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HAEMOPROTEUS BALEARICAE AND OTHER BLOOD PARASITES OF FREE-RANGING FLORIDA SANDHILL CRANE CHICKS

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ABSTRACT: We obtained blood smears from 114 Florida sandhill crane (*Grus canadensis pratensis*) chicks in Osceola and Lake Counties, Florida, USA, during 1998–2000. *Leucocytozoon grusi* was observed in 11 (10%) chicks; *Haemoproteus antigonis* was observed in eight (7%) chicks; and three (3%) chicks were infected with *Haemoproteus balearicae.* One chick infected with *H. balearicae* suffered from severe anemia (packed cell volume=13%) and was later found moribund. At necropsy this bird also had severe anemia and damage to the heart possibly due to hypoxia. This is the first report of *H. balearicae* in free-ranging North American cranes.

Key words: Anemia, Florida, *Grus canadensis pratensis, Haemoproteus antigonis, Haemoproteus balearicae, Leucocytozoon grusi,* sandhill crane.

INTRODUCTION

Sandhill cranes (*Grus canadensis*) can be found breeding, migrating, or as winter residents throughout much of North America (Tacha et al., 1992). Florida sandhill cranes (*G. canadensis pratensis*) are one of three nonmigratory subspecies of sandhill cranes and are limited in geographic distribution to Florida and southern Georgia (Tacha et al., 1992). In winter, greater sandhill cranes (*G. canadensis tabida*) also occupy this range. Additionally, two populations of whooping cranes (*G. americana*), one resident and one migratory, are currently being established in central Florida.

Four species of *Haemosporida* have been reported in cranes, *Haemoproteus antigonis, Haemoproteus balearicae, Leucocytozoon grusi,* and *Plasmodium polare*–like (Bennett et al., 1974, 1975; Telford et al., 1994). *Haemoproteus antigonis* was first described by deMello in 1935 from a Demoiselle crane (*Anthropoides virgo*) in India (see Bennett et al., 1975) and later found in Greater and Florida sandhill cranes (Forrester et al., 1974, 1975). *Haemoproteus balearicae* was first described from black crowned cranes (*Balearica pavonina*) from Africa in 1973 (Peirce, 1973). A number of unidentified *Haemoproteus* spp. have also been reported in cranes (see Bennett et al., 1975). *Leucocytozoon grusi* was described in 1974 from Florida sandhill cranes (Bennett et al., 1974). The species of *Plasmodium* was first reported in cranes when Telford et al. (1994) reported a *P. polare*–like parasite in sandhill cranes of unknown subspecies from Florida. Other reports of blood parasites from cranes include *Atoxoplasma* sp. from a sandhill crane in Florida (Bishop and Bennett, 1992).

In this report we document the prevalence of blood parasites in Florida sandhill crane chicks. This is the first report of *H. balearicae* in free-ranging North American cranes.

METHODS

Florida sandhill crane chicks were hand captured from 1998 to 2000 from eight locations in Osceola and Lake Counties, Florida, USA. The Escape Ranch (27°53′N, 80°57′W) and Hayman 711 Ranch (27°50′N, 80°59′W) were only surveyed in 1998. The Gardner-Cobb Marsh (28°2′N, 81°18′W), Overstreet Rd. (27°57′N, 81°12′W), and Pruitt Ranch (28°44′N, 81°56′W) were surveyed in 1999 and 2000. These areas were surveyed intensively three to four times per week during the breeding season (approximately March 15 through June 15) for chicks. The Disney Wilderness Preserve (28°8′N,

81°26′W), Crescent J. Ranch (28°4′N, 81°3′W), and Kissimmee Park Rd. (28°14′N, 81°19′W) were sampled one time each in 2000. Chicks were captured by hand not more than once per week. In 1999 and 2000 all chicks were marked with subcutaneous transponders (Trovan Electronic Identification Systems, Trovan, Ltd., Douglas, UK) (Dusek, 2002), and selected individuals also had radio transmitters (Advanced Telemetry Systems, Isanti, Minnesota, USA) surgically inserted (Spalding et al., 2001). In 1998 chicks were identified individually by DNA analysis after the completion of field work (Jones, 1998). Chicks were bled at each capture from the jugular vein using a 1-cc tuberculin syringe with a 28-gauge needle. Blood smears were prepared immediately and air dried. The remainder of the blood was placed in a heparinized blood collection tube. In the laboratory, slides were fixed with methanol and stained with Leucostat (Fisher Diagnostics, Pittsburgh, Pennsylvania, USA) or Giemsa (E. M. Science, Gibbstown, New Jersey, USA) and examined for blood parasites with a compound microscope at 400× for at least 20 min. Identification of parasites was made at 1,000×. Packed cell volume (PCV) was calculated using the microhematocrit method. The heparinized blood was thoroughly mixed and then used to fill a 0.08-ml hematocrit tube. Following centrifugation, the proportion of packed red cells to serum was calculated. All chicks were assigned to age classes using bill measurements (Dusek, 2002). Mean age of infected chicks was calculated by taking an average of the age classes they were assigned to. If chicks were found infected on more than one occasion, only the first age was used to calculate average age of all infected chicks.

We examined 170 blood smears from 114 individual birds during this study. Fifty-nine birds were sampled in 1998, 27 birds were sampled in 1999, and 28 birds were sampled in 2000.

Estimates of parasitemias of birds with *Haemoproteus* spp. parasites were determined by counting 1,000 erythrocytes and calculating the proportion of those with intraerythrocytic parasites. For chicks with *L. grusi* infections, the same method was used even though we did not know whether *L. grusi* developed in erythrocytes or leucocytes. If no parasitized cells were encountered during the count but infected cells had been observed during other examinations of the slide, an arbitrary infection prevalence of 0.5 infected erythrocytes/1,000 erythrocytes was assigned.

A complete postmortem examination was made of one fresh chick carcass. Sections of tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin and Giemsa.

Voucher specimens have been deposited at the US National Parasite Collection in Beltsville, Maryland (accession numbers 94497 through 94500).

RESULTS

Three species of parasites were identified from 22 sandhill crane chicks. All positive chicks were captured at study sites in Osceola County; birds from Lake County were negative. *Leucocytozoon grusi* was detected in 11 birds, only in 1998. For all chicks with *L. grusi* infections mean parasitemia was 0.05% ($n=11$), PCV was 32% ($n=11$, range 28% to 37%), and mean age at capture was 55–63 days ($n=11$, range 28–36 days to >72 days). One chick had an infection lacking mature gametocytes at its first capture, 37–45 days of age, and 11 days later had only mature gametocytes present in its blood smear.

Haemoproteus antigonis was detected in all three years in a total of eight chicks. Two birds were found infected in 1998, five birds in 1999, and one bird in 2000. Mean parasitemia of *H. antigonis*–infected birds was 0.3% ($n=8$, range 0.05% to 0.6%), mean PCV was 31% ($n=8$, range 27% to 36%), and mean age at capture was 46–54 days ($n=9$, range 19–27 days to 64–72 days).

Haemoproteus balearicae was detected in three chicks captured in 1999. Parasites in all three chicks had thin, elongated gametocytes with an incomplete margin as in the original description (Peirce, 1973). Chick E831 was captured and sampled on 29 April. This chick was encountered 1 wk later but not sampled and not seen again. It could not be determined whether or not the chick survived. At capture it was 37–45 days old. This chick had a duel infection of *H. balearicae* and *H. antigonis,* only some of which had developed to a stage where they could be differentiated. Overall parasitemia was 1.3%. Of the parasites present, the majority of them were trophozoites, but a few maturing gametocytes

of both species were present. No other blood values were measured for this chick.

Chicks 66FE and E072 were captured and sampled initially on 18 May 1999. These chicks were siblings and were located approximately 0.8 km from the site where chick E831 was captured. Chick 66FE had a PCV of 34%, while chick E072 had a PCV of 31%. No signs of clinical disease were noted in either chick. At the time of this first capture, both chicks were 1–9 days old. *Haemoproteus balearicae* was only detected in chick 66FE at this capture (parasitemia 0.3%). Both trophozoites and young gametocytes were prominent in the blood smear. Both chick 66FE and chick E072 were captured 7 days later to attach radio transmitters and were also resampled for blood. At this capture, chick 66FE was severely anemic, had a PCV of 13%, and mature gametocytes of *H. balearicae* were the most frequent stage of parasite identified on the blood smear (parasitemia 1.4%). An extended clotting time, based on experience with the same procedures on other chicks in this study, was observed at the site where the transmitter was surgically inserted and at the venipuncture site for the blood sample, resulting in mild subcutaneous hematomas at both locations. No leucocytes were observed during examination of the blood smear for this chick. Erythrocytic precursor cells, not seen in other blood smears, were common. Chick E072 had no detectable *H. balearicae* parasites, a PCV of 33%, and an apparently normal blood smear, and no clinical signs of disease were observed at this capture.

Three days following the second capture (28 May 1999), Chick 66FE was found moribund and apparently abandoned by its parents and sibling (they were out of sight and more than 200 m away). Chick 66FE died within 5 min following its discovery. The carcass was kept chilled until necropsy, approximately 3 hr later. At necropsy, the blood was very thin and watery, signs of small hematomas due to the procedures 3 days earlier were still evident, and one foot was slightly swollen. Histologically, there was evidence of early cardiomyodegeneration, and bacterial colonies were associated with inflammation and swelling on the foot. Marked extramedullary erythropoesis was present in portions of the lung. Parasite pigment granules were prominent in the spleen. No parasitic shizonts were observed in numerous tissues examined.

Chick E072 was recaptured and resampled on 8 June 1999. At this capture, it was infected with *H. balearicae* (parasitemia 9.4%), primarily trophozoites and a few young gametocytes. No clinical signs of disease were noted and it had a PCV of 26%. Except for the parasite infection, the blood smear appeared normal. Remains of this chick, apparently killed by a predator and consisting of several feather piles and the radio transmitter, were recovered 6 days later.

One chick had a dual infection of *L. grusi* and *H. antigonis;* one chick had a dual infection of *H. antigonis* and *H. balearicae.* No hematologic abnormalities were observed during scans of these blood smears.

DISCUSSION

Leucocytozoon grusi was first described from Florida sandhill cranes in Alachua County, Florida (Bennett et al., 1974) but has yet to be reported outside of Florida. We found *L. grusi* in 10% of chicks and only in 1998. Forrester and Spalding (2003) reported *L. grusi* prevalence in cranes of unknown subspecies of 11% (49 of 458) in Florida. Our estimates of *L. grusi* parasitemias in crane chicks were low, and we concluded that the infections likely had little effect on the health of any of the birds we sampled.

During the year in which *L. grusi* was detected in blood smears, crane chicks were not equipped with radio transmitters and therefore could not be relocated. Because all recaptures were opportunistic and no sick or dead birds could be located, if *L. grusi* did cause disease or in some

way contributed to mortality of crane chicks, it was not detected. In subsequent years when cranes were marked with radio transmitters, no *L. grusi* infections were detected, but crane chicks were not sampled from the locations used in 1998. Additionally, in 1999 and 2000, all study areas were subjected to extreme drought conditions that may have limited the transmission of this and other parasites.

Bennett et al. (1975) reported only two species of *Haemoproteus* in the family Gruidae, *H. balearicae* and *H. antigonis.* Bennett et al. (1975) suggested that the distribution of *H. antigonis* is "probably throughout the range of the Gruidae." Forrester and Spalding (2003) reported a prevalence of 10% (48 of 458) in sandhill cranes of unknown subspecies they examined. We found *H. antigonis* in 6% of individual Florida sandhill crane chicks. Intensities of these infections were low and probably had little impact on development or fitness of infected chicks, although no direct measure of this was done.

Haemoproteus balearicae was believed to be limited in distribution to West Africa (Bennett et al., 1975). Evidence of this parasite's movement by way of anthropomorphic factors is available. Peirce (1973) described this parasite in black crowned cranes being kept in avian collections in England. This parasite was identified also in wattled cranes (*Bugeranus carunculatus*) at the Oklahoma City Zoo, Oklahoma, USA (Halpern and Bennett, 1983). The natural ranges of both host species of *H. balearicae* are limited to Africa. In the case of the black crowned cranes, the infected birds were reported to have originated from natural populations in Africa (Peirce, 1973). Halpern and Bennett (1983) did not report on the origins of the two infected wattled cranes they examined.

We found this parasite in the blood of a crane chick between 1 day and 9 days of age (chick 66FE). Packed cell volume in this chick declined from 34% to 13% during the 7 days between the first two captures. Mean PCV of Florida sandhill cranes is approximately 33% for all age groups (Dusek, 2002). During this time period, this chick also suffered what appeared to be an almost complete loss of circulating white blood cells. No leucocytes were seen on the blood smear, but a few were observed during granulocyte counts using a hemacytometer (Dusek, 2002). Additionally, the parasitemia was higher than for any of the *H. antigonis*–infected chicks reported in this study. At necropsy, there was damage to the heart, possibly due to hypoxia secondary to severe anemia or an adrenergic response from capture stress. The finding of parasite pigment granules in the spleen indicates sequestration and destruction of infected cells, possibly accounting for the observed anemia. Additionally, increased production of erythrocytes in the lung was likely a response to the severe anemia.

Severe anemia has been associated with low parasitemia in domestic ducks infected with *Leucocytozoon simondi* (Kocan and Clark, 1966). This was later explained during a follow up study; an antierythrocytic factor was hypothesized to have caused the destruction of uninfected, as well as infected, erythrocytes brought about by acute *L. simondi* infection (Kocan, 1968). Although severe anemia has not been described as a prominent feature of *Haemoproteus* infection, the acute phase of this disease has not been well studied due to the difficulties of experimental infection. Atkinson and Van Riper (1991) suggested that a bird that was under nutritional or environmental stress could develop parasite-induced anemia as a result of the removal of a large number of infected erythrocytes.

Chick E072 was found infected with *H. balearicae* when captured between 28 days and 35 days of age (its third capture), 21 days after initial capture and 11 days after its sibling (chick 66FE) died. Its PCV was lower than at its first two captures, and it had a parasitemia of 9.4% but showed no other signs of disease. Prior to death, if this chick followed a similar progression of

disease, it is likely that it would have become lethargic and depressed due to increasing anemia, and it may not have had the physical ability to escape or the capacity to hide quickly enough from a predator. It is also possible that this chick would have suffered this fate without being infected with *H. balearicae*, as predation is frequently found to be the most important source of identifiable mortality in studies of sandhill crane chicks (DesRoberts, 1997; Ivey and Scheuering, 1997).

A third *H. balearicae*–infected chick (chick E831) was not fitted with a radio transmitter, and it is not known whether this chick survived. This single chick was positive for this parasite 20 days before the other two chicks were captured, and 4–13 days before their estimated hatch date.

Evidence concerning the effects of blood parasites on crane hosts is limited. The crowned crane from which *H. balearicae* was described had this parasite detected in blood smears over a 3-yr period (Peirce, 1973). The necropsy of the same bird determined the cause of death to be primarily syngamosis, although it also had a parasitemia of *H. balearicae* of 8% at the time of death (versus 1% reported for this same bird 4 mo earlier) (Peirce, 1973). In three other cranes examined at necropsy and included in that report, parasitemias ranged from 1% to 11.5%, but mortality was attributed to syngamosis and colisepticemia. Peirce (1973) also reported parasitemias of between 1.0% and 2.7% in six other cranes, which were examined alive. Reports of the *P. polare*–like parasite were derived from three cranes examined at necropsy (Telford et al., 1994). In two of these birds, *H. antigonis* was detected, and in one of these, *L. grusi* was also detected. A number of other disease conditions were also diagnosed in these severely debilitated birds, but death was not attributed to any single condition.

Sandhill cranes have been studied in Florida for about 30 yr, and numerous blood smears have been examined with no prior observations of this parasite (Forrester and Spalding, 2003). Our identification of *H. balearicae* in Florida would indicate that this is a recent introduction to the free-ranging Florida sandhill crane population. Numerous captive cranes exist in the central Florida area near where this parasite was found, and it is possible that this parasite may have originated from one of those facilities.

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