Summary Report for Wild Turkey Lymphoproliferative Disease Virus Project

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INTRODUCTION

Lymphoproliferative Disease Virus (LPDV) is one of three types of avian retroviruses capable of causing lymphoid tumors in both domestic and wild birds (Payne 1998). Similar lesions can occur in birds infected with avian pox, which is widespread and may be more familiar to the general public. Prior to 2009, LPDV had been found only in domestic turkeys in Europe and Israel, but had not been reported in the wild. LPDV has not been an issue in domestic poultry in the United States, but in 2009 it was identified from wild turkeys in several southeastern states. Because of its apparent similarity to avian pox and the lack of appropriate laboratory testing, it is possible that LPDV went undiagnosed or misdiagnosed in wild turkeys prior to 2009.

LPDV can cause wild turkeys to develop tumors and skin lesions. Symptomatic turkeys can also become weak, listless, emaciated, and may eventually die from the infection. However, symptomatic cases appear to be very rare in wild turkeys. There have only been eight cases of LPDV diagnosed from symptomatic wild turkeys in North Carolina. Though symptomatic cases of LPDV appear to be rare, the virus is widespread throughout the range of wild turkeys. LPDV can be diagnosed in symptomatic turkeys by histologic examination of skin lesions. Evidence of LPDV exposure can be found in non-symptomatic turkeys by examining bone marrow samples (with a Polymerase Chain Reaction (PCR) test). A positive PCR test does not mean a wild turkey has or will develop tumors, lesions or other clinical symptoms. It simply means the bird has been exposed at some point and the virus is detectable in the bone marrow. It is important to note that LPDV does not pose any known risks to humans that might consume, handle, or be in close proximity to infected turkeys.

Although several studies have examined LPDV in recent years, there is still a considerable amount of basic information that remains unknown. We don't know how the disease is transmitted from one turkey to another, how the virus affects all ages of turkeys, or how persistent it is within wild turkey populations. While direct deaths seem to be rare, we don't know if there are any less obvious effects, such as impacts on reproductive output or poult survival. Evaluating the full impact LPDV may have on turkey populations is complicated by these unknown factors, but the general consensus is that LPDV is not likely to be a major issue for wild turkey populations.

After LPDV's recent diagnosis in North Carolina, NCWRC biologists saw an immediate need to collect baseline information about the disease in the state. Therefore, NCWRC undertook efforts in both

2013 and 2015 to collect tissue samples from hunter-killed wild turkeys to test for the virus. The objectives of our study were to determine the prevalence of LPDV in our wild turkey population and better understand its temporal and geographic variation.

METHODS AND RESULTS

In 2013, NCWRC field staff participated in a large study (Thomas et al. 2015) that examined LPDV in wild turkeys in 17 states throughout the southeastern, midwestern, and northeastern United States. We opportunistically collected samples from hunters during the spring turkey season. Samples were collected throughout the state where our field staff had an opportunity to do so. From some birds we collected liver or spleen tissues, and from others we collected bone marrow samples from the lower leg. In total, we collected 226 samples (76 were bone marrow samples) that were tested for LPDV as part of this larger project. PCR testing of samples collected in 2013 was conducted by the Southeastern Cooperative Wildlife Disease Study housed at the University of Georgia. The preliminary results from this project, though not definitive, suggested that prevalence was high in North Carolina and perhaps some areas of the state had higher prevalence than others. One important discovery of the 2013 project was that bone marrow is the most sensitive for LPDV and provides the best picture of the virus' prevalence. (This became important in planning our efforts in 2015.) Full results from this project were published in 2015 by Thomas et al.

In 2015 NCWRC staff undertook a more focused effort to take a closer look at LPDV in North Carolina. We wanted to collect a larger and more representative number of samples to have a better understanding of LPDV in our state. We planned to collect 500 samples from hunter-killed turkeys during the 2015 hunting season. To ensure that turkeys from all parts of the state were well represented, we considered the turkey harvest levels for each county during the hunting season and set a goal to collect one LPDV sample for every 34 wild turkeys that were harvested per county. This approach would allow us to determine LPDV prevalence rates at regional or district levels.

During the 2015 spring turkey season, our field staff contacted hunters in a variety of ways (phone, e-mail, press release, etc.) and asked them to submit samples from the turkeys they harvested. Hunters were asked to submit a lower leg (spurs could be removed if they wished) and fill out a data sheet that stated county, sex, beard length, kill date, spur length, weight, and hunter's contact information for each sample. Hunters were encouraged to inform NCWRC biologists of their interest in this project and samples were collected from those that were willing to help. We collected 759 samples, greatly exceeding our goal of 500 samples. Total reported turkey harvest in 2015 was 17,828 birds. Thus, our efforts resulted in sampling 1 out of every 24 turkeys harvested. The vast majority of samples were from hunter-killed male turkeys taken during the spring season. We also obtained samples from 5 hunter-killed bearded hens, 4 illegally poached hens, and 20 hens that were killed by vehicles during the study period. Biologists verified the information on each data sheet and stored a small piece of tissue in

95% ethanol for future DNA work. PCR testing for the 2015 samples was conducted by the State University of New York – College of Environmental Science and Forestry.

Overall Prevalence

In total (2013 and 2015 combined) we determined the LPDV status of 829 wild turkeys from across North Carolina through PCR testing of bone marrow (Figure 1). Six additional samples were too desiccated for the lab to determine a reliable test result. Three hundred eighty two turkeys tested positive for LPDV, for an overall prevalence of 46.1 %. This is consistent with Thomas et al. (2015), who reported an overall prevalence of 47% for the southeastern United States.

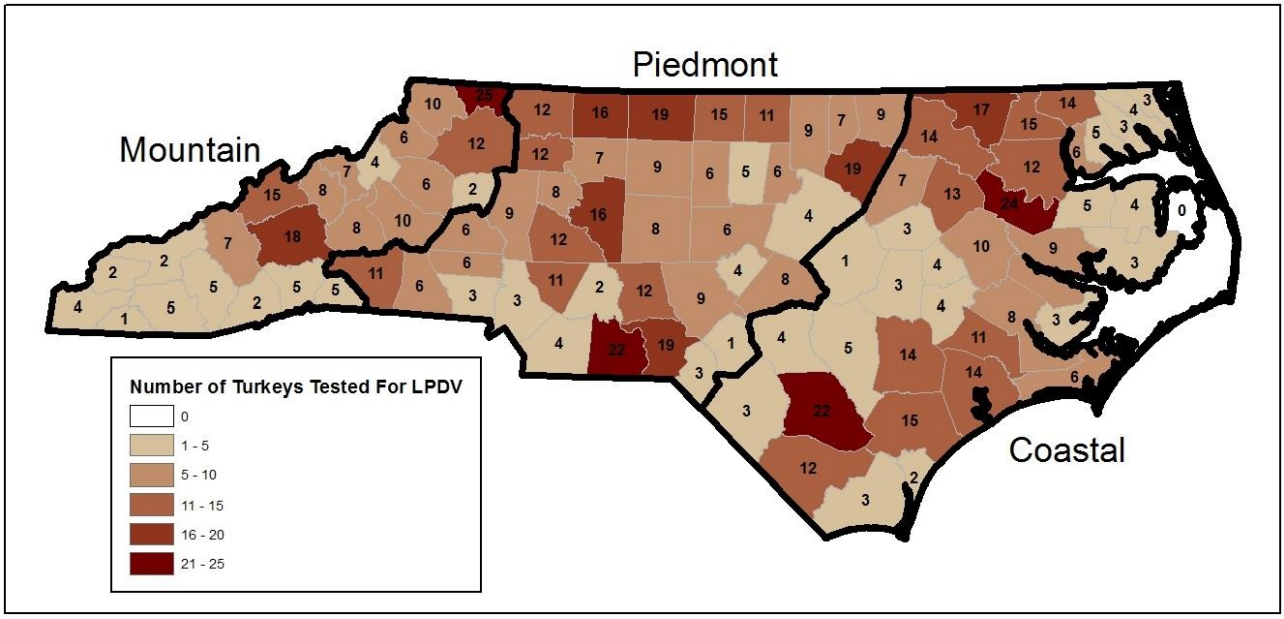

Figure 1. Number of wild turkeys tested (via PCR of bone marrow) for Lymphoproliferative Disease Virus (LPDV) in 2013 and 2015 by county.

Prevalence by Sex and Year

We examined our data to see if LPDV prevalence differed between 2013 and 2015 (Table 1) and also to see if LPDV prevalence differed between male and female wild turkeys (Table 2). A Chi-square analysis determined that the differences we observed in prevalence rate were not statistically significant between years ($\chi^2 = 1.47$, d.f.=1, p=.225) or sex ($\chi^2 = 0.06$, d.f.=1, p=.809). LPDV seems to be affecting male and female turkeys equally and at the same level in both 2013 and 2015.

Table 1. Prevalence of Lymphoproliferative Disease Virus (LPDV) of North Carolina wild turkeys by year. Testing was by Polymerase Chain Reaction (PCR) of bone marrow samples collected in 2013 and 2015, primarily from hunter-killed turkeys.

Year	Number Tested	LPDV Prevalence Percentage Positive (95% Confidence Interval)
2013	76	39.5% (28.4 – 51.4%)
2015	753	46.8% (43.1 – 50.4%)
Total	**829**	**46.1% (42.7 – 49.5)**

Table 2. Prevalence of Lymphoproliferative Disease Virus (LPDV) of North Carolina wild turkeys by sex. Testing was by Polymerase Chain Reaction (PCR) of bone marrow samples collected in 2013 and 2015, primarily from hunter-killed turkeys.

Sex	Number Tested	LPDV Prevalence Percentage Positive (95% Confidence Interval)
Female	29	48.3% (29.5 – 67.5%)
Male	800	46.0% (42.5 – 49.5%)
Total	**829**	**46.1% (42.7 – 49.5)**

Prevalence by Age

We were able to determine age for 798 turkeys. We considered male turkeys with beards less than seven inches long to be one year old and classified them as juveniles. Male turkeys with beards seven inches or longer were considered to be two years or older and classified as adults. Our tested samples consisted of 111 juveniles and 687 adults (Table 3). LPDV prevalence was significantly lower in juveniles than in adults ($\chi^2 = 13.52$, d.f=1, p=.0002). It appears that the likelihood of turkeys being exposed to LPDV depends heavily on how long they have been alive.

Table 3. Prevalence of Lymphoproliferative Disease Virus (LPDV) of male North Carolina wild turkeys by age class. LPDV testing was by Polymerase Chain Reaction (PCR) of bone marrow samples collected in 2013 and 2015, primarily from hunter-killed turkeys. Age classes were determined from beard and spur measurements provided by hunters.

Age Class	Number tested	LPDV Prevalence Percentage Positive (95% Confidence Interval
Juvenile (1 year old)	111	29.7% (21.2 – 38.2%)
Adult (2 years or older)	687	48.5% (44.8 – 52.2%)

Prevalence by Weight

Hunters measured and reported total body weight for 268 of the wild turkeys we tested. We examined the data to see if LPDV is impacting turkey weight, specifically to see if turkeys exposed to LPDV weigh less than those that do not show evidence of previous exposure (Table 4). Since juvenile turkeys weigh less than adults, we analyzed these two age groups separately. We did not include any females in this analysis. We found that on average juvenile males weigh 15.6 pounds, adult males weigh 19.6 pounds, and turkeys previously exposed to LPDV do not weigh less than those that tested negative for LPDV exposure.

Table 4. Weight in pounds of North Carolina wild turkeys by age class and Lymphoproliferative Disease Virus (LPDV) status. LPDV testing was by Polymerase Chain Reaction (PCR) of bone marrow samples collected in 2013 and 2015, primarily from hunter-killed turkeys. Weights were measured and reported by hunters.

Age Class	LPDV Status	Number weighed and tested	Average Weight Lbs. (95% Confidence Interval)	Standard Error Lbs.
Juvenile (1 year old)	Negative	22	15.2 (14.6 – 15.7)	0.29
	Positive	5	16.8 (15.5 – 17.8)	0.49
	Combined	27	15.6 (15.1 – 16.1)	0.28
Adult (2 years or older)	Negative	125	19.6 (19.2 – 20.0)	0.20
	Positive	116	19.6 (19.2 – 20.0)	0.19
	Combined	241	19.6 (19.3 – 19.9)	0.14

Prevalence by Region

We examined the data for regional differences in LPDV prevalence. A Chi-Square statistical analysis revealed that in adult wild turkeys LPDV prevalence was significantly higher in the mountain region than in either the piedmont or coastal regions (χ^2=6.97, d.f.=2, p=.031) (Table 5 and Figure 2). In juvenile turkeys, LPDV prevalence differed across regions as well (χ^2=7.63, d.f.=2, p=.022) (Table 6), with the mountain region having the highest observed prevalence rates. The observed differences in juvenile LPDV prevalence between the Piedmont and Coastal regions (χ^2=2.44, d.f.=1, p=.118), and also between Coastal and Mountain Regions (χ^2=1.17, d.f.=1, p=.279), were not statistically significant.

Table 5. Prevalence of Lymphoproliferative Disease Virus (LPDV) of **adult (2 years or older)** North Carolina wild turkeys by region. LPDV testing was by Polymerase Chain Reaction (PCR) of bone marrow samples collected in 2013 and 2015, primarily from hunter-killed turkeys. Age class was determined from beard and spur measurements provided by hunters.

Region	Number Tested	LPDV Prevalence Percentage Positive (95% Confidence Interval)	Observed differences in prevalence not statistically significant between regions with the same letter
Coastal	263	45.6% (39.5 – 51.9%)	A
Piedmont	295	46.4% (40.6 – 52.3%)	A
Mountain	129	58.9 % (50.0 – 67.5%)	B
Total	**687**	**48.5% (44.7 – 52.3%)**	

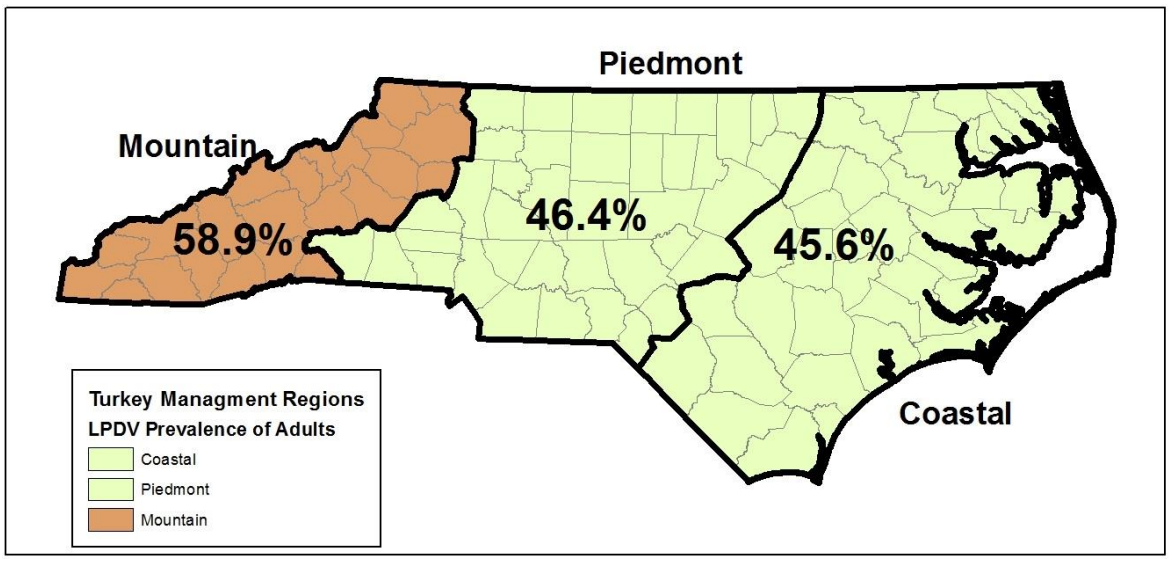

Figure 2. Lymphoproliferative Disease Virus (LPDV) prevalence in adult (2 years or older) wild turkeys. Testing was by Polymerase Chain Reaction (PCR) of bone marrow samples collected in 2013 and 2015.

Table 6. Prevalence of Lymphoproliferative Disease Virus (LPDV) of **juvenile (1 year old)** North Carolina wild turkeys by region. LPDV testing was by Polymerase Chain Reaction (PCR) of bone marrow samples collected in 2013 and 2015, primarily from hunter-killed turkeys. Age class was determined from beard and spur measurements provided by hunters.

Region	Number Tested	LPDV Prevalence Percentage Positive (95% Confidence Interval)	Observed differences in prevalence not statistically significant between regions with the same letter
Piedmont	47	17.0% (7.7 – 30.8%)	A
Coastal	31	32.3% (16.7 – 51.4%)	AB
Mountain	33	45.5% (28.1 – 63.7%)	B
Total	**111**	**29.7% (21.4 – 39.1%)**	

LPDV Correlation with Turkey Harvest

We examined our data to determine if overall LPDV prevalence is correlated with spring turkey harvest levels. Evidence of correlation would provide insight and focus future research efforts. Data for harvest and LPDV prevalence were summarized at the district level for this analysis. For turkey harvest data, we used the 3-year average (2013 - 2015) of the number of turkeys reported per square mile. Our linear regression analysis revealed that there was no relationship between overall LPDV prevalence and hunter harvest ($p=.91$, $r^2=.0016$). This analysis supports the idea that the virus is not impacting hunter harvest of turkeys.

LPDV Correlation with Productivity

We also examined our data to determine if overall LPDV prevalence is correlated with reproductive output as observed in our yearly summer wild turkey observation survey. The summer wild turkey observation surveys enlists volunteers in all counties of North Carolina to report their sightings of hens, poults, and gobblers during July and August. The survey provides a useful estimate of how successful turkeys are producing and raising poults throughout the state. For this analysis we used a 3-year average (2013-2015) of the number of poults observed per hen in the summer observation survey. Data for productivity and LPDV prevalence were summarized at the district level. Our linear regression analysis revealed that there was no relationship between overall LPDV prevalence and the number of poults observed per hen ($p=.87$, $r^2=.0033$). This analysis supports the idea that LPDV is not impacting turkey reproduction.

DISCUSSION

Our efforts, combined with the assistance of cooperating turkey hunters, have provided a great deal of insight into LPDV and wild turkeys in North Carolina. While there is still a lot we don't know about LPDV, our results support the idea that LPDV is likely not a major issue at the population level for wild turkeys. Its effect may be limited to the occasional symptomatic individual. This is good news for turkey populations, hunters, and wild turkey management.

Our work shows that LPDV is fairly prevalent through all parts of North Carolina, with somewhat higher prevalence in the mountain region. We can only speculate as to why this is the case, but it is interesting to compare our results with those reported by Thomas et al. (2015). They reported an overall LPDV prevalence rate of 47% for the southeastern United States, with higher prevalence in the northeastern United States and lower prevalence in the midwestern United States. Our overall prevalence rate of 46.1% is very similar to that, with higher prevalence in the mountain region where perhaps climate, habitat conditions, and landscape composition are more similar to the northeastern United States. If LPDV prevalence is driven by habitat and or climatic conditions then our data would support that conclusion.

The difference in LPDV prevalence between adults and juveniles is substantial and well documented in our efforts. Thomas et al. (2015) also reported a similar difference in prevalence between juvenile and adults. It may be that a turkey's likelihood of encountering LPDV is simply a function of their age. This may very well be the best news possible as it seems support the idea that exposure to LPDV is relatively common, but results in relatively few direct (i.e. symptomatic cases) deaths of wild turkeys. However, the evidence here is not conclusive. We recognize that LPDV could be having substantial effects to poults or in impacting reproduction or survival, but this is good news nonetheless.

It is worth noting that the "adult" age category includes multiple ages of individuals. As many turkey hunters know, spur length can provide an indication of a more specific age class of adult birds (i.e., 2 year old birds and those that are 3 years or older) (Steffen et al. 1990). We did examine our data in regard to age, spur length, and LPDV, but given the error rate of aging birds in this fashion (30% or greater) did not feel comfortable reporting results beyond the juvenile and adult categories. However, as a general statement from exploring the data, considering age with respect to spur length did not appear to provide additional insight beyond what we learned from examining the basic "juvenile" and "adult" categories. We make note of that here because knowledgeable turkey hunters may well wonder why we did not report results in that fashion.

Our efforts reveal that LPDV does not appear to have a negative effect on weight (which might indicate an impact on overall health) nor did we find any evidence that might suggest LPDV is impacting hunter harvest or reproduction. It is important to note that our projects were not designed to answer these specific questions so our conclusions on these topics (weight and correlations) should be viewed with caution, and further research may be needed to answer these questions in detail.

Our work did not show differences in prevalence rates between 2013 and 2015 or between males and females, suggesting that LPDV prevalence may show very little variation across sexes or between years. Our estimates came primarily from large sampling effort in 2015, while Thomas et al's (2015) came from large sampling effort in 2013. The consistency of our estimates with theirs further supports the notion of little LPDV annual variation. However, it is important to note that we had relatively few samples from 2013 (76 samples) and from females (29 samples). It is possible that LPDV may vary between years or sexes and our data set was simply not large enough to verify that.

Literature Cited

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Funds are derived from an 11 percent Federal excise tax on sporting arms, ammunition, and archery equipment, and a 10 percent tax on handguns. These funds are collected from the manufacturers by the Department of the Treasury and are apportioned each year to the States and Territorial areas (except Puerto Rico) by the Department of the Interior on the basis of formulas set forth in the Act. Funds for hunter education and target ranges are derived from one-half of the tax on handguns and archery equipment.

Each state's apportionment is determined by a formula which considers the total area of the state and the number of licensed hunters in the state. The program is a cost-reimbursement program, where the state covers the full amount of an approved project then applies for reimbursement through Federal Aid for up to 75 percent of the project expenses. The state must provide at least 25 percent of the project costs from a non-federal source.