—can be difficult to recover from the landscape, which makes fully assessing mortality from LPDV difficult (Thomas et al. 2015); however, current research suggests that death from LPDV is rare in wild birds (Allison et al. 2014). Large-scale die-offs mirroring those in commercial flocks have not been reported, and initial studies of hunter-harvested turkeys revealed that many birds test positive but exhibit no gross lesions internally or externally (Allison et al. 2014; Thomas et al. 2015). These subclinical infections make it difficult to determine the potential population-level effects of this pathogen. Decreased reproductive success and immunosuppression are characteristic of subclinical infections from other avian retroviruses (Gingerich et al. 2002; Guo et al. 2010), but it is not clear whether that is the case with LPDV (Zimber et al. 1983; Allison et al. 2014).

Disease transmission in Wild Turkeys may occur in several ways. Horizontal transmission between domestic poults was demonstrated early on (McDougall et al. 1978), and contact-related transmission is possible for wild birds because of their seasonal flocking behavior. Vertical transmission, which involves the passage of a pathogen from mother to offspring prenatally, postnatally, or perinatally (Weiss 1996), has been documented in two other avian retroviruses—avian leukosis virus and reticuloendotheliosis virus—through the shedding of infectious virions from the mother into the albumin of the egg (Witter and Salter 1989; Venugopal 1999). This route has been neither confirmed nor ruled out for LPDV (Allison et al. 2014). Vector-mediated transmission by mosquitoes or other insects is also a potential route of infection that has been demonstrated in reticuloendotheliosis virus (Motha et al. 1984), but, so far, there is no evidence this is occurring with LPDV (Allison et al. 2014).

Wild Turkey populations throughout their native range have recovered remarkably well from midcentury lows, largely because of restoration programs that depended on translocation (Tapley et al. 2007). During the past decade, however, many states have noticed a steady decrease in annual harvest estimates (Casalena et al. 2007; Hughes et al. 2007; Tapley et al. 2011), raising concern that population numbers are again falling. The discovery of LPDV has prompted questions regarding its role in the decline of this popular game species. In addition to potential impacts of LPDV on Wild Turkey populations, there are biosecurity concerns about its transmission to domestic species. To date, there have been no reported cases of LPDV in commercial flocks within the US (Thomas et al. 2015). However, given the well-documented agricultural impacts in other countries (Biggs et al. 1978; Ianconescu et al. 1983), spillover remains a concern in North America—particularly for organic and pastured flocks where direct physical contact between wild and domestic birds is more likely to occur.

Initial research on LPDV in the US characterized the national distribution of the virus (Allison et al. 2014; Thomas et al. 2015). We investigated patterns of infection on a smaller scale—New York State—to gain a more nuanced understanding of where and how LPDV is circulating. Our study had three objectives: 1) to estimate the prevalence of LPDV using hunter-harvested birds, 2) to elucidate potential predictor variables for infection, and 3) to examine the spatial patterns of the virus in New York State. This information can help game bird managers ensure that future surveillance is targeted to maximize limited resources (Ward and Carpenter 2000; Ostfeld et al. 2005) and may indicate regions where increased biosecurity measures are prudent for commercial producers.

MATERIALS AND METHODS

Sample collection

We tested 2,538 hunter-harvested turkey tarsi, gathered by the New York State Department of

Environmental Conservation (NYSDEC) in 2012 (n=871), 2013 (n=752), and 2014 (n=915). A single tarsus from each bird, collected as part of the statewide reporting process and not based on visible signs of disease, was submitted by successful fall hunters. Tarsi were placed in resealable plastic bags and mailed to the NYSDEC, along with the age, sex, and township of harvest. Tarsi were promptly frozen at –20 C until processing at the State University of New York, College of Environmental Science and Forestry, Fish and Wildlife Disease Laboratory, in Syracuse, New York.

DNA extraction and PCR

Each tarsus was cut using heavy-duty shears or a handheld hacksaw to expose the inner lumen of the bone. A sample of marrow (25 mg) was removed with stainless-steel dissection probes or forceps and placed in a sterile 2-mL screw-cap tube for storage at –20 C before DNA extraction. All instruments were disinfected in a 10% bleach solution, rinsed with water, and sterilized by flaming with ethanol before each use. We conducted LPDV testing via PCR assay as described in detail by Alger et al. (2015). Ambiguous results were rerun at a higher annealing temperature (56 C) to reduce nonspecific amplification.

Statistical analysis

Prevalence estimates with 95% Wilson confidence intervals (CI; Newcombe 1998) were calculated for all 3 study yr individually and for the entire study period as a whole. A chi-square proportion test, with a significance level of α=0.05 (Castle and Christensen 1990) was used to assess differences in prevalence estimates between the years in our study and between our overall prevalence and the estimate from a prior study also conducted with samples taken throughout New York State (Thomas et al. 2015).

We investigated three categories of explanatory variables for potential influence on the odds of LPDV infection: 1) anthropogenic, 2) land cover, and 3) demographic/population. Harvest data and translocation records were provided by the NYSDEC. The historic records from the trap-and-transfer program in New York State included data from the first translocation in 1959 through the mid-1990s when the program ended. Variables were examined at the county level and included whether or not wild birds were released during translocation, the number of releases, the number of birds released, and years since the last introduction.

To determine the change in harvest for each county, we calculated the fall harvest 10-yr (2005–15) mean and SD. We used fall harvest data because our samples were derived from fall kills. The average fall harvest was calculated for our study years (2012–14) and converted to a binary variable, where 0=within 1 SD of the 10-yr average and 1=less than 1 SD of the 10-yr average. Wild Turkey populations, and therefore harvest numbers, are subject to a large amount of annual variation (Sanford et al. 2005), so this approach was used to discern whether a drop in fall harvest estimates exceeded normal fluctuations.

To analyze land cover, ArcMap (ESRI 2014) was used to create a buffer zone of approximately 250 km^2 around the centroid of each township of harvest. Land cover data were downloaded from the 2006 National Land Cover Database (Multi-Resolution Land Characteristics Consortium, Sioux Falls, South Dakota, USA; Fry et al. 2011) into ArcMap and merged those data with the buffer zones to calculate the percentage of cover type within each zone (Kirchgessner et al. 2012). Our cover types of interest were deciduous forest, agriculture, and the ratio of agriculture to forest. Wild Turkeys have been shown to gather in large numbers through the winter months on agricultural land but remain more dispersed in areas dominated by forest cover (Porter 1992; Haroldson 1996; Fleming and Porter 2007). Our intent was to determine whether land cover configurations that favor large, concentrated groups of birds may be related to higher prevalence of infection.

Demographic variables included age (adult, juvenile), sex (male, female), and year of harvest (2012–14). Individuals considered juveniles were hatch-year birds, ranging in age from 4–5 mo, depending on the dates of hatch and harvest. Where the above data were not collected or were unable to be determined, we excluded the birds from analysis. We also included an interaction variable between sex and age to determine whether the odds of LPDV infection for males and females differed across age cohorts. Fall harvest estimates, based on a combination of direct reporting and hunter surveys documenting effort by hours spent in the woods (Sanford et al. 2005; NYSDEC 2015a, b), served as an index of abundance for each county.

To test the predictive association among all variables and the odds of LPDV infection, logistic regression analysis was used with the LPDV infection status of each bird (1=positive, 0=negative) as the dependent variable. We began with univariate analysis, and any variable with a significant relationship (Wald $P \leq 0.25$) was selected for inclusion in the global model (Hosmer and Lemeshow 2000; Le Souëf et al. 2015). Potential collinearity was assessed using Pearson's correlation coefficient (r), and if any variables were

significantly correlated ($r \geq 0.7$), the variable with the smallest effect on infection odds was excluded from the final model (Dormann et al. 2013).

Multiple logistic regression began with a global model that included all the variables selected by the univariate analysis and used a variable selection algorithm based on the work of Hosmer and Lemeshow (2000) and described by Bursac et al. (2008) to identify the most parsimonious model. We used backward selection with $P \geq 0.15$ as the exit criteria to control for confounders (Bursac et al. 2008). Once the model contained no more variables meeting the criteria for exit, we added, iteratively, each variable that was not significant in the univariate analysis to identify covariates that, although not significant on their own, might contribute to the effect of another variable in the model (Hosmer and Lemeshow 2000). Variable significance in the final model was set at $\alpha \leq 0.05$.

Odds ratios (OR) and 95% CI were reported for each variable in the final model. The OR is the probability of an event, divided by the probability of no event, and was interpreted for our study as the increase (OR>1) or decrease (OR<1) in the odds of a bird testing positive for LPDV when that variable increased by one unit. The Hosmer-Lemeshow goodness-of-fit test statistic was used to evaluate the final model (Lemeshow and Hosmer 1982). All analyses were conducted in RStudio, version 0.98.1091 (R Development Core Team 2008). Prevalence with 95% CI was calculated using the R package Binom (Sundar 2014), and the Hosmer-Lemeshow goodness-of-fit test was calculated using the MKmisc package (Kohl 2015).

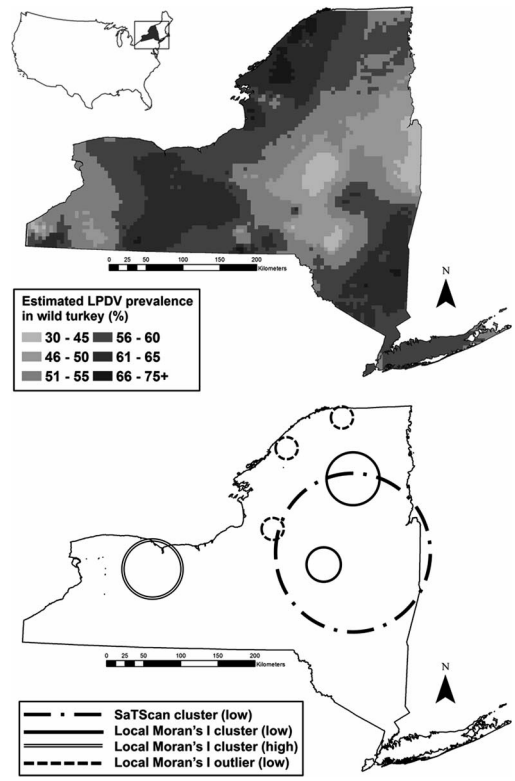

FIGURE 1. Map of New York State, USA, showing (top) kernel density estimation of lymphoproliferative disease virus (LPDV) prevalence in Wild Turkeys (*Meleagris gallopavo*) for 2012–14 and (bottom) results of Kulldorff's spatial scan statistic (SaTScan), and the Anselin Local Moran's I showing statistically significant clusters and outliers of LPDV prevalence in Wild Turkeys.

Spatial analysis

To evaluate the distribution of LPDV throughout our study area of New York State (Fig. 1), we employed both kernel density estimation and cluster analysis. Dual kernel density estimation was performed using CrimeStat, v. 4.02 (Levine 2015), which imposes a grid over the study area, then centers a moving function, called a *kernel*, over each data point to create weighted estimates for the surrounding grid cells within a predetermined bandwidth (Levine 2006). Our grid size was 5 km^2, based on maximum range estimates for Wild Turkeys (Porter 1977; Fleming and Porter 2007), and we corrected for uneven sample distribution by using an adaptive bandwidth (minimum of 20 data points/kernel), which increased in areas with fewer events and decreased in areas with many concentrated events (Davies and Hazelton 2010; Kirchgessner et al. 2013). Performing this analysis simultaneously for positive (case) and negative (control) birds allowed us to calculate the ratio of case/control densities at each location, which mathematically represented the odds of infection (event/non-event). To convert those odds into prevalence estimates (cases/total tested), we used the formula for the probability of infection (odds/1+odds). With those data, we produced a smoothed risk surface using a normal method of interpolation to estimate prevalence over the entire study area (Levine 2006; Kirchgessner et al. 2013).

To investigate the presence of statistically significant infection clusters, we used SaTScan v. 9.4.2 (Kulldorff and Informational Management Services, Inc. 2015). This software employs Kulldorff's spatial scan statistic, which looks for clusters by moving a circular window across the study area and calculating the likelihood function for each location by comparing the ratio of cases to noncases within the window to the ratio of cases to noncases outside of the window, using the Bernoulli model of binary outcomes (Kulldorff et

al. 2003). Statistical inference is tested using Monte Carlo simulations (Kulldorff 1997). The Kulldorff spatial scan statistic is popular in epidemiologic studies because of its ability to detect clusters without any prior knowledge of their size and its automatic correction for multiple comparisons, but its sensitivity to shape causes it to lose power when clusters are not compact (Kulldorff et al. 2003; Ozdenerol et al. 2005).

To compensate for that SaTScan weakness, we also used the Anselin Local Moran's I, a spatial analysis tool available in ArcMap v. 10.2.2, as a supplemental method for detecting local clusters (Abbas et al. 2015). That tool identifies areas where the point values are similar (clusters) or dissimilar (outliers) by comparing the magnitude of local vs. global indicators of spatial autocorrelation (Anselin 1995). For each township centroid, we assigned a prevalence estimate based on the ratio of positive cases to samples tested. Because of the uneven size and shape of townships across the state and the unknown distance between the centroid and actual harvest location, we increased our search radius to 32 km to ensure multiple comparison points for each township. The software made automatic adjustments for the false discovery rate that arises with multiple hypotheses testing (Caldas de Castro et al. 2006)

RESULTS

Apparent prevalence by year was 53% (459/871, 95% CI=49–56%) in 2012, 56% (422/752, 95% CI=53–60%) in 2013, and 57% (521/915, 95% CI=54–60%) in 2014. Overall estimated prevalence for all 3 yr combined was 55% (1,402/2,538, 95% CI=53–57%). There was no difference in our estimates among years; however, our overall estimate from 2012 to 2014 was significantly greater (χ^2=4.397, df =1, P=0.036) than the prevalence of 48% (132/273, 95% CI=42–54%) estimated for New York State by Thomas et al. (2015) from 2011 to 2013.

We identified 11 variables for consideration in our initial global model ($P \leq 0.25$), five of which were strongly significant ($P \leq 0.05$): 1) mean abundance index for the study years (P=0.031), 2) age ($P \leq 0.001$), 3) sex ($P \leq 0.001$), 4) the number of birds reintroduced per county (P=0.006), and 5) the ratio of agricultural land to forest within each buffer zone (P=0.020; Table 1). The number of reintroductions per county and the percentage of agricultural land cover were both highly correlated ($r \geq 0.7$) with other variables (the number of birds released and the ratio of agriculture to forest cover, respectively; Table 1) and were, therefore, excluded from further analysis. The remaining variables selected by univariate analysis for inclusion in the global model were harvest decline (P=0.118), year of harvest (P=0.093), number of years since the last reintroduction (P=0.167), and the percentage of deciduous forest (P=0.102) within each buffer zone (Table 1). Nonsignificant variables included the interaction between age and sex (P=0.823), and whether or not reintroductions occurred in each county (P=0.324; Table 1).

TABLE 1. Results of univariate logistic regression analysis for variables related to the odds of lymphoproliferative disease virus infection in Wild Turkeys (*Meleagris gallopavo*) in New York State, USA. Variables used to create initial global model shown in bold ($P \leq 0.25$). Italics indicate variables excluded from consideration because of being significantly correlated ($r > 0.7$) with another variable. Ref=referent.

Variable	Wald Z-score	P
Mean abundance index	**−2.16**	**0.031**
Harvest decline	**1.57**	**0.118**
Age		
Adult	**Ref**	**Ref**
Juvenile	**−19.73**	**<0.001**
Sex		
Male	**Ref**	**Ref**
Female	**8.93**	**<0.001**
Age×sex interaction	0.224	0.823
Year of harvest		
2012	**Ref**	**Ref**
2013	**1.22**	**0.221**
2014	**1.68**	**0.093**
Reintroductions		
No	Ref	Ref
Yes	−0.99	0.324
No. of birds released	**−3.272**	**0.001**
No. of reintroductions	**−2.77**	**0.006**
Time since last reintroduction	**−1.38**	**0.167**
Deciduous forest	**−1.63**	**0.102**
Agriculture	**1.22**	**0.222**
Ratio of agriculture to forest	**2.33**	**0.020**

Four variables remained significant in the final model after iterative reductions: 1) the ratio of agricultural to forested cover (OR=1.13, 95% CI=1.03–1.24), 2) the number of birds released in each county (OR=0.993, 95% CI=0.990–0.997), 3) age (OR=0.16, 95% CI=0.13–0.19), and 4) sex (OR=1.13, 95% CI=1.03–1.24; Table 2). Age and sex were strong predictors of LDPV infection. Prevalence estimates based on age and sex were as follows: 35% for juveniles (405/1,171, 95% CI=32–37%), 76% for adults (913/1,196, 95% CI=74–79%), 46% for males (549/1,181, 95% CI=44–49%), and 65% for females (769/1,186, 95% CI=62–68%). In our final model, the odds of a bird testing positive for LPDV decreased for juveniles and increased for females (Table 2). The Hosmer-Lemeshow goodness-of-fit statistic for the final model indicated a good fit (χ^2=9.5705, df=8, P=0.297).

The risk surface analysis revealed prevalence estimates ranging from 30% to ≥75% throughout the state (Fig. 1). The Hudson Valley/Catskills (southeast), Thousand-Islands Seaway (northwest), and central New York/Finger Lakes regions (west-central) showed areas of elevated prevalence, whereas the Adirondacks and Capital District regions (north-central), and the southern half of western New York State showed much lower prevalence (Fig. 1).

The global Moran's I statistic showed infection tended toward a clustered distribution (z=2.050, P=0.040). There was general agreement between both local statistics regarding the location of clusters, although there were some discrepancies in size. SaTScan (Kulldorff's spatial scan statistic) revealed the largest significant cluster (P=0.007) in the east-central part of the state (Fig. 1). This was a cluster of low prevalence and was located congruently with Adirondack State Park. Using the Anselin Local Moran's I, we also detected two smaller clusters of low prevalence (P=0.001) within the same area, as well as the only significant cluster of high prevalence (P≤0.001) in the northwest Finger Lakes region (Fig. 1). There were several outliers—townships with unusually low prevalence (P≤0.001) within areas of high prevalence in the Thousand-Islands Seaway region—using the Anselin Local Moran's I. All of the clusters corresponded, roughly, with the risk surface generated by the kernel density estimation (Fig. 1).

TABLE 2. Final multiple logistic regression model showing significant predictor variables for lymphoproliferative disease virus infection in Wild Turkeys (*Meleagris gallopavo*) in New York State, USA.

Variable	Odds ratio	95% Confidence interval	P
Intercept	2.13	1.63–2.79	<0.001
Abundance index	1.00	0.99–1.0	0.102
Harvest decline	1.36	0.97–1.92	0.076
Ratio of agriculture to forest	1.13	1.03–1.24	0.013
Birds released	0.993	0.990–0.997	<0.001
Age	0.16	0.13–0.19	<0.001
Sex	1.13	1.03–1.24	<0.001

DISCUSSION

Lymphoproliferative disease virus appears to be widespread in Wild Turkey populations across New York State, confirming the findings of Thomas et al. (2015). However, our estimate from 2012 to 2014 was higher (55%) than that of Thomas et al. (2015; 48%) from 2011 to 2013. Because both studies sampled similar populations in New York State (fall hunter-harvested birds), this difference is probably an artifact of sample sizes.

We did not find evidence that abundance at the county level influenced the odds of LPDV infection. Similarly, our metric of harvest decline was not a strong predictor in the final model. Because Wild Turkey populations experience significant annual fluctuations (Healy and Powell 1999), population and harvest patterns were typically observed using 3–5 yr of data (Sanford et al. 2005), meaning it is possible our study period was too brief to detect a relationship. One of the biggest questions related to LPDV in Wild Turkeys is whether the virus negatively influences population levels. Given the unknown mor-

tality rates associated with LPDV in wild birds, more work remains to determine whether this virus is affecting populations enough to be reflected in downward harvest trends.

Deciduous forest cover was a poor predictor of LPDV infection, but the ratio of agricultural land to forest cover remained significant in our final model, with an increase in the ratio of agricultural to forest land corresponding to an increase in the odds of LDPV infection (Table 2). Habitat has a key role in the movement and sustainability of Wild Turkey populations (Porter 1992; Fleming and Porter 2007). In northern states, where harsh winters may preclude adequate foraging, agricultural land can provide dietary supplementation in the form of corn left after harvest or spread in manure, and birds will often gather in large numbers on those fields (Haroldson 1996). The results of our model may indicate that areas with agricultural land encouraging those types of congregations increase the odds of LPDV infection, as opposed to forest settings where birds are more dispersed. Another possibility, however, is that we are detecting the well-demonstrated preference of Wild Turkeys for landscapes that are fragmented (Fleming and Porter 2007), and those areas have a higher prevalence of LPDV simply because they are favored by the host.

In New York State, the trap-and-transfer program, run by NYSDEC, relocated nearly 1,400 birds between 1959 and the mid-1990s. Based on our model, the odds of a turkey testing positive for LPDV was lower in counties that received more translocated birds throughout the course of that program. Half (691/1,377; 50.2%) of the birds released during this program were released to counties in the Adirondacks, the Capital Region, and the southern part of Western New York—areas with lower prevalence, based on our risk surface map (Fig. 1). However, given that the virus and host were both widespread across the state, statistical modeling alone may be insufficient to determine whether translocation significantly affected distribution of the virus.

In our final model, age and sex were both strong predictors of LPDV infection (Table 2): adults and females were more likely to be infected with LPDV than were juveniles or males. Early studies in domestic birds seem to suggest that poults from the ages of 4–16 wk are actually the group at highest risk of developing fatal disease from the virus (Biggs et al. 1978; McDougall et al. 1978). Challenge studies in nondomestic turkeys could reveal whether a similar pattern is occurring in these wild birds but, to date, have not been conducted. If, however, LPDV-related mortality is high in wild poults, that fact might be masked by a mortality rate for that age group that is close to 50% and that is highly subject to other forces, such as weather, predation, and food availability (Roberts et al. 1995). This explanation fits well with our data, considering only hatch-year birds were coded as juveniles. Assuming there are multiple opportunities for infection throughout a bird's lifetime, high mortality for poults infected with LPDV would result in uninfected hatch-year birds advancing to adulthood and experiencing further exposure.

The difference in infection odds between males and females has not, to our knowledge, been previously reported (Allison et al 2014; Thomas et al 2015). It is unlikely that difference is unique to New York State, but is, rather, a function of our large, homogenous sample pool. Thomas et al. (2015) looked for age and sex differences across all 17 states—not just New York—but many states provided samples from spring (male-only) harvests, meaning that their sample pool had a heavily skewed sex ratio (853 males and 189 females). That may explain why the prevalence estimate from their study (48%) was closer to the prevalence estimate we calculated for males alone (46%). Our findings are further supported by a recent study that tested Wild Turkey hens exclusively throughout New York State and found an estimated prevalence of 81% (Alger et al. 2015). It is unclear whether the difference between male and female prevalence is meaningful from a management standpoint. Nevertheless, it is an interesting characteristic of the virus and one that

warrants continued study to determine its biological significance.

Prevalence of LPDV in Wild Turkeys appears to be regionally variable across New York State, a finding that could be relevant for poultry producers. The various routes of Wild Turkey LPDV transmission in wild birds are not fully understood, but patterns in commercial flocks suggest direct contact among birds is the most likely avenue of potential spillover for this virus. This makes pasture-raised and organic flocks, which require access to the outdoors whenever weather is amenable (Dimitri and Greene 2002), more vulnerable than flocks on conventional farms. The organic poultry industry grew rapidly in the 1990s, and by 1997, New York State was second only to California in production (Dimitri and Greene 2002). Currently, the organic poultry industry in New York State is valued at approximately $57 billion (USDA NASS 2016). Recommended biosecurity practices for outdoor flocks include physical barriers, such as fencing or netting; vaccination against certain pathogens; and temporary confinement during local outbreaks (USDA NASS 2016). There is no vaccine available for LPDV and given its apparent endemism in Wild Turkeys, temporary confinement would be ineffectual, but ensuring adequate fencing around farms in areas of the state with known high prevalence may be prudent.

From a management standpoint, it is difficult to make sense of LPDV. Since its discovery in Wild Turkeys in 2009, there has been little evidence that the virus is having important negative impacts on turkey populations, despite its high prevalence in many states (Thomas et al. 2015). Based on surveys of hunter-harvested samples, birds appear to harbor LPDV at any age, and many are infected with no discernable signs of disease. These subclinical infections seem to imply that the virus does not pose an immediate threat to Wild Turkeys. However, decreased reproductive success and immunosuppression are both characteristics of subclinical infections from other avian retroviruses (Gingerich et al. 2002; Guo et al. 2010). If such effects are also occurring in LPDV infections, there could be long-term consequences that have yet to be demonstrated. Future research should focus on the physiologic, immunologic, and reproductive effects of infection, as well as on elucidating transmission routes; all of which are critical to understanding possible management implications associated with the virus.

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