COCCIDIOSIS OF SANDHILL CRANES (GRUS CANADENSIS) WINTERING IN NEW MEXICO

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ABSTRACT: The feces of 212 sandhill cranes (*Grus canadensis*) collected in central New Mexico from October 1982 to January 1983 and from October 1983 to January 1984 were examined to determine the prevalence of coccidial oocysts. One hundred forty-five granulomatous nodules from the viscera of 64 cranes and samples of lung, small intestine, and large intestine from 58 birds were examined by light and transmission electron microscopy for the presence of intestinal or extraintestinal coccidiosis. Of the 212 fecal samples, 160 (75%) were positive for oocysts of *Eimeria*, including *E. gruis* in 139 (66%) and *E. reichenowi* in 118 (56%) of the samples. *Eimeria bosquei* sp. n. was found in two (~1%) of the fecal samples. Subspheroid to ovoid oocysts of this new species are 19–27 × 14–19 (23.6 × 17.1) μm with ovoid sporocysts 10–14 × 7–11 (12.3 × 9.3) μm. A rough, heavily pitted outer oocyst wall, sporocyst residuum, Stieda and substieda bodies, and multiple polar bodies are present. The polar bodies, of varying sizes, always aggregate at the apex of the sporulated oocyst. An *Adelina* sp. was found in one (0.5%) crane. Coccidian developmental stages were found in the epithelium and lamina propria of the small and large intestine. Disseminated granulomatous nodules were found in the oral mucosa, esophagus, heart, descending aorta, liver, small intestine, mesenteries, and parietal peritoneum. Unique cell types resembling coccidian asexual and sexual stages were observed by light and electron microscopy in some of the nodules.

INTRODUCTION

It is generally agreed that *Eimeria gruis* and *E. reichenowi* are the only valid species of coccidia naturally infecting sandhill cranes (*Grus canadensis*) and other species of cranes (Yakimoff and Matschoulsky, 1935; Pande et al., 1970; Pellérdy, 1974; Courtney et al., 1975; Forrester et al., 1978; Carpenter et al., 1979, 1980, 1984; Novilla et al., 1981). Experimentally, *E. gruis* and/or *E. reichenowi* can cause disseminated visceral coccidiosis (DVC) in addition to intestinal stages typically associated with eimerian coccidia (Carpenter et al., 1979, 1980, 1984; Novilla et al., 1981). The extraintestinal stages are grossly evident as small, white, raised granulomatous nodules or as microscopic inflammatory foci, and DVC has been implicated as the primary cause of death in captive sandhill and whooping cranes (*Grus americana*) (Carpenter et al., 1980). DVC has also been reported in wild sandhill cranes (Carpenter et al., 1984).

Because of the severe pathogenicity and mortality associated with infections of *Eimeria* spp. in captive sandhill and whooping cranes, and because a lack of strict host specificity may allow for eimerians to be transmitted from sandhills to the endangered whoopers (or other species of cranes), the consequences to crane management practices have come into consideration in recent years (Koga, 1955; Courtney et al., 1975; Forrester et al., 1978; Carpenter et al., 1979, 1980, 1984; Carpenter and Novilla, 1980; Novilla et al., 1981).

There have been only three studies reporting the prevalence of coccidian oocysts in the feces of wild cranes (Courtney et al., 1975; Forrester et al., 1978; Carpenter et al., 1984). The object of our study was to determine the prevalence of intestinal coccidiosis and DVC in the wild sandhill crane population wintering in New Mexico.

MATERIALS AND METHODS

Feces and/or samples of tissues were taken in the field from 214 cranes killed in Doña Ana and Luna counties during controlled hunts

sponsored by the New Mexico Department of Game and Fish from October 1982 to January 1983 and from October 1983 to January 1984. Intestinal contents were stored in 2.5% aqueous (w/v) potassium dichromate ($K_2Cr_2O_7$) solution. Upon return to the lab, fecal suspensions were filtered through 40- and 60-mesh brass screens, and the filtrate was placed into 14 cm petri dishes for 5 days at ambient temperature (~22 C) to allow oocysts, if present, to sporulate. The filtrates were then stored at 5 C until they could be examined by light microscopy. Oocysts were concentrated for study on coverslips by flotation in sugar solution (Duszynski et al., 1982).

During the 1982-1983 season, white, raised nodules found in the oral mucosa of cranes were excised and placed into Bouin's fixative (Humason, 1979). Upon return to the lab, the tissues were washed in 50% ethanol, dehydrated in a graded series of ethanol, cleared with Histosol® (National Diagnostics, 198 Route 206 South, Somerville, New Jersey 08876, USA), and embedded in Paraplast Plus® at 56 C. Five to 7 μm sections were cut and stained with either Erlich's or Harris' hematoxylin and eosin. Oocysts from flotation samples and histological slides were examined and photographed with a Zeiss Universal photomicroscope. Measurements were made with an ocular micrometer, and all measurements, unless otherwise stated, are in μm with the range followed by the mean in parentheses.

During the 1983-1984 season, cranes were examined at necropsy more extensively in the field. Portions of lung, small intestine, and large intestine just distal to the cecal diverticula were sampled from each bird. White, raised nodules from the remaining viscera, if present, were also removed. All of these tissues were placed in cold (4 C) Karnovsky's fixative (Dungworth et al., 1976) at pH 7.2, then washed in 0.1 M sodium cacodylate buffer. Tissue samples used for light microscopy were processed as above. Tissues to be used for transmission electron microscopy were cut into small cubes (~1 mm³), postfixed with 1% (w/v) osmium tetroxide (OsO_4) in 0.1 M sodium cacodylate buffer for 1 hr, dehydrated in a graded series of ethanol, treated with propylene oxide, embedded in Epon 812 or LX-112 (Ladd, Research Industries, Inc., P.O. Box 1005, Burlington, Vermont 05402, USA), and polymerized at 62 C for 72 hr. Ultrathin sections were cut with a Sorvall MT-5000 ultramicrotome and mounted on formvar-coated 200-mesh copper grids. Sections were stained with lead citrate and uranyl acetate and examined in an AE1 EM6-B transmission electron microscope.

Sandhill cranes were identified to subspecies based on wing chord, tarsus, and culmen postnares measurement standards established by the New Mexico Department of Game and Fish (Downer, 1983).

Statistical differences between subspecies, sex, and age of cranes parasitized were calculated using the chi-square distribution.

RESULTS

A total of 214 birds were examined in this study, but because of the nature of the collections (controlled hunts in several localities simultaneously) consistent data sets could not be collected for all birds. This resulted in the following subsets of data: (1) feces were collected from 212 birds; (2) tissues were taken from 64 birds; (3) 140 birds were identified to subspecies based on body measurements; (4) the sex of 180 birds was determined; and (5) the maturity of 188 birds was categorized as either adult or juvenile.

Sporulated oocysts of *Eimeria* spp. were found in 160 of 212 (75%) fecal samples. *Eimeria gruis* (Fig. 1) was seen in 139 (66%), and *E. reichenowi* (Fig. 2) in 118 (56%) of the fecal samples examined. Extreme polymorphism was evident among the *E. reichenowi*-type oocysts observed (Parker and Duszynski, 1986). Oocysts of *Eimeria bosquei* sp. n. (Figs. 3-6) were found in two of 212 (~1%) fecal samples, and oocysts of an *Adelina* sp. (Fig. 7) were seen in one of 212 (~0.5%) samples. Of the 160 positive samples, 39 had *E. gruis* only, 21 had *E. reichenowi* only, 97 had oocysts of both species, two had oocysts of *E. gruis* and *E. bosquei* sp. n. and one had oocysts of *E. gruis* and *Adelina* sp.

Eimeria bosquei sp. n.
(Figs. 3-6)

Description (all measurements are in micrometers)

Oocysts subspheroid to ovoid; oocyst wall of uniform thickness, approximately 1.5, composed of two layers: yellow-brown, heavily pitted outer layer, about two-thirds of total thickness and smooth inner layer
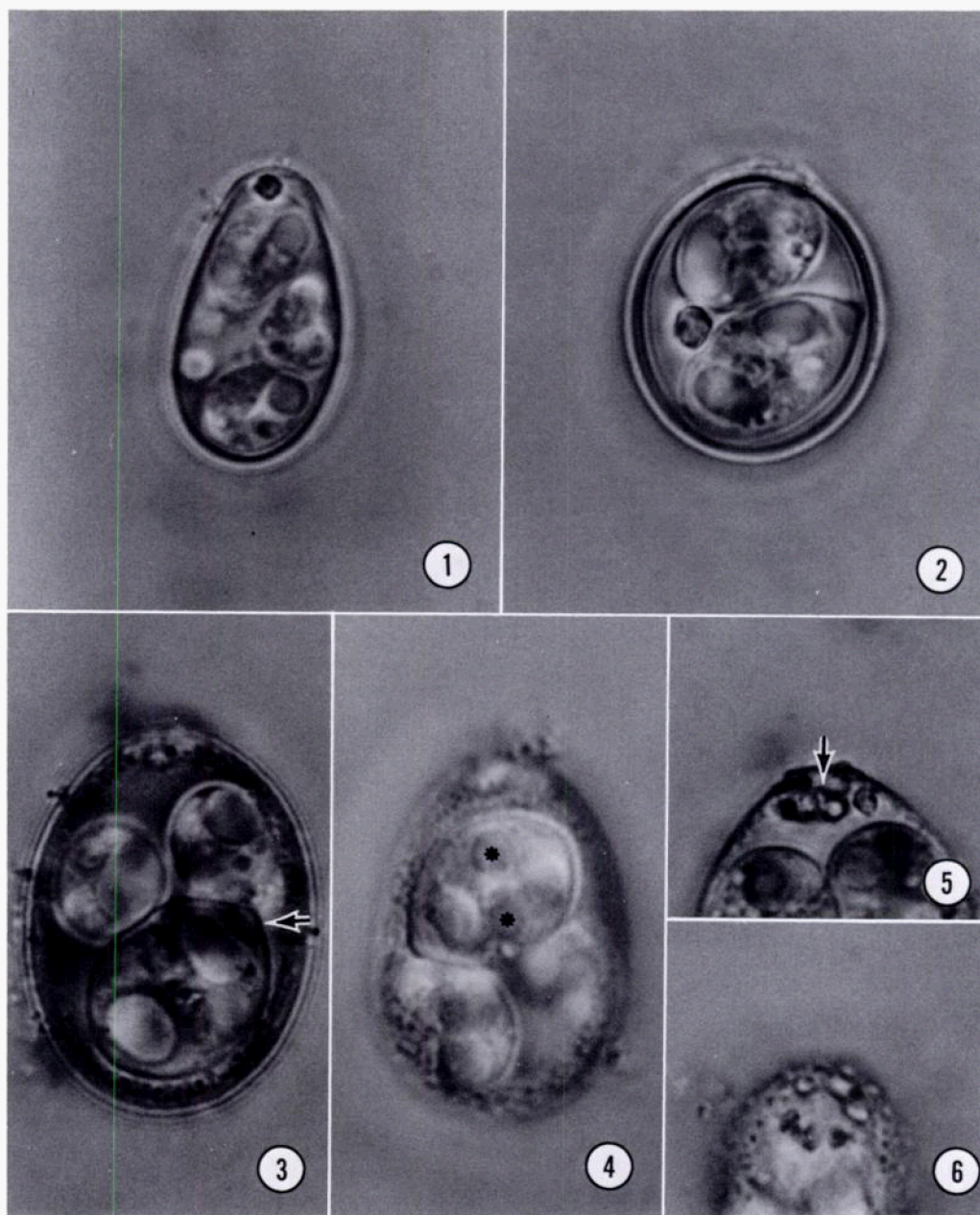
FIGURES 1–6.   Photomicrographs of sporulated oocysts of *Eimeria* spp. from sandhill cranes in New Mexico. ×2,100. 1. *E. gruis.* 2. *E. reichenowi.* 3–6. *E. bosquei* sp. n. 3. Note Stieda and substieda bodies (arrow). 4. Note sporozoite refractile bodies (*) and rough oocyst wall. 5. Multiple polar bodies at apex of oocyst (arrow). 6. Apex of oocyst. Note circular pattern of polar bodies in this oocyst.
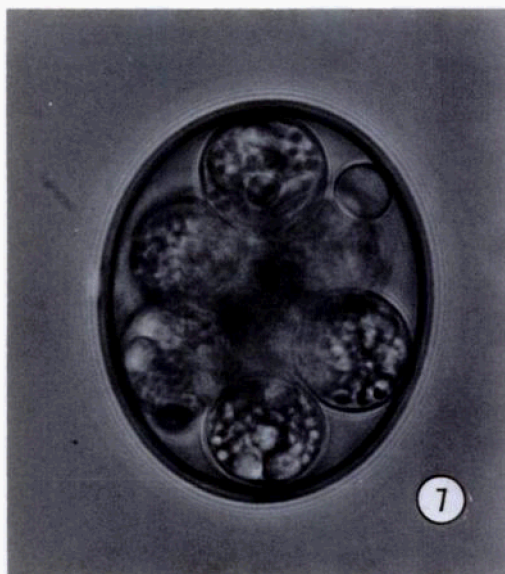
FIGURE 7.   Photomicrograph of sporulated oocyst of *Adelina* sp. from a sandhill crane in New Mexico. ×1,270.

that appears bluish-green (achromatic objective); micropyle and oocyst residuum absent; sporulated oocysts ($n = 100$) 19–27 × 14–19 (23.6 × 17.1) with L:W ratio 1.13–1.61 (1.38); multiple polar bodies of varying sizes present in circular to random pattern (Figs. 5, 6), always at apex of oocyst; sporocysts ovoid 10–14 × 7–11 (12.3 × 9.3) with L:W ratio 1.15–1.51 (1.33); Stieda and substieda bodies present (Fig. 3); sporozoites curled around central granular residuum; large posterior refractile body and a smaller anterior one present in each sporozoite (Fig. 4).

Taxonomic summary

Diagnosis: Oocysts of this eimerian are easily distinguished from those of other eimerians described previously from cranes by their size and shape and the unique arrangement of multiple polar bodies at one pole.

Host: Grus canadensis Linnaeus, 1758, Museum of Southwestern Biology, NK 10263 (lesser sandhill) and NK 10287 (subspecies not determined).

Sporulation time: Unknown, but most oocysts sporulated after 5 days at room temperature.

Site of infection: Unknown, oocysts recovered from intestinal contents.

Prevalence: Found in two of 212 (~1%) fecal samples from *G. canadensis*. Samples from both birds contained numerous oocysts.

Etymology: The specific name is derived from the Bosque del Apache National Wildlife Refuge in central New Mexico, a common wintering area of sandhill cranes.

The prevalence of coccidial oocysts in the crane population subsets (subspecies, sex, and maturity) is shown in Table 1. There was no significant difference in the percent of infected cranes among these population subsets or among the population subsets of cranes infected with *E. gruis* only, *E. reichenowi* only, or both species of *Eimeria* (Table 1). Most cranes, however, were infected with both *E. gruis* and *E. reichenowi*, and more cranes that harbored single species infections had *E. gruis* only (Table 1). This pattern was consistent among all host population subsets.

Histologically, coccidian asexual and sexual stages were observed in 24 of 58 (41%) intestinal tracts sampled. Meronts and gamonts were sporadically found in intestinal epithelial cells and in the lamina propria of the small and large intestine. Developing oocysts were also observed in intestinal tissue, but they were crenated, making identification to species impossible.

One hundred forty-five white, raised granulomatous nodules from 64 cranes measured 0.5–5 mm in diameter (Fig. 8). Nodules were found in the oral mucosa, liver, small intestine, heart, esophagus, parietal peritoneum, descending aorta, and mesenteries. Nodules were most prevalent in the oral mucosa and liver (Table 2). Cranes of both subspecies, sexes, and maturity categories had the granulomas.

Fifty-four of the 64 cranes (84%) with

TABLE 1. Prevalence of oocysts of *Eimeria gruis* and *E. reichenowi* in different subsets of crane populations wintering in New Mexico.

Host population subset	No. infected/ no. examined (%)	No. of birds (% of total subset) infected with		
		E. gruis only	*E. reichenowi* only	*E. gruis* and *E. reichenowi*
Grus canadensis canadensis (lesser)	60/90 (67)	18 (20)	8 (9)	34 (38)
G. c. tabida (greater)	40/50 (80)	8 (16)	4 (8)	28 (56)
Male cranes	82/108 (76)	22 (20)	10 (9)	50 (46)
Female cranes	57/72 (79)	9 (13)	7 (10)	41 (57)
Adult cranes	130/170 (76)	27 (16)	17 (10)	86 (51)
Juvenile cranes	16/18 (89)	4 (22)	1 (6)	11 (61)

oral or visceral nodules had eimerian oocysts in their feces, eight (12%) had no oocysts in their feces, and two (3%) had no fecal samples taken. Most of the 54 cranes with visceral nodules and coccidian oocysts in their feces were infected with both *E. gruis* and *E. reichenowi* (Table 3).

Histologically, the nodules were encapsulated by dense connective tissue, with the surrounding tissues appearing essentially normal (Fig. 8). Granulomas in the oral mucosa and esophagus were restricted to the lamina propria (Fig. 8). Peripheral, heavy lymphoid infiltration was present and lymphoid nodules were often associated with the granulomas. The nodules contained collagenous fibers, fibroblasts, reticular cells, macrophages, lymphocytes, granulocytes, and plasma cells. Nodules in the liver and heart were gen-

erally lymphoid nodules containing lymphocytes, macrophages, and reticular cells and fibers. Nodules of the small intestine were peripheral to the outer wall of the tunica muscularis. Nodules of the peritoneum and mesenteries were confined by a thin serosal layer, and the nodule found in the aorta was in the tunica adventitia. Cell types similar to those described above in the oral mucosa nodules were found in the nodules of the small intestine, peritoneum, mesenteries, and aorta.

Throughout the granulomas, coccidian asexual and sexual stages were observed (Figs. 9–14). These developmental stages were seen in 28 of 42 (67%) oral mucosa nodules, nine of 24 (38%) liver nodules, one of seven (14%) small intestine nodules, and in all five nodules found in the esophagus, peritoneum, and aorta. No coccidian developmental stages were observed in the heart nodules or mesenteric nodule.

In the oral mucosa granulomas, spheroid to ovoid meronts measuring 5–15 μm

TABLE 2. Prevalence and distribution of visceral nodules in sandhill cranes from New Mexico.

Site of nodule	Cranes with nodules/ cranes sampled (%)
Oral mucosa	42/64 (67)
Liver	24/58 (41)
Small intestine	7/58 (12)
Heart	6/58 (10)
Esophagus	2/58 (3)
Peritoneum	2/58 (3)
Aorta	1/58 (2)
Mesenteries	1/58 (2)

TABLE 3. Prevalence of oocysts of *Eimeria* spp. in sandhill cranes from New Mexico with visceral nodules.

Oocysts present	No. infected/ no. sampled (%)
E. gruis and *E. reichenowi*	39/54 (72)
E. gruis only	8/54 (15)
E. reichenowi only	5/54 (9)
E. gruis and *E. bosquei* sp. n.	2/54 (4)
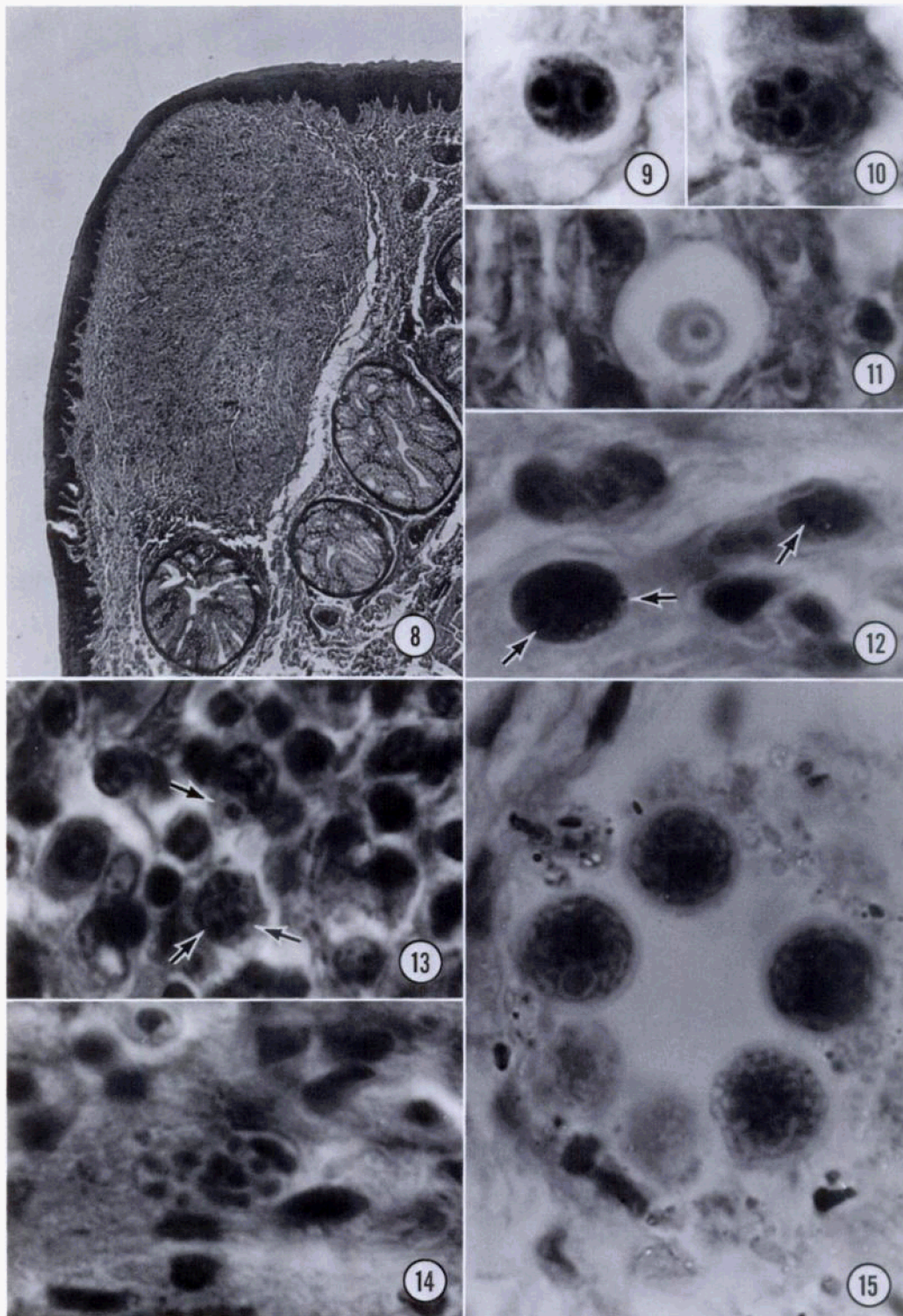
with one to eight basophilic nuclei were present in parasitophorous vacuoles of macrophages and other cells (Figs. 9, 10). Other spheroid to elongate developmental stages measuring 5–20 μm contained dark basophilic inclusion bodies and a frothy, vacuolated cytoplasm (Fig. 12). By transmission electron microscopy, these dark inclusion bodies were osmiophilic, and lighter-staining inclusions were also observed (Figs. 16, 17). More than one nucleus was present in some of these cell types (Fig. 17). Macrogamonts were also observed in the oral mucosa (Fig. 11). Coccidian developmental stages similar to those described above were also seen in nodules of the esophagus, small intestine, peritoneum, and aorta. In liver granulomas, merozoites or early meronts were observed in reticular cells and macrophages (Fig. 13), and meronts were also seen among hepatocytes (Fig. 14). Although no nodules were found in the lungs, macrogamonts were seen in the lung tissue of one crane (Fig. 15).

DISCUSSION

Eimeriid coccidia typically parasitize the intestinal tract of vertebrates (Levine, 1982). Although most eimerian species are thought to develop exclusively within the host's enterocytes, reports of extraintestinal development are numerous (Desser, 1978; Duszynski et al., 1979; Lima, 1979; Carpenter et al., 1979, 1980, 1984; Novilla et al., 1981; Wehage, 1983; Hawkins et al., 1984; Kogut and Long, 1984; Morrison and Hawkins, 1984). Thus, extraintestinal development of Eimeria spp. may be more prevalent than traditionally believed (Marquardt, 1981; Overstreet, 1981).

Although E. gruis and E. reichenowi have been reported from sandhill cranes (Courtney et al, 1975; Carpenter et al., 1979, 1984; Novilla et al., 1981) and other crane species (Yakimoff and Matschoulsky, 1935; Pande et al., 1970; Pellérdy, 1974; Forrester et al., 1978; Carpenter et al., 1980), this is the first report of coccidiosis in sandhill cranes wintering in New Mexico. The prevalence of eimerians in these cranes indicates that a large percentage of sandhill cranes in the wild is infected.

Eimeria bosquei sp. n. has oocysts that are easily distinguished structurally from E. gruis and E. reichenowi. Although this new species was found in only two cranes, both samples contained millions of sporulated oocysts which suggests that it is not a spurious parasite of cranes.

Endogenous stages of Adelina species have been reported from various invertebrate hosts (Levine, 1982; Purrini, 1984). Barnard et al. (1974) were unable to infect cotton rats with oocysts of Adelina species found in feces of cotton rats, and they assumed the oocysts were from an invertebrate that the rat had eaten. Courtney et al. (1975) described a species of Adelina from the feces of five sandhill cranes. They believed that it was a spurious observation and declined to give it a specific name. The species of Adelina reported in this study closely resembles the Adelina reported by Barnard et al. (1974) and Courtney et al. (1975), and this coccidian was found in only one crane, in small numbers. Thus, we feel it is premature to describe the parasite at the present time because we believe, as do others, that it is probably a parasite of invertebrates.

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FIGURES 8–15. Extraintestinal developmental stages of coccidia in tissues of sandhill cranes in New Mexico (all stains are H&E). 8. Histological section of a typical oral granuloma. ×55. 9–12. Coccidian stages in granuloma of oral mucosa. ×2,000. 9. Early binucleate meront. 10. Meront with four nuclei. 11. Macrogamont. 12. Unique cell types in parasitophorous vacuoles. Note vacuolated cytoplasm and dark inclusion bodies (arrows). 13, 14. Coccidian stages in liver nodules. ×2,000. 13. Note merozoites (arrows) in mononuclear phagocytes. 14. Meront among hepatocytes. 15. Group of macrogamonts in lung tissue. ×2,000.
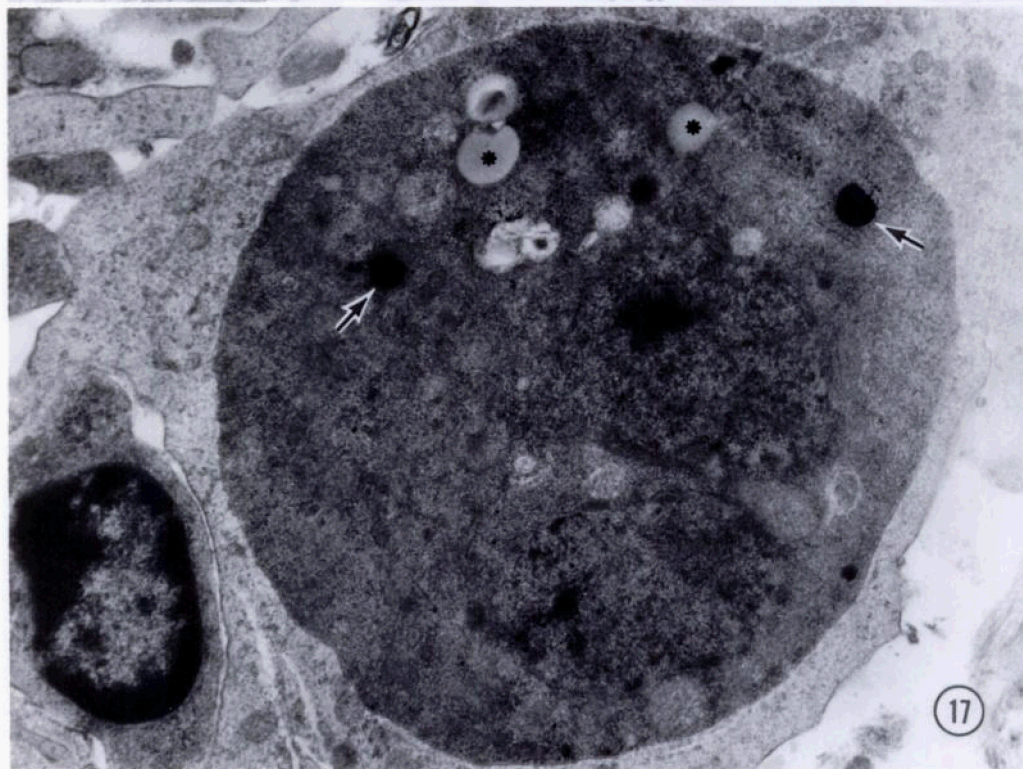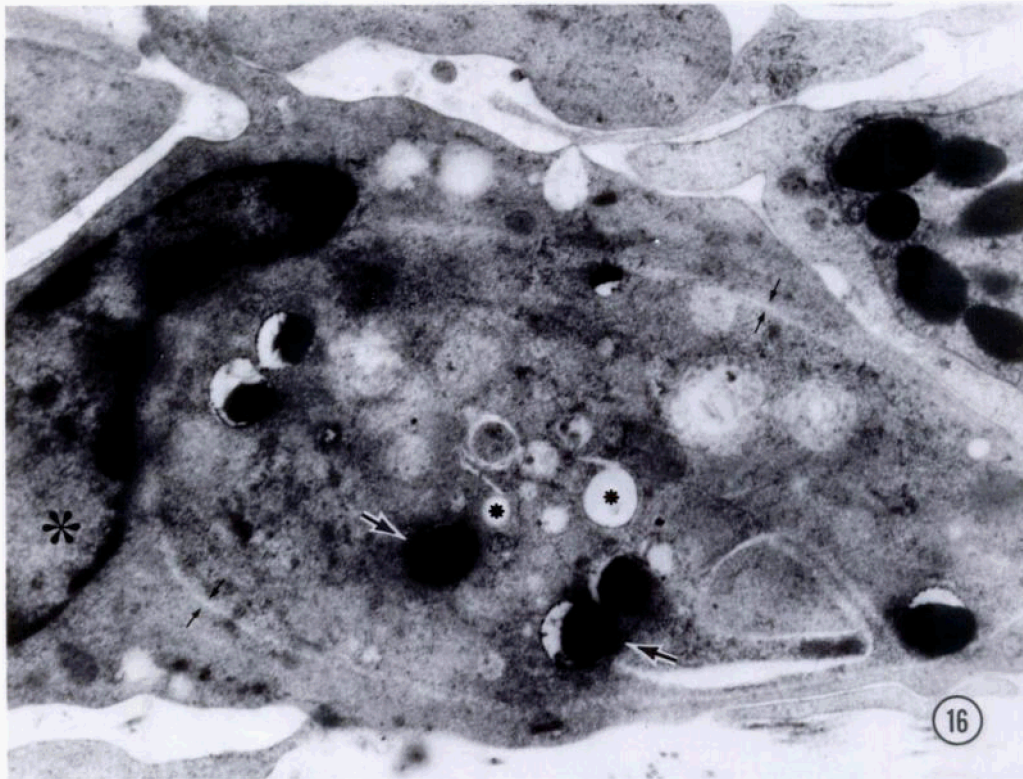
Traditionally, lesser and greater sandhill cranes have been categorized as distinct subspecies based on morphological traits, migratory habits, and other characteristics, although their migratory routes often overlap (Walkinshaw, 1973; Aldrich, 1979; Johnsgard, 1983). Tacha (1981), however, stated that sandhill crane subspecies are rapidly being degraded due to inter-subspecific breeding in mid-continental North America. Because lesser and greater sandhill cranes are so closely related, it is not surprising that there was no significant difference in prevalence of coccidia between these two subspecies (Table 1). Nor was there significant difference of coccidians between male and female cranes. Although a higher percentage of juvenile cranes harbored intestinal coccidia, the difference between juvenile and adult cranes also was not statistically significant (Table 1).

The presence of typical coccidian developmental stages in the intestinal tract indicates that coccidian organisms are able to complete their life cycle in the small and large intestine of sandhill cranes. Histological examination of intestinal tissues revealed much postmortem degeneration of the intestinal epithelium, but few coccidian developmental stages. Samples were randomly taken from various sites along the intestinal tract, but if the parasites were highly site specific, the portion of intestine where most of the developmental stages occurred may not have been sampled. This may explain why only a few intestinal stages were found. However, developmental stages were also seen in the lamina propria, and in tissues where the epithelial layer was well preserved, only a few developmental stages were found.

Eimeria gruis and *E. reichenowi* have been implicated as contributing agents to severe disease and mortality in sandhill and whooping cranes (Carpenter et al., 1979, 1980, 1984) and DVC has been experimentally produced in cranes inoculated with pooled oocysts of *E. gruis* and *E. reichenowi* (Novilla et al., 1981; Carpenter et al., 1984). In these studies, Carpenter, Novilla, and their co-workers reported granulomatous nodules throughout the viscera and provided light- and electron-micrographs as evidence of the coccidian nature of the pathogenic organisms. Similar granulomas were found in wild cranes during this study, and the presence of cell types resembling coccidian developmental stages in many of the nodules indicates that DVC is present in wild cranes. The prevalence of *E. gruis* and *E. reichenowi* in the feces of birds with visceral nodules suggests that one or both of these eimerian parasites are the cause of these granulomas, and this is the first report of DVC in wild cranes from New Mexico.

In wild sandhill cranes, however, the granulomas were not as numerous or as widely distributed in the body as they were when produced experimentally or found in captive cranes. Carpenter et al. (1984) and Novilla et al. (1981) infected birds with thousands of oocysts, and captive birds are often contained in areas where feces, and therefore oocysts, are abundant. Many oocysts may be required to produce the numbers of granulomas and the severity of lesions reported in captive and experimentally infected cranes, and free-ranging cranes may not encounter such large numbers of oocysts. Apparently, there is a positive correlation between the number of oocysts ingested and the

← FIGURES 16, 17. Transmission electronmicrographs of unique cell types (similar to those in Fig. 12) from oral granulomas in sandhill cranes in New Mexico. 16. Note parasitophorous vacuole (small arrows), osmiophilic inclusion bodies (large arrows), cytoplasmic vacuoles (small asterisks), and host cell nucleus (large asterisk). ×19,346. 17. Osmiophilic inclusion bodies (arrows) and lighter staining inclusions (asterisks). Note that parasite is binucleate. ×14,357.

number of nodules produced (Carpenter et al., 1984). Stress may also play a role in the number of nodules present in captive birds.

By light microscopy, the cell types (Fig. 12) observed in the granulomas found in wild cranes resemble intracellular protozoan organisms. By electron microscopy, the presence of the organism within a parasitophorous vacuole in the host cell indicates its parasitic nature (Fig. 16). The dark osmiophilic inclusion bodies resemble wall forming bodies, and the lighter staining inclusions (Fig. 17) resemble lipid or carbohydrate bodies often associated with coccidian macrogamonts (Scholtyseck, 1973; Chobotar and Scholtyseck, 1982).

The extraintestinal stages reported here elicit a marked cellular immune response and hyperplasia of fibrous tissue. It is not known whether the parasites die, remain active, or become dormant after being encapsulated in the granuloma.

The apparent lack of severe pathologic changes exhibited by intestinal and extraintestinal coccidiosis in sandhill cranes wintering in New Mexico may indicate that coccidia, particularly *Eimeria* species, are not serious pathogens of cranes in the wild. Nonetheless, a high percentage of wild sandhill cranes is infected with eimerians that may, under the right conditions, produce severe DVC. These cranes remain a source for transmission of eimerian oocysts among other cranes in the wild, including the endangered whooping cranes. Therefore, a more complete understanding of the biology of the *Eimeria* spp. of cranes is essential for proper management of cranes.

ACKNOWLEDGMENTS

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