# SURVEY OF GROSS AND HISTOPATHOLOGIC FINDINGS IN TWO WINTERING SUBPOPULATIONS OF SANDHILL CRANES (*ANTIGONE CANADENSIS*)

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# Survey of Gross and Histopathologic Findings in Two Wintering Subpopulations of Sandhill Cranes (*Antigone canadensis*)

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ABSTRACT: Sandhill Cranes (*Antigone canadensis*) of the midcontinent population (MCP) and Rocky Mountain population (RMP) are migratory game birds with stable populations that travel between Canada and the southern US and Mexico. In the winters of 2012–14, we performed gross and histologic examinations of 43 hunter-harvested Sandhill Cranes in Texas (MCP) and New Mexico (RMP) to assess the impact of disease on populations. Lesions were significantly more common in the MCP relative to the RMP, likely reflecting differential environmental exposure to pathogens and parasites. Grossly, liver nodules and esophageal granulomas were present in 8–39% of birds. In feces from over half of birds, we found coccidian oocysts with mitochondrial gene sequences identical to those of *Eimeria gruis* and *Eimeria reichenowi* previously obtained from sympatric Whooping Cranes (*Grus americana*). Over one-quarter of birds had liver and cardiac lesions suggestive of disseminated visceral coccidiosis. We documented proliferative colitis due to *Cryptosporidium* in a wild Sandhill Crane. Additionally, several endoparasites were found in histologic sections from several cranes, including a bird with respiratory trematodiasis and two birds with *Tetrameres* sp. in the proventriculus associated with ductal ectasia. In addition to describing lesions and parasites that impact Sandhill Crane health, these pathology data may also be relevant for the conservation of endangered Whooping Cranes using a surrogate species approach.

*Key words:* *Antigone canadensis*, *Eimeria*, pathology, Sandhill Crane.

Sandhill Cranes (*Antigone canadensis*) of the midcontinent population (MCP) and the Rocky Mountain population (RMP) migrate yearly from summer breeding grounds in the northwestern US, western Canada, Alaska, and eastern Siberia to wintering grounds in Texas, New Mexico, California, Arizona, and Mexico (Dubovsky 2016). A popular species with birdwatching enthusiasts and recreational hunters, there is little information regarding disease prevalence in the wild population. Known agents of disease in captive and wild Sandhill Crane populations include *Eimeria* sp. (disseminated visceral coccidiosis), parasitic helminths, Haemosporida, inclusion body disease virus, West Nile virus, and mycotoxins (Docherty and Romaine 1983; Forrester and Spalding 2003; Hansen et al. 2008). The MCP consists of the Lesser (*A. c. canadensis*) and Greater (*A. c. tabida*) subspecies and the RMP consists of the Greater subspecies only (Dubovsky 2016). Our purpose was to document necropsy findings from cross-sections of migratory Sandhill Cranes in Texas and New Mexico to provide baseline health status data on these robust populations which can be evaluated with future studies of the birds in changing environmental conditions.

The Lesser and Greater Sandhill Cranes from the MCP that were examined included 24 wintering near Canyon, Texas (34°58′46″N, 101°55′33″W), and seven wintering near Francitas, Texas (28°51′35″N, 96°20′19″W). The Greater Sandhill Cranes from the RMP that were examined included 12 wintering near Socorro, New Mexico (34°3′42″N, 106°53′58″W). All birds were collected between November 2012 and January 2014. Birds were necropsied within 6 h of death. Birds were weighed and aged based on plumage, and sex was determined by visualization of the gonads. A systematic necropsy was performed and samples from tongue, larynx, trachea, esophagus, crop, proventriculus, ventriculus, duodenum, pancreas, jejunum, ileum, cecum, colon, spleen, liver, lungs, heart, thyroids, kidneys, adrenal glands, gonads, bursa (when present), skeletal muscle,

TABLE 1. Sex, age, and weight range of hunter-killed Sandhill Cranes (*Antigone canadensis*) examined by necropsy for diseases by harvest site in Texas, USA (midcontinent subpopulation) and New Mexico, USA (Rocky Mountain subpopulation), winter 2012–14.

| Harvest site | Sex | Age | Weight range (kg) |
|---|---|---|---|
| Canyon, Texas | 16 male<br>5 female<br>3 unknown | 5 adult<br>19 juvenile | 2.3–4.0 |
| Francitas, Texas | 4 male<br>3 female | 6 adult<br>1 juvenile | 3.1–4.5 |
| Socorro, New Mexico | 7 male<br>4 female<br>1 unknown | 11 adult<br>1 juvenile | 4.0–6.5 |

TABLE 2. Histologic lesions in wintering Sandhill Cranes (*Antigone canadensis*) examined by necropsy for diseases, from the midcontinent population (MCP; $n$=31) and the Rocky Mountain population (RMP; $n$=12), winter 2012–14.

| Histologic diagnosis | No. (%) | |
|---|---|---|
| | MCP | RMP |
| Necrotizing hepatitis | 12 (39) | 0 |
| Lymphohistiocytic hepatitis | 7 (23) | 0 |
| Submucosal esophageal granulomas | 9 (29) | 0 |
| Myocarditis | 11 (36) | 0 |
| Enteritis secondary to intestinal coccidiosis | 18 (58) | 1 (8) |
| Enteritis without intestinal coccidiosis | 0 | 4 (33) |
| Air sac and tracheal granulomas | 4 (13) | 0 |
| Proliferative colitis | 2 (7) | 0 |
| Proventricular tetramariasis | 2 (7) | 0 |

sciatic nerve, and brain were collected from each bird and preserved in 10% neutral buffered formalin and trimmed for microscopic examination. Feces were collected when present in the lower intestinal tract and subjected to fecal flotation for the identification of coccidian oocysts as previously described (Bertram et al. 2015).

Of the 43 harvested birds, adults ($n$=22) and juveniles ($n$=21) were equally represented, and the majority (63%) were male while 28% were female with the remainder of unknown sex (Table 1). Gross lesions included multifocal, tan, 2–3-mm nodules in the livers of 39% (12/31) of birds harvested in Texas and 8% (1/12) of birds harvested in New Mexico. Submucosal esophageal nodules interpreted as granulomas were identified in 26% (8/31) birds from Texas and a single bird from New Mexico (8%, 1/12; $\chi^2$=10.9, df=2, $P$=0.004). Additionally, coccidian oocysts consistent with *Eimeria gruis* and *Eimeria reichenowi* were noted in 74% (17/23) and 57% (4/7) fecal samples from birds harvested in Texas and New Mexico, respectively. The oocysts had identical mitochondrial gene sequences to those we previously obtained from *Eimeria* spp. in voided wild Whooping Crane (*Grus americana*) feces (Bertram et al. 2015). There was no association between the presence of granulomas grossly and the presence of oocysts in the feces ($\chi^2$=0.068, df=1, $P$=0.794). Additionally, a juvenile bird from the Canyon, Texas, harvest had a presumably traumatic amputation of the right tarsometatarsus.

A total of nine histologic diagnoses were associated with the cranes (Table 2); in comparison to the Texas birds, birds in the New Mexico population had fewer histologic lesions. The lower prevalence of both gross and microscopic findings in the RMP relative to the MCP likely reflects differential environmental exposure to pathogens and parasites. Random, mild to moderate, multifocal necrotizing hepatitis or lymphohistiocytic hepatitis were noted in 39% and 23% of birds, respectively, all from the MCP. Although experimental and natural coccidian infections have resulted in granulomatous or necrotizing hepatitis depending on the stage of infection, lesions in naturally infected birds are not as severe as those seen in experimental models (Novilla and Carpenter 2004), and we did not note intralesional coccidia in the hepatic lesions. Eleven birds—all from the MCP—had multifocal mild histiocytic and heterophilic myocarditis with cardiomyofiber loss and necrosis. The heart and liver lesions are suggestive of disseminated visceral coccidiosis caused by *E. gruis* and *E. reichenowi* (Courtney et al. 1975; Novilla and Carpenter 2004). A previous survey of coccidiosis in New

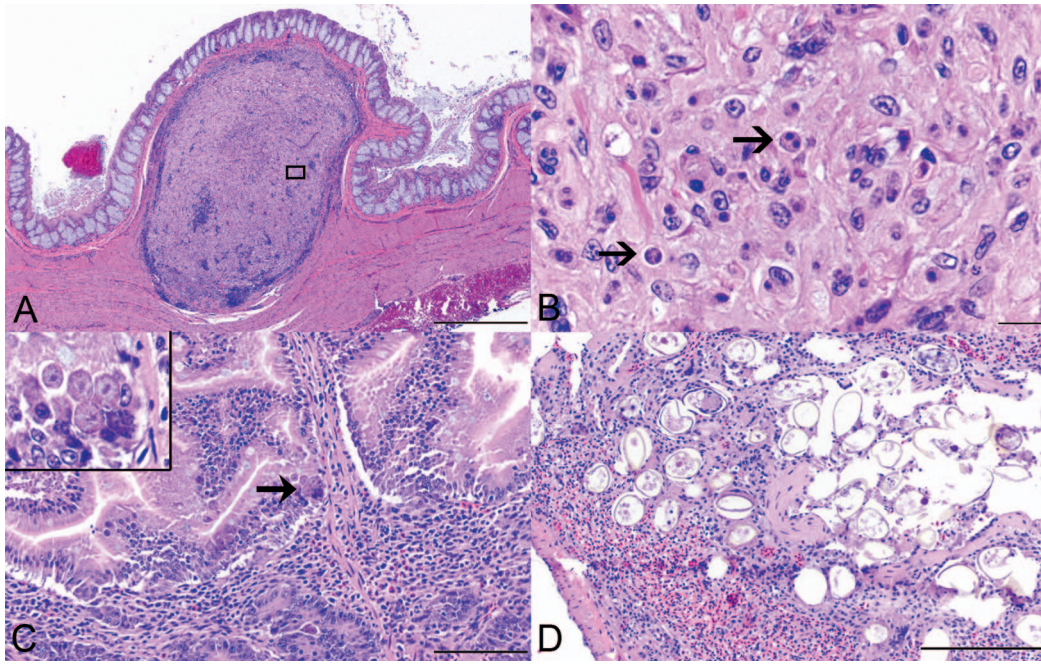


FIGURE 1. Histopathology of hunter-harvested Sandhill Cranes (*Antigone canadensis*) from Canyon, Texas, USA, 2014. (A) Esophagus: The submucosa is expanded by granuloma composed of composed of epithelioid macrophages, heterophils, lymphocytes, plasma cells, and fewer multinucleated giant cells. H&E. Black box indicates the area magnified in 1B. Bar=1 mm. (B) Higher magnification of the esophageal granuloma depicted in 1A, macrophages occasionally contain cytoplasmic 5–10-μm meronts (arrows) consistent with *Eimeria* spp. H&E. Bar=20 μm. (C) Small intestine: Enterocytes are expanded by apically located intracellular gametocytes (arrow). The mucosa is hyperplastic, and the lamina propria is infiltrated by heterophils and fewer lymphocytes. H&E. Bar=100 μm. Inset shows higher magnification of an intraepithelial macrogametes depicted by the arrow. (D) Lung: Expanding and replacing air capillaries are numerous nonoperculated, 80–100-μm trematode ova with a 3–4-μm anisotropic yellow shell; ova contain basophilic granular material and occasional miracidium. The ova are surrounded by a moderate mixed inflammatory infiltrate of macrophages, lymphocytes, plasma cells, and fewer heterophils. H&E. Bar=200 μm.

Mexico Sandhill Cranes identified meronts in nine of 24 liver nodules and in none of the heart lesions (Parker and Duszynski 1986).

Multifocal granulomas composed of a large number of epithelioid macrophages, rare multinucleated giant cells, and fewer lymphocytes, which were surrounded by a thin layer of concentric bands of fibrous connective tissue, were identified in the air sac and submucosa of the esophagus, proventriculus, and trachea of 13 MCP birds (Fig. 1A, B). Epithelioid macrophages within the granulomas had a peripherally displaced nucleus and two to four intracellular, 5- to 10-μm meronts consistent with *Eimeria* sp. (Fig. 1B, inset). Although a single RMP bird had gross submucosal esophageal nodules that were similar in appearance to those of the MCP cranes with histologic *Eimeria*-containing granulomas, no histologic lesions were noted in the examined sections of the affected RMP bird's gastrointestinal tract.

Intestinal stages of coccidia, including intraepithelial gametocytes (macrogametes and microgametes) and extracellular oocysts, were identified in 58% of the MCP and 8% of the RMP (Fig. 1C). Intraepithelial and extracellular coccidial life stages were often accompanied by minimal to mild inflammatory infiltrate of eosinophils, heterophils, and lymphocytes with erosion of the mucosa. Additionally, four birds (33%) of the RMP

had mild to moderate eosinophilic and lymphoplasmacytic enteritis without evidence of intraepithelial protozoa in the examined sections. Additional microscopic diagnoses in the MCP population include multifocal mild proliferative colitis with numerous round, basophilic, 3–5-µm organisms, consistent with *Cryptosporidium* sp. This is the first report of *Cryptosporidium* sp. in a wild Sandhill Crane, although cloacal cryptosporidiosis has been reported in a captive White-naped Crane (*Antigone vipio*; Kim et al. 2005).

Several endoparasites were observed within histologic sections. A single bird in the Texas population had a focally extensive severe eosinophilic and lymphoplasmacytic bronchopneumonia, tracheitis, and air sacculitis with intralesional trematode ova (Fig. 1D). Ova were nonoperculated, 80–100-µm with a 3–4-µm anisotropic yellow shell and contained basophilic granular material and occasional developing miracidium. The genus and species of the trematode were not definitively identified; however, several species of trematodes have been identified in North American Sandhill Crane populations and include *Orchipedum jolliei*, *Prohyptiamus grusi*, and *Echinostoma revolutum* (Iverson et al. 1983; Gaines et al. 1984). *Orchipedum jolliei* is the most commonly identified trematode in Sandhill Cranes and is found in the trachea (Iverson et al. 1983; Gaines et al. 1984). The individual bird with respiratory trematodiasis was infected with 93 *O. jolliei* in the trachea (Bertram 2016). Based on the anatomic location of infection and heavy worm burden, the intralesional trematode eggs are presumably *O. jolliei*. In addition, another bird from the MCP had mild, multifocal granulomatous air sacculitis with intralesional trematode ova. Ductal ectasia with intraductal nematodes consistent with a *Tetrameres* sp. were found in the proventriculi of two adult Texas birds. *Tetrameres grusi* has been identified in Sandhill Cranes in Florida and Texas (Bush et al. 1973; Gaines et al. 1984) and is reported to have a high prevalence in wild Sandhill Cranes compared to other nematode parasites (Gaines et al. 1984).

We documented the disease burden of helminth and coccidian parasites in two populations of Sandhill Cranes. Recent interest in Sandhill Crane morbidity factors is driven by continuing concern for the endangered Whooping Crane, as Sandhill Cranes have been used as a surrogate species to identify potential risk factors for the endangered Whooping Crane (Spalding et al. 2008; Bertram et al. 2017). Since the MCP of Sandhill Cranes is sympatric with Whooping Cranes, this report highlights disease entities, including *E. gruis* and *E. reichenowi*, to which Whooping Cranes may potentially be exposed as well.

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