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Necropsy Findings and Arbovirus Surveillance in Mourning Doves from the Southeastern United States

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ABSTRACT: Mourning doves (*Zenaida macroura*) are the most abundant and widespread native member of the columbid family, as well as a major migratory game species, in the United States. However, there is little information on mortality factors in mourning doves. Records of necropsy accessions at the Southeastern Cooperative Wildlife Disease Study (SCWDS) from 15 southeastern states, from 1971 through 2005, were reviewed. One hundred thirty-five mourning doves were submitted from nine states during the 35-yr period. Trichomonosis constituted 40% ($n=54$) of all diagnoses and was the most frequent diagnosis. Toxicoses and avian pox constituted 18.5% ($n=25$) and 14.8% ($n=20$) of all diagnoses, respectively. Remaining diagnoses included trauma, suspected toxicosis, *Ascaridia columbae* infection, suspected tick paralysis, and undetermined. Adults were observed more frequently with trichomonosis (94.1%) and toxicoses (68%) as compared to juveniles, but a gender predisposition was not apparent for either disease. Age and gender predilections were not apparent for cases of avian pox. The majority of the trichomonosis and avian pox cases were observed in the spring-summer, whereas the majority of the toxicosis cases were observed in the winter-spring. Additionally, the Georgia Department of Human Resources–Division of Public Health and West Virginia Department of Health and Human Resources submitted 809 mourning doves to SCWDS from 2001 through 2005 for West Nile virus surveillance efforts. West Nile virus was isolated from 2.1% ($n=17$) and eastern equine encephalitis virus (EEEV) was isolated from 0.2% ($n=2$) of the submitted birds.

Key words: Columbid, diseases, mourning dove, southeastern United States, *Zenaida macroura*.

Mourning doves (*Zenaida macroura*) are the most abundant native columbid and represent the most popular avian game species within the United States. Populations have fluctuated throughout the United States and have declined over much of the eastern United States during the last few decades (Dolton and Rau, 2003). Previous morbidity and mortality investigations in mourning doves have disclosed several diseases that may have potential population implications (Conti, 1993; Forrester and Spalding, 2003). Trichomonosis, caused by the protozoan *Trichomonas gallinae*, is considered the most important disease in mourning doves, and several large epidemics have been reported (Stabler, 1954; Conti, 1993; Forrester and Spalding, 2003). One of the largest outbreaks occurred in 1950 and 1951 in multiple southeastern states. During this 2-yr outbreak, in Alabama alone, an estimated 50,000 to 100,000 mourning doves died of trichomonosis (Haugen and Keeler, 1952). Avian pox, toxicoses, and trauma have been identified as other diseases with potential population implications (Conti, 1993; Forrester and Spalding, 2003). In this review we examined mourning dove diagnoses from the Southeastern Cooperative Wildlife Disease Study (SCWDS) necropsy records and investigated whether common causes of morbidity and mortality were associated with gender, age, or seasonal patterns. Additionally, records of mourning doves submitted from two states for West Nile virus (WNV) surveillance from 2001 through 2005 were reviewed.

Records of mourning dove necropsy accessions received at SCWDS from 1971 through 2005 were examined; doves were from 15 southeastern states. Accessions were subjected to a complete necropsy, which included both gross and histopathologic examination of all major organs. The findings were categorized into cause of mortality, age, gender, season of mortality, and state of origin. A chi-

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TABLE 1. Diagnostic findings in 135 mourning doves from the southeastern United States (1971–2005).

Diagnosis	No. of doves (%)	No. of mortality events (%)	States[a]	Frequency[b]
Trichomonosis	54 (40)	39 (47)	AL, FL, GA, NC, SC, TN, VA, WV	18
Toxicosis	25 (18.5)	7 (8.4)	GA, SC, VA, WV	7
Suspected toxicosis	22 (16.2)	6 (7.2)	GA, SC, WV	6
Avian pox	20 (14.8)	17 (20.4)	GA, SC, VA, WV	10
Undetermined	6 (4.4)	6 (7.2)	FL, GA, VA	6
Trauma	5 (3.7)	5 (6.0)	GA	5
A. columbae infection	2 (1.5)	2 (2.4)	FL, GA	2
Suspected tick paralysis	1 (<0.1)	1 (1.2)	GA	1

[a] AL = Alabama; FL = Florida; GA = Georgia; NC = North Carolina; SC = South Carolina; TN = Tennessee; VA = Virginia; WV = West Virginia.

[b] Number of years disease diagnosed during the 35-yr retrospective study.

squared test (alpha=0.05) was used to determine if significant differences existed between the categories of interest. In addition to necropsy accessions, the Georgia Department of Human Resources–Division of Public Health and West Virginia Department of Health and Human Resources submitted dead mourning doves to SCWDS from 2001 through 2005 as part of their WNV surveillance programs. The virus isolation records were examined for mourning doves from which arboviruses were isolated. Unlike complete necropsies, WNV submission protocols consisted only of extraction of a brain sample for virus isolation without histopathological examination of tissues. Therefore, the cause of death could not be directly attributed to virus infection. During 2001 gross findings were recorded for birds submitted for WNV testing; however, these findings were not recorded for the subsequent years.

From 1971 through 2005, 135 mourning doves from nine southeastern states (Alabama, Florida, Georgia, Louisiana, North Carolina, South Carolina, Tennessee, Virginia, and West Virginia) were submitted to SCWDS for necropsy. A significantly greater number of adult (X^2=33.3, df=1, P<0.001) and male (X^2=6.02, df=1, P<0.02) mourning doves were submitted as compared to juvenile and female doves, respectively. Trichomonosis, avian pox, and toxicoses were the three most frequently diagnosed diseases, and they constituted 73% (n=99) of all diagnoses. Of these three diseases, trichomonosis was diagnosed most frequently (X^2=13.4, df=2, P<0.005) followed by toxicoses and avian pox (Table 1). The number of mortality events for each of the three diseases was analyzed, and trichomonosis was the most frequent event followed by avian pox and toxicosis (Table 1). A significantly greater number of mortality events were due to trichomonosis (X^2=15.4, df=2, P<0.001), and a significantly lesser number were due to toxicoses (X^2=9.3, df=2, P<0.01). Mortality events of avian pox were not significantly different from trichomonosis or toxicoses events (X^2=0.76, df=2, P>0.05). When suspected toxicoses were included with trichomonosis, avian pox, and confirmed toxicoses, the combined diagnoses constituted 89.6% (n=121) of all diagnoses. Other diagnoses included trauma, *Ascaridia columbae* infection, suspected tick paralysis, and undetermined (Table 1).

Trichomonosis was diagnosed by identifying flagellated protozoa consistent with *Trichomonas* spp. on wet mount, culture, or histologic preparations demonstrating intralesional protozoa. Trichomonads were cultured in Diamond's media supplemented with 10% horse serum (Diamond, 1957). Trichomonosis was diagnosed more frequently in adults (n=51, 94%), as were

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toxicoses (n=21, 84%), but a gender predisposition was not apparent for either disease. Of the identified organic chemicals causing confirmed mortality in doves, two mortality events were due to carbofuran, and one event each was due to famphur and toxaphene. The remaining doves diagnosed as toxicosis had nondiagnostic findings on chemical analysis, and the diagnosis was based solely on >50% decrease in brain cholinesterase (ChE) concentrations as compared to brain ChE concentrations for control doves. For doves diagnosed with avian pox, no age or gender predisposition was apparent. Ninety-eight percent of the avian pox diagnoses were made from identifying characteristic eosinophilic inclusion bodies of avian pox virus on impression smears or histologic examination. The remaining avian pox diagnoses were made solely by observations of gross lesions.

More mourning doves were submitted in the spring and summer months (X^2=9.6, df=1, P<0.0025) than in the autumn and winter months. Toxicoses were diagnosed more frequently in the spring (90%; X^2=32.6, df=3, P<0.001) than in other seasons, whereas avian pox was diagnosed more frequently in the summer (90%; X^2=28.8, df=3, P<0.001). Trichomonosis was diagnosed more often in the spring and summer months (70%; X^2=4.4, df=1, P<0.05) than in autumn and winter months.

West Nile virus was isolated from 2.1% (n=17), and eastern equine encephalitis virus (EEEV) was isolated from 0.2% (n=2) of the 809 doves submitted from Georgia and West Virginia. Virus isolation and reverse-transcriptase polymerase chain reaction for EEEV and WNV were performed as previously described (Gottdenker et al., 2003; Allison et al., 2004). Gross findings recorded for doves submitted in 2001 indicated that 43.4% (n=36) had lesions consistent with trichomonosis, 19.3% (n=16) had lesions consistent with trauma, and 3.6% (n=3) had lesions consistent with avian pox. These findings must be viewed with caution, because confirmatory diagnostic testing was not conducted.

Trichomonosis was the most frequently diagnosed disease in this retrospective study. Mourning doves may transmit or acquire *T. gallinae* through feeding crop milk to nestlings, billing courtship during mating, or by ingestion of contaminated food or water (Kocan, 1969; Kocan and Herman, 1971). The high frequency of trichomonosis diagnoses in the present review is consistent with previous reports of diseases in mourning doves (Conti, 1993; Forrester and Spalding, 2003). Population impacts of trichomonosis outbreaks in mourning doves are variable, and, generally, the disease is observed in juveniles more than adults (Haugen and Keeler, 1952; Conti and Forrester, 1981; Ostrand et al., 1995). Schulz et al. (2005) monitored the annual variation of *T. gallinae* from hunter-killed mourning doves without clinical trichomonosis and found that *T. gallinae* was isolated from essentially an equal portion (5.5%) of hatch-year and after-hatch-year birds. We observed trichomonosis in 48% (n=51) of the adults and in 20% (n=3) of the juveniles, which may be due to a significantly greater number of adults being submitted.

Rock pigeons (*Columba livia*) are the natural host for *T. gallinae*, and most pigeons harbor this protozoan but rarely have clinical disease (Stabler, 1954; Tudor, 1991). *Trichomonas gallinae* is known to have a wide spectrum of virulence, but the factors that control virulence are incompletely known (Honigberg et al., 1971; Honigberg, 1979). Infection with an avirulent strain or survival of infection with a virulent strain of *T. gallinae* provides columbids with protective immunity, resulting in individuals that are refractory to clinical disease (Stabler, 1948; Kocan, 1972). Therefore, previously infected pigeons and doves may serve as inapparent carriers of virulent strains of *T. gallinae* and are potential sources of infection for

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naive birds (Stabler, 1954). Pigeons and doves also may serve as a source of infection for raptors, in which the disease can have focal population impacts (Boal et al., 1998).

Brains and gastrointestinal contents were collected from dead mourning doves and frozen at −20 C for brain ChE assays as described by Hill (1988). When available, brains from healthy mourning doves were used for control specimens. Following the initial brain ChE assay, the samples were incubated at 37 C for 18 hr to evaluate enzyme reactivation. Reactivation of ChE is characteristic of a carbamate poisoning, but not an organophosphorus poisoning (Smith et al., 1995).

Chemical analyses of the gastrointestinal contents were performed as previously described in White et al. (1989) and Holstege et al. (1994) at the Cooperative Extension Service (College of Agriculture, University of Georgia, Athens, Georgia, USA) or the University of Pennsylvania Veterinary Diagnostic Laboratory (College of Veterinary Medicine, Kennett Square, Pennsylvania, USA). Gastrointestinal contents were screened for organic compounds including organophosphorus and carbamate pesticides. In general, the list of screened compounds included 3-hydroxy carbofuran, aldicarb, aldicarb sulfone, aldicarb sulfoxide, bendiocarb, carbaryl, carbofuran, carbosulfan, chlorpyriphos, diazinon, disulfoton, famphur, fenthion, formetanate HCl, methiocarb, methomyl, mexacarbate, oxamyl, parathion, primicarb, propoxur, terbufos, thiodicarb, toxaphene, and trimethacarb.

Confirmed toxicosis cases were diagnosed by significantly decreased brain ChE concentration alone or in conjunction with identification of a specific compound. Diagnoses of suspected toxicosis were based on a history strongly suggestive of poisoning, ingesta in gastrointestinal tract, and unremarkable necropsy findings, but with negative toxicological findings or detection of a compound at nondiagnostic levels in addition to <50% decrease in brain ChE concentrations as compared to ChE concentrations for control doves.

Confirmed toxicoses were observed most frequently in the months of late winter and early spring, which may coincide with application of pesticides on lawns, fields, and planted crops. Additionally, several of the case histories indicated possible intentional poisoning. Carbofuran was the most frequently identified compound in our retrospective study, followed by famphur and toxaphene. A 20-yr retrospective summary from the National Wildlife Health Center disclosed that 18 mortality events, involving 302 individual columbids, were due to anticholinesterase pesticides (Fleischli et al., 2004). Of the identified compounds in this study, three mortality events were due to carbofuran, two events were due to fenthion, one event was due to famphur, and 12 events were due to unspecified anticholinesterase pesticides. Heavy metal toxicosis has been a concern in doves, especially with the use of lead shot at dove fields (Schulz et al., 2006). However, there were no cases of heavy metal toxicoses in mourning doves in our records.

Avian pox was diagnosed more frequently in the summer, which would be expected because the virus is believed to be predominately transmitted by blood-feeding arthropods (Akey et al., 1981). In Florida cases of avian pox in wild turkeys (*Meleagris gallopavo*) have been documented primarily in September through December, which corresponds to the peak of two mosquito populations known to transmit avian pox virus (Akey et al., 1981). Because avian pox infections often are easily recognized by visual examination, field biologists may not submit these carcasses for laboratory testing; thus, avian pox may be underrepresented in our necropsy accessions.

The two doves diagnosed with *A. columbae* infection had more than 100 parasites each within the intestinal tract.

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Previous reports documented that *A. columbae* occurred in 2.0%–25.8% of examined doves, with infections generally consisting of fewer than 30 parasites (Barrows and Hayes, 1977; Conti and Forrester, 1981; Lee et al., 2004). Interestingly, a study comparing the relative helminth intensities in mourning doves and the interrelationships with the introduced white-winged dove (*Zenaida asiatica*) in Florida disclosed a higher intensity of helminths in mourning doves in areas where white-winged doves were present (Conti and Forrester, 1981). Bean et al. (2005) reported that helminth intensity in the introduced Eurasian collared dove (*Streptopelia decaocto*) was similar to that of the white-winged doves, but significantly higher than that of mourning doves. However, it is unknown if Eurasian collared doves directly affect mourning dove helminth intensities in areas where the two species are sympatric. The two doves diagnosed with *A. columbae* infection originated in areas in which Eurasian collared doves are present (Romagosa and Labisky, 2000).

Two ticks identified as *Ixodes brunneus* were removed from the dove diagnosed with suspected tick paralysis. *Ixodes brunneus* infection has been associated with tick paralysis syndrome in birds; however, definitive diagnosis of the syndrome requires removal of the ticks and regression of clinical signs (Luttrell et al., 1996), which was not possible because the bird was dead upon submission. However, histologic and gross examination did not disclose additional causes of mortality.

West Nile virus was isolated from 2.1% of submitted doves, and EEEV was isolated from 0.2% of submitted doves. Tesh et al. (2004) reported that 2.2% ($n=6$) of mourning doves, 2.1% ($n=1$) of rock pigeons, and 2.6% ($n=1$) of Inca doves (*Columbina inca*), tested as part of dead bird surveillance in Texas, were positive for WNV. In a separate investigation, 12.3% ($n=30$) of free-ranging, healthy mourning doves, 28.9% ($n=153$) of rock pigeons, and 26.8% ($n=15$) of common ground doves (*Columbina passerine*) from Georgia were seropositive to WNV (Gibbs et al., 2006). Comparably, 4.3% ($n=14$) of blue jays (*Cyanocitta cristata*) and 9.7% ($n=3$) of American crows (*Corvus brachyrhynchos*) were WNV seropositive. The relatively low virus isolation rates from dead bird submissions in conjunction with high seroprevalence rates from clinically healthy birds suggest that mourning doves may be relatively less susceptible to WNV-induced disease in comparison to corvids and several other passerine species (Gibbs et al., 2006). Forrester and Spalding (2003) found that <1% of tested doves were positive for EEEV, which is consistent with our findings.

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