DOI: 10.7589/2019-05-124

Journal of Wildlife Diseases, 56(3), 2020, pp. 673–678

Postmortem Evaluation of Reintroduced Migratory Whooping Cranes (*Grus americana*) in Eastern North America

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ABSTRACT: We reviewed necropsy records of 124 Whooping Cranes (*Grus americana*) recovered following reintroduction of 268 individuals from 2001 to 2016 in the eastern US. Causes of death were determined in 62% (77/124) of cases facilitated by active monitoring that limited decomposition and scavenging artifact. The greatest proportions of mortality were caused by predation (0.468; 95% confidence interval 0.356–0.580; 36/77), collision with power lines or vehicles (0.260; 0.162–0.358; 20/77), and gunshot (0.169; 0.085–0.253; 13/77). Six deaths were attributed to infection (0.078; 0.018–0.138; 6/77), including bacterial and fungal etiologies. Lead analysis of 50 liver samples yielded two results with elevated concentrations (3.65 and 10.97 ppm wet weight), and 10 bone samples from partial carcasses lacking suitable liver tissue resulted in one elevated result (48.82 ppm dry weight). These data indicate that underlying subclinical or clinical lead toxicosis may be a factor in up to 5% of deaths attributed to predation or impact trauma. Brain cholinesterase activity testing indicated no exposure to organophosphate or carbamate pesticides (mean±SD=17.32±2.90 μmol/min/g, 31/71). The causes of death and potential underlying factors summarized in this study constitute the first definitive mortality survey of migratory Whooping Cranes based on a high carcass recovery rate. Causes of death by infectious etiologies remained comparatively rare in this study, and occurred as single cases with no evidence of sustained transmission among reintroduced Whooping Cranes.

Key words: Brain cholinesterase, *Grus americana*, gunshot, lead poisoning, mortality, reintroduction, Whooping Crane.

Whooping Crane (*Grus americana*) reintroduction efforts in the eastern US have been ongoing since 1993 to satisfy downlisting criteria for the Endangered Species Act (Canadian Wildlife Service and US Fish and Wildlife Service 2005). In 2001, work began to establish a migratory population of Whooping Cranes east of the Mississippi River using multiple chick rearing, migration, and release methods (Hartup 2019b). This Eastern Migratory Population (EMP) received extensive monitoring and study of reproduction, spatial ecology, habitat selection, and survivorship (Urbanek et al. 2010; Mueller et al. 2013). Cole et al. (2009) evaluated postmortem findings early in the study and identified predation and anthropogenic trauma from gunshot and power line collisions as leading causes of death. The study summarized 17 mortalities following release of 80 cranes from 2001 to 2006 and determined cause of death in 11. In addition, few infectious disease and toxicology test results were reported. Postmortem data accumulated at a much greater rate with increased numbers of released cranes in the last decade. The purpose of this report was to provide a more comprehensive review of postmortem findings from captive-reared and released EMP Whooping Cranes from 2001 to 2016 using the same methodology as Cole et al. (2009).

Recovery of carcasses was high because of use of telemetry for monitoring: of 268 captive-reared cranes released in this period, a total of 166 cranes were known or suspected to have died and 124 carcasses were recovered (75% recovery rate). Because of low numbers and slower recoveries of carcasses, findings from wild-hatched EMP cranes were not included in this study. Postmortem evaluation included a field assessment, necropsy, and screening tests to determine cause of death as previously described (Cole et al. 2009). Diagnostic and field data collected for this study are available in Yaw et al. (2019). Tests were performed to the extent possible de-

TABLE 1. Summary of deaths from infectious causes in Whooping Cranes (*Grus americana*) of the reintroduced Eastern Migratory Population in 2001–16.

Bird ID	Sex[a]	Age at death (days)	System	Infectious etiology	Additional comments
12-02	M	5,132	Gastrointestinal	*Clostridium perfringens* type A toxins	Not previously diagnosed in Gruiformes.
3-03	F	3,038	Musculoskeletal	*Staphylococcus aureus*	Infectious arthropathy. Bacteria and unidentified fungal hyphae observed microscopically.
2-05	F	846	Cardiovascular	Bacteria, multiple species	Suspect sepsis. Low-pathogenicity avian influenza virus isolated from intestine; *Ribeiroia ondatrae* found in esophagus and proventriculus in low numbers.
42-07	F	1,460	Gastrointestinal	*Clostridium perfringens*	Suspect septicemia may be secondary to enteritis caused by *Echinoparyphium* sp. Mild parasitemia of *Plasmodium* sp.
1-09	F	572	Respiratory	*Aspergillus fumigatus*	Clinical history of respiratory abnormality (cough and moist rales) during chick rearing; untreated.
55-13	M	188	Respiratory	*Aspergillus fumigatus* and *Mucor/Rhizopus* sp.	Delayed first migration departure. Leg injury 1 mo prior that resolved.

[a] M = male; F = female.

pending on carcass condition and availability. A Student's *t*-test was used to compare the mean age of death of female and male cranes. Point estimates of the proportion of each cause of death in the EMP were calculated with reference to the number of mortalities where a cause was determined. Comparison of point estimates of causes of death between Cole et al. (2009) and the current study was performed using two-proportion *z*-tests (Snedecor and Cochran 1980). Statistical significance was established at $P<0.05$.

Recovered carcasses consisted of 68 females and 56 males. The mean±SE age at death was 1,048±92 d (median=639 d). There was no statistical difference in age at death between females and males ($P=0.399$). A total of 33% (41/124) of the carcasses were intact, but 67% (83/124) of the carcasses were incomplete due to scavenging or predation. Dead cranes were recovered in 10 states throughout the EMP range, with concentrations of mortalities in the summer range of Wisconsin (56%, 70/124) and winter range of Florida (18%, 23/124). Mortalities also occurred in Indiana ($n=9$), Illinois ($n=6$), Kentucky ($n=4$), Georgia ($n=3$), Alabama ($n=3$), Michigan ($n=3$), Tennessee ($n=2$), and South Carolina ($n=1$).

Cause of death was determined in 62% (77/124) of the cases reviewed. The largest proportion of all causes of death was from predation (0.468; 95% confidence invterval 0.356–0.580; 36/77); about 53% (19/36) of predation cases occurred in Wisconsin. Power line and vehicle collisions (including automobile, truck, and aircraft) comprised impact trauma events (0.260; 0.162–0.358; 20/77). Gunshot deaths (0.169; 0.085–0.253; 13/77) occurred throughout the range of the EMP, including cases from Wisconsin ($n=1$), Michigan ($n=1$), Indiana ($n=4$), Kentucky ($n=2$), Alabama ($n=2$), and Georgia ($n=3$). The proportions of these three causes of death ($P=0.108$, $P=0.216$, $P=0.503$, respectively) were not statistically different than those calculated from results of Cole et al. (2009).

Six deaths were attributed to infection (0.078; 0.018–0.138; 6/77), including bacterial and fungal etiologies (Table 1). All six

TABLE 2. Parasites listed in necropsy reports of reintroduced Whooping Cranes (*Grus americana*) from the Eastern Migratory Population in 2001–16.

Parasite	Blood (*n*=37)	Visceral organs[a] (*n*=44)	Lung (*n*=41)	Esophagus (*n*=32)	Ventriculus (*n*=41)	Proventriculus (*n*=36)	Small intestine (*n*=44)	Ceca (*n*=33)	External (*n*=21)
Acuaria sp.				1					
Amidostomum sp.							1		
Apatemon sp.							2		
Ascaridia pterophora							1		
Avioserpens taiwana					1				
Capillaria sp.		1			13		2	2	
Clinostinum sp.							1		
Cotylurus sp.							1		
Cyathostoma sp.			1						
Echinoparyphium sp.				1			2	2	
Echinostoma sp.					1		13	5	
Eimeria sp.							1		
Epomidostomum sp.						1			
Hymenolepididae sp.							1		
Hypoderaeum sp.				1			1		
Leucocytozoon sp.	1								
Plasmodium spp.	2								
Ribeirola ondatrae				1		1			
Strigeidae sp.							4		
Schistophorus sp.					2				
Strongyloides sp.							1		
Tetrameres sp.					1	6			
Zygocotyle lunata								1	
Cestodes[b]							1		
Nematodes[b]		1		3	3	2	7	1	
Trematodes[b]		2		1			3		
Mites[b]									1
Coccidia[b]		3					1		

[a] Liver, spleen, kidney.

[b] Not identified to genus.

carcasses were recovered in Wisconsin. Results of routine parasite and microbiology tests are provided in Tables 2 and 3, respectively. West Nile virus (WNV) PCR tests of liver (*n*=41), central nervous system (*n*=31), bone marrow (*n*=18), and feather pulp (*n*=8) were negative. Serological results for WNV were complicated by prior vaccination of the released cranes and are not reported here; no case exhibited histologic lesions consistent with WNV disease. Matrix reverse-transcription PCR tests for avian influenza virus from tracheal and cloacal swab samples (*n*=43) were negative. All serum neutralization tests for antibodies to inclusion body disease of cranes (crane herpesvirus) were negative (*n*=25).

A single case of chronic exertional myopathy was attributed to translocation, and has been summarized elsewhere (Hanley et al. 2005, case 3). No additional cases of exertional myopathy have occurred since capture guidelines and prevention strategies were promulgated by the Whooping Crane Health Advisory Team to the International Whooping Crane Recovery Team in 2005 following that incident. Lastly, a single case of egg yolk coelomitis leading to death was identified in

TABLE 3. Bacteria isolated from small and large intestinal aerobic culture swabs at necropsy of reintroduced Whooping Cranes (*Grus americana*) from the Eastern Migratory Population in 2001–16.

Small intestine (n=37)	No. isolates	Large intestine (n=35)	No. isolates
Bacillus sp.	4	*Bacillus* sp.	3
Corynebacterium sp.	1	*Camobacterium mobile*	1
Enterococci sp.	8	*Corynebacterium* sp.	1
Streprococcus sp.	2	*Enterococci* sp.	14
		Streptococcus sp.	3
Acinetobacter sp.	1	*Vagococcus* sp.	1
Aeromonas sp.	4		
Citrobacter sp.	4	*Aeromonas* sp.	7
Edwardsiella tarda	8	*Citrobacter* sp.	3
Enterobacter sp.	3	*Edwardsiella tarda*	6
Escherichia coli	20	*Enterobacter* sp.	3
Escherichia fergusonii	1	*Escherichia coli*	24
Hafnia alvei	2	*Escherichia fergusonii*	1
Klebsiella oxytoca	1	*Hafnia alvei*	2
Klebsiella pneumoniae	1	*Klebsiella oxytoca*	1
Klebsiella sp.	1	*Klebsiella pneumoniae*	1
Moellerella wisonsensis	1	*Klebsiella* sp.	1
Morganella morganii	1	*Morganella morganii*	1
Myroides sp.	1	*Plesiomonas shigelloides*	7
Paenibacillus sp.	1	*Proteus* sp.	3
Plesiomonas shigelloides	8	*Pseudomonas* sp.	1
Proteus sp.	1	*Salmonella javiana*	1
Shewanella putrefaciens	1	*Salmonella thompson*	1
Vibrio alginolyticus	1	*Salmonella litchfield*	1
Vibrio parahaemolyticus	1	*Salmonella* sp.	2
		Vibrio parahaemolyticus	1

an adult female. This crane had an intact, shelled egg in the distal oviduct, as well as a moderate amount of yolk within the coelomic cavity and a marked inflammatory response.

Toxin screening of the cases was limited to lead and brain cholinesterase activity analyses. Lead analysis of 50 liver samples yielded concentrations above detection threshold (>0.25 ppm wet weight) in two samples (3.65 and 10.97 ppm wet weight). Both cranes died from power line collisions. No liver lead reference data is available for Whooping Cranes; the concentrations are consistent with subclinical and clinical liver lead levels, respectively, in bald eagles (*Haliaeetus leucocephalus*; Yaw et al. 2017). In addition, 10 bone samples (humerus) from partial carcasses lacking suitable liver tissue were tested for lead using established protocols (Pattee et al. 1981). One sample result (48.82 ppm dry weight) was consistent with clinical toxicity; this crane died from predation. Two samples were below detection threshold. The mean±SD bone lead concentration for the remaining samples was 1.7±1.3 ppm dry weight (n=7), consistent with background bone lead levels in bald eagles (Yaw et al. 2017).

Brain cholinesterase activity was determined from 32 fresh carcasses to assess potential exposure to organophosphate or carbamate pesticides; results from 15 decomposed carcasses were excluded because of potential bias from degradation (Hill 1988). One outlying value >3 SD above the initial mean was disregarded. The mean±SD brain cholinesterase activity was 17.32±2.90 μmol/min/g (median=16.60 μmol/min/g, n=31). No

results indicated either exposure (20% reduction) or intoxication (50% reduction) compared to a previous US Geological Survey, National Wildlife Health Center (Madison, Wisconsin, USA) reference standard for Whooping Cranes from 2005 (mean±SD, 15.40±1.80 μmol/min/g, n=10).

The causes of death and identification of potential underlying factors summarized in this study constituted the first definitive mortality survey of migratory Whooping Cranes based on a high carcass recovery rate. Pearse et al. (2019) were able to assign cause of death to only two cases among 14 carcasses recovered from 19 deaths recorded from 68 cranes marked in the Central Flyway population. Similar limitations confronted the studies of Lewis et al. (1992) and Stehn and Haralson-Strobel (2014) that relied on opportunistic observations of dead cranes during aerial surveys and yielded smaller sample sizes.

Modeling of the EMP using data from 2001 to 2010 indicated initially high survival rates that are similar to those of the self-sustaining Central Flyway population (Servanty et al. 2014). Uncertainty exists, however, over lifetime reproductive output sufficient for population persistence, highlighting the need to ensure survival of breeding adults (Converse et al. 2019). Frequent predation of cranes on summering grounds in Wisconsin may reflect increased risks from complete remigial molt (leaving cranes flightless for up to 4–6 wk) or inadequate habitat conditions (e.g., lack of water for roosting) in which to evade predators (Urbanek et al. 2010). Impoundment management that produces a mosaic of cover types and depth of water for roosting on managed lands may provide benefits to both nesting and subsequently, molting, cranes. Modifications in captive rearing to promote wildness may also improve threat recognition and predator avoidance (e.g., parent rearing; Hartup 2019a).

The frequency of death by vehicle or power line collision requires ongoing evaluation of sites to explore prevention strategies. Yet, the data in our study indicated that underlying subclinical or clinical lead toxicosis may be a factor in up to 5% of deaths attributed to predation or impact trauma. Gunshot deaths from vandalism in the EMP have been persistent throughout the period of reintroduction and have occurred across its range. None of the cases were associated with misidentification during a legal crane or waterfowl hunting season. Project directors have renewed engagement in public outreach and antipoaching efforts with several state natural resource agencies since 2015 to reduce this cause of mortality.

Causes of death by infectious etiologies remained comparatively rare and occurred as single cases with no evidence of sustained transmission among Whooping Cranes. Primary bacterial or fungal disease is not suspected in any case, as the infections reported are typically secondary to underlying debilitation, trauma, or other processes. Parasites may have contributed to mortality in one case of *Echinoparyphium* sp.–associated enteritis. Evidence of effects of viral etiologies in the reintroduced cranes was negligible.

Despite EMP Whooping Cranes coinciding with intensive agricultural regions throughout their annual cycle (Urbanek et al. 2010), our study did not find evidence of exposure to organophosphate or carbamate pesticides based on brain cholinesterase testing. The brain cholinesterase data from this study represented a species-specific and geographic reference that may be used in future investigations.

Successful establishment of a self-sustaining migratory Whooping Crane population in the eastern US will depend on many factors including adult survival. This follow-up study demonstrated the importance of long-term, multiagency population monitoring efforts and reconfirmed that infectious diseases are not a significant driver of mortality. Evaluating lead poisoning as a comorbidity factor in Whooping Cranes is warranted with future investigations.

The authors thank the Whooping Crane Eastern Partnership operational field teams for assistance with this study, and acknowledge the collective knowledge and contributions of the pathologists, epidemiologists, laboratorians, and technicians that worked

on the referenced case reports at the US Geological Survey, National Wildlife Health Center, University of Florida College of Veterinary Medicine, and US Fish and Wildlife Service Forensics Laboratory. We also thank G. Doss and J. Doll for assistance with data verification. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the US Government.

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Submitted for publication 13 May 2019.
Accepted 5 December 2019.