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Haemosporidian infection in captive masked bobwhite quail (*Colinus virginianus ridgwayi*), an endangered subspecies of the northern bobwhite quail

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ABSTRACT

The avian haemosporidian parasites (phylum Apicomplexa) are taxonomically diverse and cosmopolitan in distribution; infecting most bird families. Sources of concern are reports of clinical haemosporidian infections in birds kept as part of zoo and aviary collections. Recently, severe and acute mortality episodes have been reported in masked bobwhite quail (*Colinus virginianus ridgwayi*), an endangered subspecies from the American Southwest. Two hundred and five eggs of the captive flock held in Arivaca, Arizona, were hatched at a zoo in the American Southwest. Thirty-four sub-adult or adult animals had lesions associated with tissue phases of haemoparasites, especially vasculitis, ventricular leiomyositis and ulcerative pododermatitis. Molecular techniques applied to blood collected from the zoo's last twelve remaining animals resulted in the detection of a *Plasmodium juxtanucleare*-like and *Haemoproteus* sp. parasites. A Raven (*Corvus corax*), in a contiguous exhibit, was positive for the same *P. juxtanucleare*-like parasite, but remained asymptomatic for three years following detection. These findings indicate that other birds in the exhibit within the zoo premises could act as reservoirs. We conclude that haemosporidian infections could be a factor in the demise of the captive masked bobwhite quails housed at the zoo. We suggest that active surveillance for haemoporidian parasites should be incorporated as a precaution to *ex situ* conservation efforts of susceptible endangered species.

1. Introduction

Avian haemosporidian infections have been increasingly studied as model systems for investigating different aspects of the host–parasite interactions in ecology, evolution, and conservation biology. The avian haemosporidian parasites (phylum Apicomplexa) are taxonomically diverse, cosmopolitan in distribution, and infect most bird families (Valkiūnas, 2005). The three commonly reported avian hemoprotozoa belong to the genera *Plasmodium*, *Haemoproteus*, and *Leucocytozoon*. Regardless of their broad host and geographic distributions, information on their pathogenicity is almost completely based on laboratory experiments with domesticated birds (canaries, chickens, ducklings, pigeons, turkey poults) (Valkiūnas, 2005). However, some haemosporidioses are well

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studied and thoroughly documented in zoos and rehabilitation centers. Based on such assessments, we know that these parasites are associated with a broad spectrum of clinical disease manifestations, from asymptomatic to acutely fatal (Cardona et al., 2002; Valkiūnas, 2005; Ferrell et al., 2007; Grim et al., 2008). Among haemosporidian parasites, *Plasmodium* is responsible for most frequently observed outbreaks of especially severe malaria in domestic and wild birds in zoos and aviaries (Valkiūnas, 2005; Fix et al., 1988; Cranfield et al., 1990; Ferrell et al., 2007; Alley et al., 2008; Olias et al., 2011). However, some species of *Haemoproteus* can be highly pathogenic and cause severe myositis in avian hosts (Cardona et al., 2002; Olias et al., 2011). For example, chronic outbreaks of *Haemoproteus lophortyx* infection have been reported in captive bobwhite quail (Cardona et al., 2002).

Recently, severe and acute mortality episodes have been reported in masked bobwhite quail (*C. virginianus ridgwayi*, Odontophoridae) at breeding facilities. Masked bobwhite quail, a subspecies of the Northern bobwhite, is the only endangered quail in North America. Although the birds never had an extensive distribution in the U.S., they were plentiful and common in Northern Mexico during the 1860s (Brown and Ellis, 1977). Excessive grazing pressure, coupled with extended drought during the early 1890s, deteriorated the grasslands of Southern Arizona and caused the masked bobwhite population to shrink (Brown, 1900; Tomlinson, 1972). This endangered sub-species is currently restricted to one captive population in the Buenos Aires National Wildlife Refuge (Altar Valley, Southern Arizona) and two known native populations in private ranches in north-central Sonora, Mexico (Kuvlesky et al., 2000). The current captive flock of masked bobwhite quail may represent the only hope for this highly endangered subspecies (Hernández et al., 2006).

In this study, we explore the possibility that the recent mortality episodes in masked bobwhite quail at the zoos could be related, in part, to outbreaks of Haemosporidian infections. In this investigation we report both *Haemoproteus* sp. and *Plasmodium juxtanucleare*-like parasites for the first time in captive masked bobwhite quail hatched and raised in a zoo. We describe new pathological signs such as vascular damage, especially in the ventriculus and skin associated with the haemosporidian infection.

2. Materials and methods

2.1. Samples description

In 2006, a Southwest zoo received a total of 206 fertile masked bobwhite quail eggs for hatching. Two dozen masked bobwhite quail were placed on exhibit, and the rest of the animals were placed in separate and isolated wire mesh holding facilities. The birds placed on exhibit were housed with other native regional wildlife in large connected flight cages. These birds were in contact with a flock of 14 thick billed parrots (*Rhynchopsitta pachyrhyncha*). A small stream ran through the exhibit. A contiguous flight cage housed two bald eagles (*Haliaeetus leucocephalus*), two black vultures (*Cathartes aura*), two common ravens (*Corvus corax*), and an American porcupine (*Erethizon dorstatum*). Gambel's quail (*Callipepla gambelii*), and two golden eagles (*Aquila chrysaetos*) were exhibited in other remote parts of the zoo.

The Zoo keepers noted that many quails appeared weak, listless and unresponsive while still in adequate body condition, and many had ulcerative skin lesions on the dorsum of the feet and around the tarsometatarsal-phalangeal joint. Blood smears of clinically ill birds consistently showed numerous large intra-erythrocytic haemosporidian parasites. Affected birds would be found dead soon after initial clinical signs. Necropsy would usually reveal generalized pallor. Tissues from five dead masked bobwhite quail were fixed in 10% neutral buffered formalin and submitted for histologic examination to two separate laboratories. By March 2007 only 10 adults quail remained alive; thus, the success rate of this breeding facility was only 5% from the original 206 fertile eggs. Those 10 adults were the subject of this investigation in an effort to understand why such failure in maintaining this endangered species in that particular location.

2.2. Cytology and histopathology

Histopathologic and cytologic investigation was conducted at Northwest ZooPath and at the Arizona Veterinary Diagnostic Laboratory on five of the dead quail. Following necropsy, sections of brain, heart, lung, liver, kidney, spleen, pancreas, alimentary tract at all levels, reproductive tract, and skin of the feet were fixed in 10% neutral buffered formalin, processed routinely, sectioned at 5 μm and stained with hematoxylin and eosin (HE). Blood films from each bird were stained with Wright–Giemsa technique.

2.3. Detection of haemosporidian parasites by polymerase chain reaction (PCR)

Zoo animals close to the exhibited masked bobwhite quail were bled as part of their annual medical exam. A total volume of 0.25–0.5 ml of whole blood was collected in EDTA from each animal and frozen prior to testing. Blood samples were drawn from the remaining ten masked bobwhite quail, six Gambel's quail (*C. gambelii*, Odontophoridae), two common raven (*C. corax*, Corvidae), four thick-billed parrot (*R. pachyrhyncha*, Psittacidae), one bald eagle (*H. leucocephalus*, Accipitridae), and two golden eagles (*A. chrysaetos*, Accipitridae). In addition, we collected and analyzed blood samples from 38 masked bobwhite quail at the Buenos Aires National Wildlife Refuge. Three blood samples of the greater roadrunner (*Geococcyx californianus*) were also analyzed; one from the zoo, and two from Liberty Wildlife, a wildlife rehabilitation center in the same region. All samples were frozen at −4.0 °C and banked for future testing.

Using 100 μl of thawed blood samples from each of the birds, deoxyribonucleic acid (DNA) was extracted using QiAamp® DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany). Each DNA sample was screened for haemosporidian parasites by using a nested polymerase chain reaction (PCR) protocol that targets mitochondrial markers in the parasite. Mitochondrial markers were chosen