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Regional Pathogen Surveillance of Free-Ranging Wild Turkeys (*Meleagris gallopavo*) in North Carolina, USA

Adam C. Edge,[1,2] Christopher E. Moorman,[3] Krishna Pacifici,[3] David J. Moscicki,[3] Nicole M. Nemeth,[1,4] Mark G. Ruder,[1] Elizabeth Kurimo-Beechuk,[1] Marcelo H. Jorge,[1,5] Christopher A. Cleveland,[1,6] Kayla B. Garrett,[1,5,6] Melanie R. Kunkel,[1] Alec T. Thompson,[1,6] Kayla G. Adcock,[1,6] Christopher D. Kreh,[7] Hannah M. Plumpton,[7] Bret A. Collier,[8] Sonia M. Hernandez,[1,5,6] and Michael J. Yabsley[1,5,6,9]

[1] Southeastern Cooperative Wildlife Disease Study, University of Georgia, 589 D. W. Brooks Drive, Athens, Georgia 30602, USA
[2] Current address: Wildlife & Forestry Division, Tennessee Wildlife Resources Agency, 5107 Edmondson Pike, Nashville, Tennessee 37211, USA
[3] Department of Forestry and Environmental Resources, North Carolina State University, 2820 Faucette Drive, Raleigh, North Carolina 27695, USA
[4] Department of Pathology, University of Georgia, 501 D. W. Brooks Drive, Athens, Georgia 30602, USA
[5] Daniel B. Warnell School of Forestry and Natural Resources, University of Georgia, 180 E. Green Street, Athens, Georgia 30602, USA
[6] Center for the Ecology of Infectious Diseases, University of Georgia, 203 D. W. Brooks Drive, Athens, Georgia 20602, USA
[7] North Carolina Wildlife Resources Commission, 1722 Mail Service Center, Raleigh, North Carolina 27699, USA
[8] School of Renewable Natural Resources, Louisiana State University Agricultural Center, 101 Efferson Hall, Baton Rouge, Louisiana 70803, USA
[9] Corresponding author (email: myabsley@uga.edu)

ABSTRACT: Wild Turkeys (*Meleagris gallopavo*, hereafter turkeys), an important North American game species, have experienced declines throughout their eastern range. Growing concern over turkey population sustainability has renewed interest in investigating potential disease threats. We conducted pathogen surveillance in turkeys in three North Carolina, USA ecoregions—Mountains, Piedmont, and Coastal Plain—in 2020–22 to provide baseline data relevant to the southeastern USA. We collected samples from 586 live free-ranging turkeys plus 22 recaptured individuals (n=608: 194 males, 414 females; 159 juveniles, 449 adults) to test for exposure to or infection with selected pathogens. Molecular testing revealed infections with *Haemoproteus* spp. (57%), lymphoproliferative disease virus (LPDV; 46.8%), *Mycoplasma* spp. (39.8%), *Leucocytozoon* spp. (8.8%), and reticuloendotheliosis virus (REV; 3.4%). We detected antibodies to *Toxoplasma gondii* (21.3%), West Nile virus (WNV; 15.4%), and avian influenza virus (2.0%). No turkey coronavirus, *Plasmodium*, *Borrelia*, or *Salmonella* infections were detected. There were no prevalence differences between sexes, except for REV (females=5%, males=1%). Prevalence was higher in adults than in juveniles for LPDV (adult=52%, juvenile=33%), WNV (adult=19%, juvenile=6%), *Haemoproteus* (adult=60%, juvenile=49%), *T. gondii* (adult=24%, juvenile=14%), and *Leucocytozoon* (adult=11%, juvenile=3%). Prevalence of LPDV differed significantly across ecoregions, with the highest prevalence in the Piedmont (62%), followed by the Mountains (51%) and the Coastal Plain (27%). Prevalence of WNV antibodies was higher in the Piedmont (24%) than in the Mountains (8%). *Haemoproteus* and *Leucocytozoon* prevalence increased over a regional gradient, with detections of 24% and 0% in the Mountains, 65% and 6% in the Piedmont, and 85 and 21% in Coastal Plain, respectively. *Mycoplasma* spp. prevalence was higher in the Mountains (45%) and the Coastal Plain (47%) than in the Piedmont (27%). Our data highlighted sex-, age-, and region-based differences in prevalence for several pathogens, thereby enabling managers to tailor management strategies and researchers to investigate effects of these pathogens on turkey survival and movement.

Key words: Ecoregions, free-ranging wildlife, *Meleagris gallopavo*, pathogens, surveillance, Wild Turkey.

INTRODUCTION

Following the successful restoration of Wild Turkeys (*Meleagris gallopavo*, hereafter turkeys) throughout their range in the latter 20th century (Kennamer et al. 1992), recent trends reveal population declines across the central and eastern USA (Chamberlain et al. 2022). Because of the economic and social importance of turkeys as a game species in

North America (Keck and Langston 1992), considerable investments have been made to study potential mechanisms of population decline, including pathogens (Byrne et al. 2015; Eriksen et al. 2015; Londe et al. 2023). Disease can cause direct mortality, increase predation susceptibility, or indirectly suppress reproductive success (Davidson et al. 1985; Atkinson et al. 1988b; Payne 1998; Davidson and Wentworth 1992). The spillover risk of pathogens shared between domestic poultry and free-ranging turkeys due to expansion of agricultural landscapes in an increasingly globalized world only exacerbates these concerns (MacDonald et al. 2019a; Adcock et al. 2024).

Lymphoproliferative disease virus (LPDV), an oncogenic avian retrovirus, was first detected in a free-ranging turkey in the USA in 2009 and it is now recognized as widespread in free-ranging turkey populations in eastern North America (Allison et al. 2014). Nevertheless, population-level effects of infection are poorly understood (Thomas et al. 2015; MacDonald et al. 2019a; Niedringhaus et al. 2019; Adcock et al. 2024). Although LPDV-associated neoplasia (lymphoma) in wild birds is less common than in domestic turkeys, LPDV may affect reproduction and promote coinfections due to immunosuppression (Niedringhaus et al. 2019; Cox et al. 2022). The source of LPDV is unknown, and although LPDV has been reported in domestic turkeys in Europe and the Middle East, it has not been reported in USA domestic turkeys (Biggs et al. 1978; Ianconescu et al. 1983). Thus, this virus is a concern for domestic turkey producers in the USA should spillover from wild birds occur. Another spillback risk of concern is highly pathogenic avian influenza virus (HPAIV), especially after the introduction of AIV subclade 2.3.4.4 in the USA. In 2021, HPAIV spillover killed 41 free-ranging turkeys in Wyoming, USA (Malmberg et al. 2023), suggesting potential risk to wild birds from increasingly popular backyard poultry. However, many risk factors associated with pathogens in wild populations remain unclear, highlighting the importance of continued monitoring and investigation.

During the turkey restoration era starting in the latter half of the 19th century in the USA, numerous parasites and pathogens were detected (Davidson et al. 1982, 1985; Castle and Christensen 1984; Hopkins et al. 1990; Davidson and Wentworth 1992). Examination of 139 sick or dead turkeys from southeastern USA states during 1972–84 revealed cases of avian pox (22%), histomoniasis (10%), malnutrition (9%), organophosphate toxicosis (3%), bacterial septicemia (3%), pododermatitis (2%), and rarely (1%) lymphoproliferative disease (before LPDV discovery), salmonellosis, aspergillosis, toxoplasmosis, and crop trichomoniasis (Davidson et al. 1985). Similar results were reported from 76 turkey mortalities in Florida, USA, from 1969 to 1990, although avian malaria (*Plasmodium* spp. infection) cases were also detected (Forrester 1992). These data are important but biased because only sick or dead birds were tested. To improve understanding of pathogen prevalences, more recent studies have tested hunter-harvested turkeys (Oates et al. 2005; Scott et al. 2010; Thomas et al. 2015; MacDonald et al. 2019a; Kreh and Palamar 2022; Haynes et al. 2024). These studies have had increased sample sizes and spatial distribution, but many were sex and age biased (National Wild Turkey Federation 2023).

Sampling live free-ranging birds across demographic groups (sex and age) is needed to best assess population-level pathogen effects (Alger et al. 2015). Thus, our objective was to expand on a previous LPDV study (Kreh and Palamar 2022) and evaluate the prevalence and distribution of multiple turkey pathogens across demographics and ecoregions of North Carolina, USA. Unlike in many other USA states, the turkey population in North Carolina has remained stable, with reported abundance estimates of 260,000 birds in 2014 and 265,000 in 2019 (Seamster 2016; Chamberlain et al. 2022). Harvest numbers have also been consistently high over recent years, fluctuating between 25,000 and 30,000 birds harvested per year (North Carolina Wildlife Resources Commission 2021).

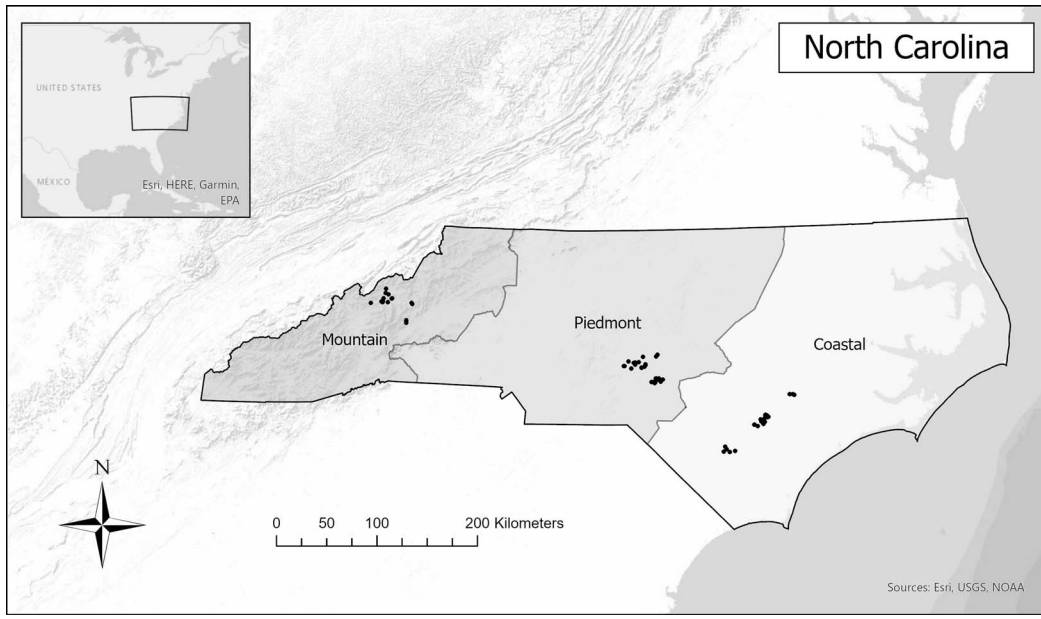

FIGURE 1. Map showing sampling sites and Mountains, Piedmont, and Coastal Plain ecoregions of North Carolina, USA, where live free-ranging Wild Turkeys (*Meleagris gallopavo*) were sampled for pathogens in 2020–22. Maps were created using ArcGIS software (Esri, Redlands, California, USA), and county lines for delineating the regions were obtained from the North Carolina Department of Transportation (NCDOT 2024).

Additionally, the elevational gradient across the three major North Carolina ecoregions provides variation across study sites and enables identification of broadly applicable trends. Thus, North Carolina is an ideal state in which to assess baseline pathogen parameters.

MATERIALS AND METHODS

Study area and sample collection

We analyzed samples collected from turkeys captured on private properties in the three major North Carolina ecoregions: Mountains (24,374 km^2), Piedmont (48,842 km^2), and Coastal Plain (55,412 km^2; Fig. 1), as part of a project investigating turkey space use and movement (Moscicki 2020). Details on the study area are provided in the Supplementary Material. We captured turkeys via rocket-nets at sites baited with corn (*Zea mays*) in winter (January–March) 2020–22, as described previously (Moscicki 2024). We recorded capture location, county, region, sex, and age (i.e., juvenile or adult, based on the 9th and 10th primary feathers; Pelham and Dickson 1992). We collected biologic samples from all birds, when possible, including whole blood, serum, cloacal and oropharyngeal swabs, and fresh feces. Blood samples were collected from the brachial vein, placed in EDTA and additive-free tubes, and kept cold. Additive-free tubes were centrifuged within 6 h to collect serum. Samples were frozen at −20 °C until analysis. We opportunistically recaptured and resampled birds in subsequent years. Capture and sampling protocols were reviewed and approved by the North Carolina State University Animal Care and Use Committee (19-739-01, 19-739-02, 19-739-04).

Testing procedures

We tested samples for previous exposure to and current infection with select viruses, bacteria, and protozoan parasites. We screened whole blood samples by using PCR to identify infections with LPDV; reticuloendotheliosis virus (REV); and *Borrelia*, *Haemoproteus*, *Plasmodium*, and *Leucocytozoon* spp. (Supplementary Material Table S1). We tested serum samples to determine exposure to AIV, *Toxoplasma gondii*, and flaviviruses (including West Nile virus [WNV]) by using blocking ELISA (bELISA) kits (IDEXX AI Ab Test, IDEXX Laboratories, Portland, Maine, USA), modified agglutination test, and plaque

TABLE 1. Regional demographics for 608 Wild Turkeys (*Meleagris gallopavo*) captured (including 22 recaptures) in Mountains, Piedmont, and Coastal Plain ecoregions of North Carolina, USA, 2020–22.

	Mountains		Piedmont		Coastal plain		
Age	Male	Female	Male	Female	Male	Female	Total
Juvenile	31	36	15	31	22	24	159
Adult	49	100	42	108	35	115	449
Total	80	136	57	139	57	139	608

reduction neutralization test, respectively (Dubey and Beattie 1988; Brown et al. 2010; Kunkel et al. 2022a; Roy et al. 2022). Samples from 2022 that were AIV bELISA antibody positive were further tested for clade 2.3.4.4b H5 antibodies by virus neutralization (Stallknecht et al. 2022, 2024). We submitted cloacal swabs to the Poultry Diagnostic and Research Center (Athens, Georgia, USA) to test for turkey coronavirus (TCoV) by using reverse-transcriptase PCR (Supplementary Material Table S1). We submitted feces to the Athens Veterinary Diagnostic Laboratory (Athens, Georgia, USA) for *Salmonella* spp. culture. We tested for *Mycoplasma* spp. by using PCR screening of oropharyngeal swabs (Supplementary Material Table S1). To determine *Mycoplasma* species, PCR amplicons were extracted from a 0.8% agarose gel by using a QIAGEN Gel Extraction Kit (Germantown, Maryland, USA) following manufacturer's directions. Purified DNA fragments were submitted for bidirectional Sanger sequencing (Genewiz, South Plainfield, New Jersey, USA). Sequences were edited and assembled using Geneious 10.2.6 (Biomatters Limited, Auckland, New Zealand).

Statistical analysis

We used the 'stats' package in program R (version 4.3.2) to complete all statistical analyses (R Core Team 2023). We calculated 95% confidence intervals for each pathogen prevalence value using the Wilson score-test interval (Brown et al. 2002). We used a Fisher's exact test to determine relationships between sex, age, and ecoregion in relation to pathogen prevalence, because of low sample sizes in some categories (McDonald 2014). Additionally, we used a pairwise Fisher's exact test for post hoc examination of pathogen prevalence differences between the three ecoregions (McDonald 2014). Significance was determined with an α of 0.05. An adjustment for multiple comparisons was not conducted because we wanted to explore differences specific to each pathogen, some of which share similar transmission risks (e.g., vectorborne or direct transmission). Thus, some statistically significant findings could be false positives.

RESULTS

During the 3-yr study, we captured 586 individual turkeys and recaptured 22 turkeys, for a total of 608 samples (2020=196, 2021=188, 2022=224). Captures were evenly distributed throughout ecoregions (Mountains=216, Piedmont=196, Coastal Plain=196). The overall sex ratio of captures was approximately 1:2, including 194 males and 414 females, with an age structure of 159 juveniles and 449 adults (Table 1). Overall, 482 (79.3%) of the 608 samples were positive for at least one pathogen.

Viruses

The most commonly detected virus was LPDV, with 235 (46.8%) of 502 birds being PCR positive (Table 2). There was no significant difference in LPDV prevalence between sexes (male=42%, female=49%; P=0.150), but adults (52%) had a higher prevalence than juveniles (33%; P<0.001). Regionally, LPDV prevalence was significantly higher in the Piedmont (62.0%) than in the Mountains (50.9%; P=0.048) and the Coastal Plain (27.4%; P<0.001; Table 3). Turkeys captured in the Mountains had a significantly higher prevalence than those from the Coastal Plain (P<0.001). Antibodies to flaviviruses were detected in 71 (15.4%) of 461 birds, with most detections (67/71, 94%) attributed to WNV;

TABLE 2. Overall prevalence of selected pathogens from 608 Wild Turkeys (*Meleagris gallopavo*) captured (including 22 recaptures) throughout North Carolina, USA, 2020–22, ordered from highest prevalence to lowest prevalence.

Pathogen	Category	No. positive (total)	% prevalence	95% CI[b]
Haemoproteus spp.	Protozoan	285 (500)	57.0	52.6–61.3
Lymphoproliferative disease virus	Virus	235 (502)	46.8	42.5–51.2
Mycoplasma spp.	Bacteria	218 (548)	39.8	35.8–43.9
Toxoplasma gondii	Protozoan	98 (460)	21.3	17.8–25.3
West Nile virus[a]	Virus	71 (461)	15.4	12.4–19.0
Leucocytozoon spp.	Protozoan	44 (499)	8.8	6.6–11.6
Reticuloendotheliosis virus	Virus	17 (502)	3.4	2.1–5.4
Avian influenza virus	Virus	9 (453)	2.0	1.0–3.7
Turkey coronavirus	Virus	0 (502)	0	0–0
Plasmodium spp.	Protozoan	0 (500)	0	0–0
Borrelia spp.	Bacteria	0 (500)	0	0–0
Salmonella spp.	Bacteria	0 (42)	0	0–0

[a] Includes four detections within the family *Flaviviridae* assumed to be West Nile virus.
[b] CI = confidence interval calculated using the Wilson score-test interval.

antibodies in the remaining 4 birds were also assumed to be due to WNV exposure. There was no sex-related difference in WNV seroprevalence (P=0.892), but adults had a significantly higher seroprevalence than juveniles (P<0.001). Ecoregion differed significantly (P<0.001), with turkeys in the Piedmont (24%) having significantly higher WNV seroprevalence than those in the Mountains (8%; P<0.001), whereas turkeys in the Coastal Plain (16%) did not significantly differ from those of the other two ecoregions. Prevalence of REV was low, with 17 (3.4%) of 502 turkeys testing positive. Significantly more females than males tested REV positive (P=0.017), with no difference between ages (P=0.085) or ecoregions (P=0.304). Only 9 (2.0%) of 453 birds tested positive for AIV antibodies. There were no differences in AIV seroprevalence by sex (P=0.633), age (P=0.085), or ecoregion (P=0.304). Classical TCoV was not detected in any samples.

Bacteria

Detection prevalence of *Mycoplasma* spp. was relatively high (218/548, 39.8%; Table 2). Of 218 detections, sequence analysis identified *Mycoplasma gallopavonis* in 162 (74.3%), *Mycoplasma gallinaceum* in 44 (20.2%), *Mycoplasma iners* in 2 (0.9%), and undetermined species in 10 (4.6%). There was no relationship between sex (P=0.778) or age (P=0.194) and *Mycoplasma* spp. prevalence. However, there was a significant regional difference (P<0.001), with turkeys in the Mountains (44.9%; P<0.001) and the Coastal Plain (47.2%; P<0.001) having significantly higher *Mycoplasma* spp. prevalence than those from the Piedmont (26.6%; Table 3). We did not detect *Borrelia* or *Salmonella* spp. from 500 blood samples and 42 fecal samples, respectively.

Protozoan parasites

We detected a high *Haemoproteus* spp. prevalence (285/500, 57%; Table 2), with no significant difference between sexes (male=58%, female=56%; P=0.772) but a significantly higher prevalence in adults (60%) than juveniles (49%; P<0.049). Regional *Haemoproteus* spp. detections differed significantly (P<0.001), with increasing prevalence from west to east (Mountains=24%, Piedmont=65%, Coastal Plain=84%; Table 3). *Haemoproteus* prevalence in the Coastal Plain was significantly higher than in both the Piedmont (P<0.001) and the Mountains (P<0.001), and prevalence in the Piedmont was

TABLE 3. Number and prevalence (prev) of positive viral, bacterial, and protozoan infections from 608 Wild Turkeys (*Meleagris gallopavo*; including 22 recaptures) captured in Mountains, Piedmont, and Coastal Plain ecoregions of North Carolina, USA, 2020–22.

		2020		2021		2022		Total	
Pathogen[a]	Region	No. (*n*)[b]	% prev	No. (*n*)	% prev	No. (*n*)	% prev	No. (*n*)	% prev
LPDV	Mountains	25 (62)	40.3	19 (50)	38.0	45 (63)	71.4	89 (175)	50.9
	Piedmont	40 (65)	61.5	27 (49)	55.1	34 (49)	69.4	101 (163)	62.0
	Coastal	0 (25)	0.0	13 (62)	21.0	32 (77)	41.6	45 (164)	27.4
WNV	Mountains	7 (62)	11.3	3 (44)	6.8	3 (62)	4.8	13 (168)	7.7
	Piedmont	18 (51)	35.3	9 (46)	19.6	7 (47)	14.9	34 (144)	23.6
	Coastal	0 (24)	0.0	16 (61)	26.2	4 (60)	6.7	20 (145)	13.8
REV	Mountains	4 (62)	6.5	0 (50)	0.0	2 (63)	3.2	6 (175)	3.4
	Piedmont	2 (65)	3.1	6 (49)	12.2	0 (49)	0.0	8 (163)	4.9
	Coastal	1 (25)	4.0	2 (62)	3.2	0 (77)	0.0	3 (164)	1.8
AIV	Mountains	1 (62)	1.6	0 (44)	0.0	1 (61)	1.6	2 (167)	1.2
	Piedmont	3 (46)	6.5	0 (47)	0.0	0 (46)	0.0	3 (139)	2.2
	Coastal	0 (23)	0.0	0 (62)	0.0	4 (62)	6.5	4 (147)	2.7
Mycoplasma spp.	Mountains	13 (81)	16.0	43 (69)	62.3	41 (66)	62.1	97 (216)	44.9
	Piedmont	7 (70)	10.0	16 (51)	31.4	23 (52)	44.2	46 (173)	26.6
	Coastal	6 (27)	22.2	29 (68)	42.6	40 (64)	62.5	75 (159)	47.2
Haemoproteus spp.	Mountains	7 (62)	11.3	6 (50)	12	28 (62)	45.2	41 (174)	23.6
	Piedmont	54 (65)	83.1	27 (49)	55.1	25 (48)	52.1	106 (162)	65.4
	Coastal	16 (25)	64.0	51 (62)	82.3	71 (77)	92.2	138 (164)	84.1
Leucocytozoon spp.	Mountains	0 (62)	0.0	0 (50)	0.0	0 (61)	0.0	0 (173)	0.0
	Piedmont	4 (65)	6.2	2 (49)	4.1	3 (48)	6.3	9 (162)	5.6
	Coastal	8 (25)	32.0	9 (62)	14.5	18 (77)	23.4	35 (164)	21.3
Toxoplasma gondii	Mountains	14 (61)	23.0	7 (43)	16.3	18 (61)	29.5	39 (165)	23.6
	Piedmont	12 (51)	23.5	3 (47)	6.4	10 (49)	20.4	25 (147)	17.0
	Coastal	6 (24)	25.0	14 (62)	22.6	14 (62)	22.6	34 (148)	23.0

[a] LPDV = lymphoproliferative disease virus; WNV = West Nile virus; REV = reticuloendotheliosis virus; AIV = avian influenza virus. There were no detections of turkey coronavirus, *Plasmodium* spp., *Borrelia* spp., or *Salmonella* spp.
[b] Number positive (total samples tested).

also significantly higher than in the Mountains ($P < 0.001$). Prevalence of *Leucocytozoon* was low, with 44 (8.8%) of 499 birds testing positive. Similar to *Haemoproteus*, there was no relationship of *Leucocytozoon* prevalence to sex (male=6%, female=10%; P=0.180) but significant age (P<0.050) and regional (P<0.001) differences. Adults (11%) had higher *Leucocytozoon* prevalence than juveniles (3%), with increasing prevalence west to east (Mountains=0%, Piedmont=5.6%, Coastal Plain=21.3%). *Leucocytozoon* prevalence was significantly higher in the Coastal Plain than the Mountains (P<0.001) and the Piedmont (P<0.001). Prevalence in the Piedmont was also higher than in the Mountains (P=0.001). *Plasmodium* was not detected in any turkeys. Antibodies against *T. gondii* were found in 98 (21.3%) of 460 of birds, with a significant difference by age (adult=24%, juvenile=14%; P=0.046) but not sex (male=15%, female=23%; P=0.337) or ecoregion (Mountains=24%, Piedmont=17%, Coastal Plain=23%; P=0.302; Table 2).

Recaptures and retesting

We recaptured 22 turkeys, with a mean time between capture events of 466 d (range, 319–745 d). Most recaptures had no pathogen status change (i.e., remained positive or negative; Table 4). New detections were noted for *Mycoplasma* spp. (47%), LPDV (38%), and *T. gondii* (23%). A low percentage of recaptures

TABLE 4. Change in pathogen testing results from initial capture to recapture for 22 recaptured Wild Turkeys (*Meleagris gallopavo*) in Mountains, Piedmont, and Coastal Plain ecoregions of North Carolina, USA, 2020–22. Because of sample availability, not all 22 birds were tested for all pathogens at each time point.

Pathogen	No. sampled	No change (%)	Change from negative to positive (%)	Change from positive to negative (%)
Avian influenza virus	453	13 (100)	0	0
Reticuloendotheliosis virus	502	15 (94)	1 (6)	0
West Nile virus	461	12 (92)	1 (8)	0
Leucocytozoon spp.	499	13 (87)	0	2 (13)
Haemoproteus spp.	500	12 (80)	2 (13)	1 (7)
Lymphoproliferative disease virus	502	9 (56)	6 (38)	1 (6)
Toxoplasma gondii	460	7 (54)	3 (23)	3 (23)
Mycoplasma spp.	548	8 (53)	7 (47)	0

became negative at the subsequent testing period, including for *T. gondii* (23%), *Leucocytozoon* spp. (13%), *Haemoproteus* spp. (6.7%), and LPDV (6.2%).

Coinfections and codetections

Coinfections and/or antibody codetections were common throughout our samples and were detected in 318 (52.3%) of 608 turkeys, with cases of two (170/318, 53.5%), three (115/318, 36.2%), four (31/318, 9.7%), or five (2/318, 0.6%) pathogens per bird. The three pathogens with the highest prevalence also had the highest rates of coinfection: *Haemoproteus* spp. (n=399, number of unique coinfections) followed by LPDV (n=348) and *Mycoplasma* spp. (n=267; all codetections are provided in Supplementary Material Table S2). Given recent interest in LPDV, coinfection and/or codetections occurred with, in descending order, *Haemoproteus* (n=137), *Mycoplasma* (n=86), *T. gondii* (n=50), WNV (n=40), *Leucocytozoon* (n=18), REV (n=10), and AIV (n=7). Overall, LPDV was rarely detected alone (14%, n=202) and commonly detected with at least one other pathogen (86%): turkeys with LPDV also had one (85/202, 42.1%), two (91/202, 45%), three (24/202, 12%), or four (2/202, 1%) coinfections and/or codetections.

DISCUSSION

Our examination of live free-ranging turkey pathogen exposure and infection provides important information on population-level disease risk. Overall, we found that pathogen exposure or infection of apparently subclinically infected turkeys in North Carolina was common; we detected at least one pathogen from nearly 80% of turkeys, plus many codetections. Sampling live birds enables testing for pathogens and parasites that infrequently cause disease and may be missed by examining only sick or dead birds submitted for diagnostic evaluation (Nusser et al. 2008; Haynes et al. 2024); however, it limits the ability to study some known parasites of concern, such as intestinal parasites (e.g., *Eimeria* spp., *Heterakis gallinarum* as a vector of *Histomonas meleagridis*) and trichomonads (Greenawalt et al. 2020; Adcock et al. 2025).

Concern about viral infections in turkeys has garnered much research attention, given recent population declines and concerns related to emerging viruses and transmissibility across the wild bird-domestic bird interface (Allison et al. 2014; Niedringhaus et al. 2019; Kunkel et al. 2022a; Malmberg et al. 2023). Widespread oncogenic retroviral infections in turkey populations have been of particular interest since the initial documentation of LPDV infection and disease in the USA

(Niedringhaus et al. 2019). Both REV and LPDV can induce tumor formation (neoplasia) that may cause immunosuppression and has been associated with fatal neoplasia in wild birds (Ley et al. 1989; Hayes et al. 1992; Allison et al. 2014; Stewart et al. 2019; Adcock et al. 2024). Overt clinical disease caused by REV and LPDV in free-ranging populations is relatively rare, but the potential underlying effects are still unknown (Payne 1998; Thomas et al. 2015; Niedringhaus et al. 2019). Documented REV prevalence in turkeys has been highly variable, from low (5–11%) in free-ranging birds in Texas and Kentucky, USA (Stewart et al. 2019; Haynes et al. 2024), to relatively high (>40%) among mortality submissions from the eastern USA (Adcock et al. 2024). The low REV prevalence (3.4%) that we detected was similar to prevalences from other studies of free-ranging healthy turkeys (Stewart et al. 2019; Haynes et al. 2024). By contrast, documented LPDV prevalences have been higher (46–72%) throughout regions of North America (Thomas et al. 2015; Alger et al. 2017; MacDonald et al. 2019a; Kreh and Palamar 2022; Adcock et al. 2024). Our LPDV prevalence of 47% was similar to that of a previous study (46%) conducted in North Carolina on birds harvested in 2013 and 2015 (Kreh and Palamar 2022). Similar to other studies, we found a higher LPDV prevalence in adults than in juveniles (Thomas et al. 2015; Alger et al. 2017; Kreh and Palamar 2022). This might be explained by the sampling of uninfected hatch-year birds following high poult mortality (Alger et al. 2017) and/or the longer period (i.e., increased opportunities) of viral exposure for adults (Thomas et al. 2015). The variation in LPDV prevalence among ecoregions (highest in the Piedmont, followed by the Mountains and last the Coastal Plain) is not fully understood. Alger et al. (2017) found that an increased ratio of agricultural to forest lands increased the odds of LPDV infection in New York, USA, but most North Carolina agricultural lands are in the Coastal Plain (US Department of Agriculture 2016). By contrast, Shea et al. (2022) found that turkeys in Maine, USA, were approximately 10 times more likely to be infected by LPDV with every 10% increase in forest cover; this generally aligns with our study because forest cover is higher in the North Carolina Piedmont and Mountains (US Department of Agriculture 2016). However, LPDV distribution in turkey populations probably relates to numerous factors and should be further examined.

We only detected a low AIV antibody prevalence (2%) in different years and ecoregions, similar to Davidson et al. (1988) and Jennelle et al. (2017), who failed to detect AIV antibodies in turkeys from the southeastern USA and Minnesota, USA, respectively. Low pathogenic AIVs (specifically H7 strains) can cause disease in domestic turkeys (Spackman et al. 2010), but the recent detection of H5N1 HPAIV in numerous wild bird and mammal species in the USA, including an outbreak involving 41 turkeys in Wyoming, has raised concern about this pathogen in wild populations (Malmberg et al. 2023). Although AIV prevalence in our study was low, and no individuals had been exposed to clade 2.3.4.4b H5, turkeys should be monitored closely because continued infections of wild and domestic birds in the USA represent a spillover risk to turkey populations, especially in regions with high-density commercial operations or farms with free-ranging birds. The low prevalence that we found in turkeys also suggests that if they were exposed, they would lack population immunity and may be more susceptible to severe disease.

Another virus of concern, WNV, has been linked to declines in multiple wild bird species (Kunkel et al. 2022b). However, experimental infection trials have indicated that WNV is probably not a significant risk to domestic or wild turkey populations (Swayne et al. 2000; Kunkel et al. 2022a). Overall, we found low WNV seroprevalence (15.4%) in North Carolina turkeys, with antibodies more commonly detected in adults than in juveniles. The higher WNV prevalence that we found in the Piedmont than in the Mountains was expected because western North Carolina is thought to have low suitability for *Culex* spp. vectors, in

part due to higher elevations (Gorris et al. 2021). Previous WNV studies in turkeys have documented highly variable antibody prevalence (from 0% to >67%) in eastern USA states (Kunkel et al. 2022b). Although WNV does not appear to be highly pathogenic for turkeys, isolated cases, coinfections, or other factors may predispose individual birds to disease (Kunkel et al. 2022b).

We detected a relatively high *Mycoplasma* spp. prevalence (40%) in turkeys, including the species *M. gallopavonis*, *M. gallinaceum*, and *M. iners*. These species are typically considered nonpathogenic to poultry and unlikely to impact turkeys; similar or higher prevalences have been reported in turkeys in eastern North America (e.g., South Carolina, USA [87.7%]; Ontario, Canada [98.7%]; Luttrell et al. 1992; Beylefeld et al. 2018; MacDonald et al. 2019b). A study of Rio Grande (*Meleagris gallopavo intermedia*) and Merriam's (*Meleagris gallopova merriami*) turkeys in western USA states (Arizona, Colorado, New Mexico, North Dakota, Oklahoma, and Texas) previously detected a similar *Mycoplasma* spp. prevalence (30%); isolates there included known pathogenic species (i.e., *M. gallisepticum* and *M. synoviae*), suggesting that apparently healthy birds can be carriers (Fritz et al. 1992). Infections by *Mycoplasma* spp. (*M. gallisepticum*, *M. synoviae*, *M. iowae*, and *M. meleagridis*) have been reported to negatively influence egg production and cause respiratory infection in domesticated birds (Lancaster and Fabricant 1988; Rocke et al. 1988); however, wild birds typically show no clinical signs (MacDonald et al. 2019b). Additionally, synergistic effects from coinfections may increase disease severity (Rhoades 1981; Naylor et al. 1992). Some *Mycoplasma* spp. detections in turkey populations have been related to the presence of industrial or backyard poultry operations (MacDonald et al. 2019b), a relationship that may explain the higher prevalence that we found in turkeys from the North Carolina Coastal Plain because this ecoregion has many high-density commercial poultry farms (Environmental Working Group 2019). However, there are no known commercial poultry farms near our capture locations in the Mountains, so the higher *Mycoplasma* spp. prevalence there may not be explained by interactions with domestic poultry. Other factors (e.g., density, intraspecific interactions) may be important for *Mycoplasma* spp. transmission.

Expectedly, we failed to detect *Salmonella* spp., which have rarely been reported in turkeys (Davidson et al. 1985; Hopkins et al. 1990; MacDonald et al. 2018). Craft et al. (2022) suggested that a more diverse microbiome of wild turkeys (versus captive turkeys) may provide resistance to colonization and invasion by *Salmonella* spp. and other pathogenic bacteria. The absence of *Borrelia* spp. in our study may relate to the season in which samples were collected and local tick demographics (Jordan et al. 2009; Scott et al. 2010; Cleveland et al. 2020). Higher *Borrelia* spp. prevalences have been documented in turkeys harvested in spring and fall (Jordan et al. 2009; Cleveland et al. 2020; Scott et al. 2010), and although our sampling period (January–March) corresponds to activity of adult *Ixodes scapularis*, a *Borrelia* spp. vector, adult *I. scapularis* rarely feed on birds (Eisen 2025). During our study, only one tick (*Amblyomma americanum* nymph) was collected in 2020, suggesting that tick parasitism during winter in North Carolina may be uncommon.

Several protozoan parasites have been reported to cause disseminated disease in both wild and domestic turkeys, including *H. meleagridis*, *Leucocytozoon smithi*, *T. gondii*, and *Sarcocystis* spp. (Atkinson and Forrester 1987; Quist et al. 1995; Teglas et al. 1998; Dubey et al. 2000). Experimental infection of domestic turkeys with *H. meleagridis* resulted in significantly reduced poult development and mortality in cases of high parasitemia (Atkinson et al. 1988b). Disease has also been reported in free-ranging turkeys, but based on data from domestic turkeys, it is probably most severe in poults, an age group less commonly submitted for diagnostic evaluation. *Haemoproteus* spp. prevalence (57%) was high in our study,

similar to that of other studies in which *Haemoproteus* spp. were among the most frequently detected blood parasites in turkeys (Forrester et al. 1974; Lynch et al. 2025). The second most common blood parasite that has been reported in turkeys, *Leucocytozoon* spp., was detected in approximately 9% of our turkeys, a value that is relatively low compared with documented prevalences in Mississippi and Louisiana, USA (Stacey et al. 1990; Lynch et al. 2025). In domestic turkeys, *L. smithi* causes significant mortality, although only sporadic cases have been reported in wild turkeys (Forrester et al. 1974; Davidson and Wentworth 1992; Forrester and Spalding 2003). *Haemoproteus* and *Leucocytozoon* spp. are transmitted by biting midges (*Culicoides* spp.; Atkinson et al. 1988a) and blackflies (*Simulium* spp.; Greiner and Forrester 1979), respectively, perhaps explaining the gradient of detections of both pathogens from the mountains to the coast in North Carolina. Hemoparasite prevalence can relate to host density, vector abundance, and environmental conditions, with more blood-sucking arthropods typically occurring in locations with warmer, wetter weather (Atkinson et al. 1988a).

Nearly a quarter (21%) of turkeys in our study had antibodies against *T. gondii*, a value that is within the range that has been reported in turkeys in the southeastern USA (10–71%; Lindsay et al. 1994; Quist et al. 1995). Testing of 13 recaptured turkeys found seroconversion in 3 birds and seroreversion in 3 birds. Although *T. gondii* results in chronic infections, seroreversion has been noted to occur sporadically in naturally infected domestic and wild species (Sandström et al. 2013; Ramey et al. 2019; Olsen et al. 2021). Experimentally, *T. gondii* did not cause disease in domestic turkeys and persistent infections developed (Dubey et al. 1993; Bangoura et al. 2013; Zöller et al. 2013); however, sporadic fatal systemic infections have been reported in wild turkeys (Howerth and Rodenroth 1985; Quist et al. 1995). This parasite is transmitted to turkeys via ingestion of food or water contaminated by felid (domestic cat [*Felis catus*] or bobcat [*Lynx rufus*]) feces. These hosts are common across North Carolina, potentially explaining the lack of regional prevalence differences (Lindsay et al. 1994). Finally, *T. gondii* is zoonotic and impacts the health of many domestic and wildlife species and *T. gondii* strains from turkeys in Pennsylvania, USA, were similar to strains from numerous wildlife species in North America (Dubey et al. 1993; Cerqueira-Cézar et al. 2019).

In our study, all three protozoan parasites were found more commonly in adults than in juveniles. This mostly conforms to previous *Leucocytozoon* spp. research reporting higher prevalence in adults (Eve et al. 1972; Hopkins et al. 1990) or no difference between age classes (Forrester et al. 1974; Stacey et al. 1990). *Haemoproteus* spp. have been detected more often in juveniles (Hopkins et al. 1990) or evenly between age classes (Eve et al. 1972; Forrester et al. 1974; Stacey et al. 1990). Fewer data are available on *T. gondii* prevalence in turkeys, but higher prevalence in adults is consistent with that of other bird species (e.g., Chen et al. 2015; Dubey et al. 2021; Lopes et al. 2021; Wyckoff et al. 2024).

Our comprehensive baseline of turkey pathogen exposure and infection across demographics and ecoregions in North Carolina should enable better demographic- and region-specific management of turkey populations. Although birds sampled in this study were apparently healthy, many had infections with pathogens that can cause clinical disease under some circumstances. Future work should investigate whether factors such as survival, movement, and reproductive success are impacted by infection or coinfection with these pathogens. Finally, most of these pathogens are expected to cause more significant mortality among poults, for which data are generally lacking. Additional data on pathogen-associated poult mortality and the effects of these pathogens on fine-scale movement, survival, and reproductive effects would help

elucidate the possible role of disease in turkey population declines.

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SUPPLEMENTARY MATERIAL

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