Journal of Wildlife Diseases, 46(2), 2010, pp. 499–506

AVIAN BOTULISM: A CASE STUDY IN TRANSLOCATED ENDANGERED LAYSAN DUCKS (*ANAS LAYSANENSIS*) ON MIDWAY ATOLL

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ABSTRACT: Laysan Ducks are endemic to the Hawaiian archipelago and are one of the world's most endangered waterfowl. For 150 yr, Laysan Ducks were restricted to an estimated 4 km^2 of land on Laysan Island in the northwestern Hawaiian Islands. In 2004 and 2005, 42 Laysan Ducks were translocated to Midway Atoll, and the population increased to approximately 200 by 2007. In August 2008, mortality due to botulism type C was identified, and 181 adult, fledgling, and duckling carcasses were collected from August to October. Diseased birds were found on two islands within Midway Atoll at multiple wetlands; however, one wetland contributed most carcasses. The epidemic was discovered approximately 14–21 days after the mortality started and lasted for 50 additional days. The details of this epidemic highlight the disease risk to birds restricted to small island populations and the challenges associated with managing newly translocated endangered species. Frequent population monitoring for early disease detection and comprehensive wetland monitoring and management will be needed to manage avian botulism in endangered Laysan Ducks. Vaccination may also be beneficial to reduce mortality in this small, geographically closed population.

Key words: *Anas laysanensis*, botulism, *Clostridium botulinum*, endemic island waterfowl, Laysan teal, waterfowl disease.

INTRODUCTION

Islands are characterized by isolated, nonmigratory, endemic, and often endangered populations of fauna and flora highly susceptible to catastrophic disease and other stochastic events. One example is the Laysan Duck (*Anas laysanensis*), a Hawaii endemic anatid that was historically present on all the Hawaiian islands (Cooper et al., 1996). This duck is listed as critically endangered (IUCN, 2009) and was restricted for about 150 yr to only 4 km^2 on Laysan Island (25°46′N, 171°44′W). Concern about the species' extinction risk led to translocation of a group of birds to the islands of Midway Atoll in 2004 and 2005 (US Fish and Wildlife Service, 2004; Reynolds et al., 2008).

Laysan Island is unique among the Hawaiian Islands in having a large, shallow, hypersaline lake and no introduced mammals. Laysan Ducks forage on aquatic invertebrates, terrestrial arthropods, seeds, algae, and succulent vegetation and use freshwater seeps around the lake to raise ducklings (Moulton and Weller, 1984; Moulton and Marshall, 1996; Reynolds et al. 2006). Laysan Ducks do not migrate or disperse from Laysan Island, and they are vulnerable to extinction from stochastic events (US Fish and Wildlife Service, 2004). Laysan Island seldom supports more than 320–600 adult Laysan Ducks, probably due to density-dependent population limitations (Seavy et al., 2009).

In August–October 1993, an epidemic of emaciation complicated by infection with the nematode *Echinuria uncinata* (Echinuriasis) was detected in Laysan Ducks from Laysan Island (Work et al., 2004). *Echinuria* spp. can cause significant disease, particularly in birds experiencing crowding stress (Ould and Welch, 1980), such as during drought, when birds are concentrated around freshwater sources, presumably enhancing chances of exposure and infection (Cornwell, 1963). On Laysan, low rainfall, poor food resources, and crowding around limited freshwater seeps were believed to be contributory factors (Work et al., 2004). The epidemic was catastrophic and resulted in a population decline of nearly 75% (Reynolds, 2002). Since then, Laysan Duck mortality has been systematically monitored, and, after 10 yr, population abundance recovered to pre–die-off levels (Seavy et al., 2009). Adult Laysan Ducks normally experience low mortality, and their population dynamics are very sensitive to changes in the mortality rates of adult females (Reynolds and Citta, 2007). Avian botulism was first identified in one Laysan Duck from Laysan Island in spring 2003 (Reynolds and Work, 2005); two other cases were found on Laysan Island in 2007 and 2008.

Translocation of wildlife can be a useful tool in the conservation of rare or endangered species; however, concerns about disease in translocated wildlife have been well documented (Woodford, 1993; Cunningham, 1996). Because diseases can have a disproportionate demographic effect, particularly for isolated, endangered species (Thorne and Williams, 1988; Work et al., 2000), risk assessments for disease should be an inherent part of any wildlife translocation plan and should include measures to minimize the risk of moving pathogens. This includes developing a well-known health history of the source and destination wildlife populations through necropsy surveys, quarantine, clinical examinations, vaccination, and management protocols for animals at the release site (Viggers et al., 1993). Furthermore, translocated populations should be systematically monitored after release to assess the likelihood of new or additional disease risks.

Prior to movement to Midway Atoll (Midway), wild Laysan Ducks selected for translocation from Laysan Island to Midway were given a physical exam, parasite treatment (200 $\mu g\ kg^{-1}$ of ivermectin), and met certain body condition scores (Reynolds et al., 2008). In preparation for the arrival of ducks to Midway, wetland and nesting habitat was created or enhanced (Reynolds and Klavitter, 2006). In 2004 and 2005, 42 Laysan Ducks were translocated from Laysan to Midway, and a breeding population was successfully established, increasing to >200 postfledglings by 2007 (Reynolds et al., 2008). In 2008, catastrophic mortality occurred in Laysan Ducks on Midway. In this paper, we describe the epidemic, identify its cause, and discuss management implications of this event for remote island ecosystems.

MATERIALS AND METHODS

Midway Atoll (28°12′N, 177°22′W) is located 1,930 km from Honolulu, Hawaii and is composed of three islands: Sand (452 ha), Eastern (136 ha), and Spit (6 ha; Fig. 1). The atoll is protected as a National Wildlife Refuge (NWR) within the Papahanaumokuakea Marine National Monument. Midway Atoll is a highly altered ecosystem that has had relatively continuous human presence since 1859 and significant military activity for more than 50 yr. Prior to the epidemic, there were >17 diverse small wetlands on the atoll, totaling 1.8 ha, including a rainwater Catchment consisting of a 0.9-ha concrete-lined shallow body of water that holds up to 2.4 million l of water (80% of the standing freshwater on Midway) and various intermittently to continually flooded forested and palustrine wetlands. Eleven of these wetlands were created prior to translocations of Laysan Ducks to establish additional habitat for the ducks. Three existing wetlands were enhanced to provide additional open-water habitat. Wetlands were charged by groundwater or surface flow and ranged from 18 to 408 m^2 with depths of ~0.1–1.5 m. The edges of wetlands ranged from shallow feathered mud flats to steeper slopes with dense native (*Cyperus laevigatus* and *Cyperus polytachos*) and non-native (*Cyperus alternifolius*) sedges. Water levels could only be manipulated in two wetlands (Tarmac and Catchment). Since the translocations in 2004 and 2005, Laysan Ducks have been documented using all of the wetlands on Sand and Eastern islands (Fig. 1).

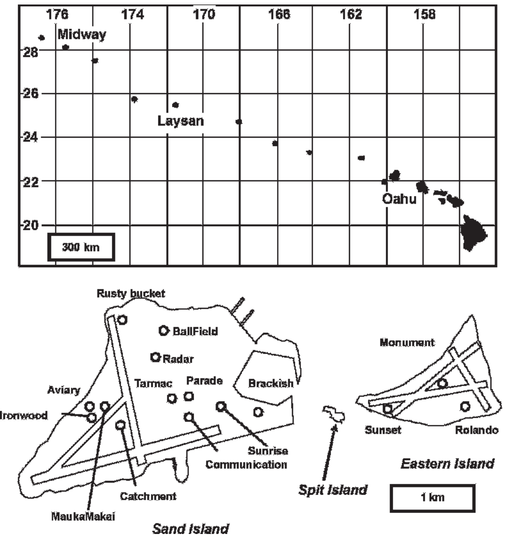

FIGURE 1. Main and northwestern Hawaiian islands (top) and locations of wetlands on Sand and Eastern Islands (below) on Midway Atoll National Wildlife Refuge (not to scale).

On 11 August 2008, unusual numbers of Laysan Duck carcasses were observed around the catchment wetland. Because avian botulism was suspected from field signs (protruberant nictitating membrane, flaccid paralysis of legs, wings, and neck), all wetlands on Midway were searched to remove all carcasses in an attempt to break the carcass-maggot cycle typical of botulism epizootiology (Rocke and Bollinger, 2007) and to collect specimens for diagnostic evaluation. Carcasses collected were grossly classified as ducklings, hatch-year (HY consisting of birds from fledging to independence; ≥65–70 days), or adults (>1 yr old). We refer to birds moved from Laysan to Midway as "founders." Carcasses were subjectively classified as fresh, moderately, or markedly decomposed based on smell, presence of maggots, and integrity of the carcass. Necropsies of ducks included determination of weight followed by a complete external and internal examination. Representative tissues including brain, skeletal muscle, lung, liver, kidney, proventriculus, spleen, heart, pancreas, and small intestines were fixed in 10% formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin for microscopic examination. Hearts and clotted blood were stored frozen (−20 C) and assayed for botulinum toxin type C using the mouse cross protection test (Smith, 1980). Approximately 20 cm^3 of live fish (*Gambusia affinis*), 2 cm^3 of midge larvae (Diptera: Chironomidae), and four dragonfly larvae (Odonata: Libellulidae) were collected from three wetlands (Rusty Bucket, Catchment, and Monument) and frozen for future assays for botulinum toxin to identify potential routes of exposure.

Based on necropsy findings, duck carcasses were classified into the following diagnostic categories: 1) Confirmed botulism cases were those with documented presence of botulinum toxin type C in heart blood using the mouse protection test; 2) suspected botulism were cases showing no evident gross lesions or ducks with only red discoloration of the lungs but not all tested for botulism; 3) septicemia were cases with histologic evidence of bacteria within internal organs associated with inflammation; 4) trauma were cases with gross evidence of broken bones, severe bruising of muscle, or ruptured liver associated with acute hemorrhage; and 5) undetermined were cases for which cause of death was unknown.

Numbers of sick and dead ducks and location of those recoveries were recorded daily. Gross and microscopic pathology lesions were categorized according to frequency of occurrence from most to least.

RESULTS

In total, 181 Laysan Duck and 12 Northern Pintail (*Anas acuta*) carcasses were recovered on Midway Atoll from 11 August to 11 October 2008 (Fig. 2). In addition, one each Least Tern (*Sterna antillarum*), Ruddy Turnstone (*Arenaria interpres*), Wandering Tattler (*Heteroscelus incanus*), and Lesser Scaup (*Aythya affinis*) were recovered but not necropsied. Although discovery of the unusual mortality occurred on 11 August, based on the stage of decomposition of animals recovered the first day, we suspect the epidemic started 2–3 wk earlier. Type C botulism was confirmed in 13/16 (81%) cases tested by the mouse protection assay. The time line of emergency response actions and the summary of carcass recoveries are given in Figure 2.

We estimate the entire epidemic lasted 40–50 days based on stage of decomposition of recovered carcasses. As the epidemic progressed, the percentage of birds recovered from Eastern Island increased

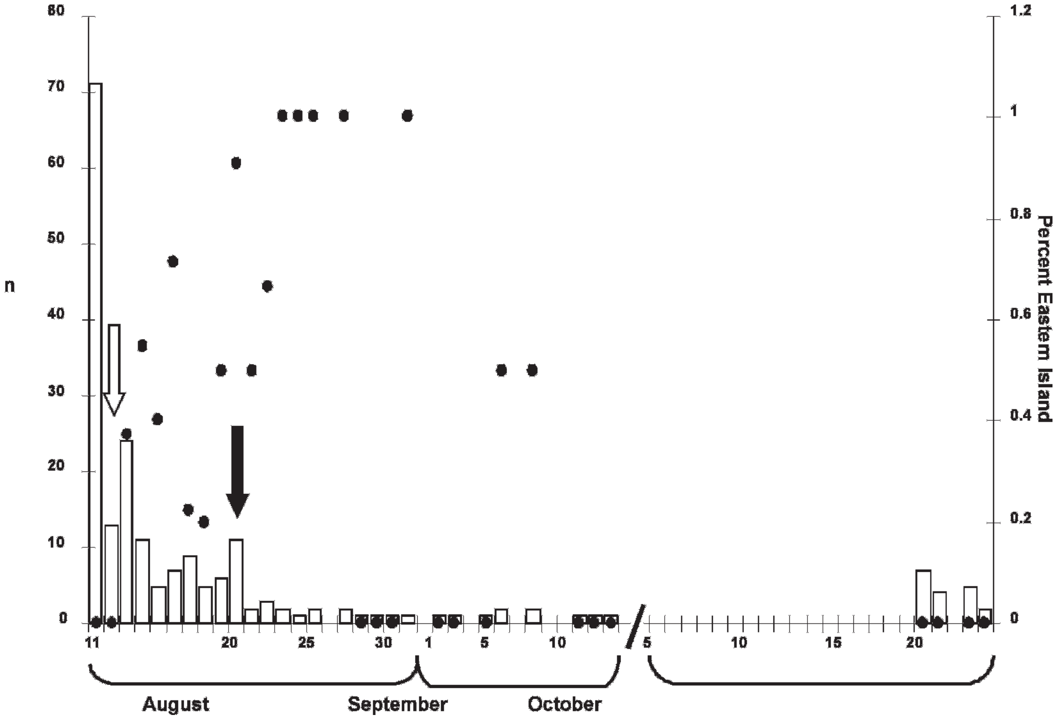

FIGURE 2. Epizootic curve for type C botulism in waterfowl on Midway Atoll 12 August–25 October 2008. Bars are numbers (left axis) of sick or dead ducks. First bar is day epizootic was discovered. White arrow indicates day Tarmac was drained; black arrow indicates day Catchment was drained (3 days required for draining). Catchment was flooded on days 0 and 3. A second smaller die-off of migratory Northern Pintails and Laysan Ducks occurred in late October after 18-day hiatus. Dots show percentage (right axis) of sick or dead ducks on Midway Atoll that were found on Eastern Island.

and reached a plateau during days 10–20, after which recoveries of carcasses decreased (Fig. 2). Recovery of sick and dead birds was the highest on the day of discovery of the epidemic. The Catchment wetland had the highest number of Laysan Duck carcasses (Fig. 3). Clinical signs manifested by ducks included inability to use their wings, difficulty in holding up their head, prolapse of nictitating membrane, dehydration, cloacal impaction, and general weakness. Eighty-four of 181 Laysan Ducks and 6 of 12 pintails were necropsied. Sixty-four Laysan Ducks came from Sand Island, 17 came from Eastern, and the remainder came from unrecorded locations; all Northern Pintails came from Sand Island. Sex breakdown for necropsied Laysan Ducks was 34 female, 28 male, and 22 unknown. Sex breakdown for necropsied pintails was 4 female and 2 male. Age breakdown for necropsied Laysan Ducks was 41 adult, 31 hatch year, 8 duckling, and 4 unknown. All Northern

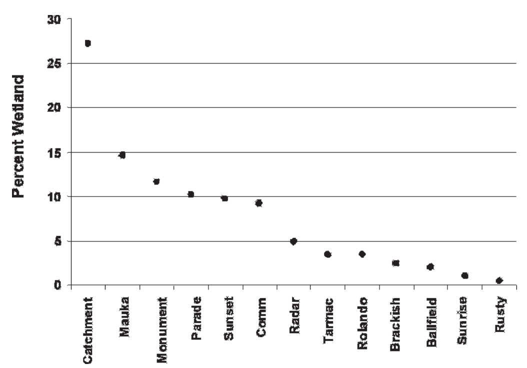

FIGURE 3. Percent of sick or dead ducks recovered by wetland site (see Fig. 1) on Midway Atoll 12 August–25 October 2008. Monument, Sunrise, and Rolando are on Eastern Island.

Pintails were nonbreeding migratory adults or subadults.

Fifty-two Laysan Duck carcasses were in good to excellent body condition; 17 were in fair body condition; 7 were in poor body condition; the remaining 8 were unknown. For Northern Pintails, the breakdown for body condition was 1 good, 4 fair, and 1 poor. The most common gross lesion for all ducks was diffuse red discoloration of the lungs (27 ducks), mild bruising of the pectoral musculature (2 ducks) and fibrinous coelomitis or pericarditis (1 duck each). On microscopy, the most common lesion ($n=60$ ducks) was mild to marked infiltrates of homogeneous eosinophilic material within airways and perivascular connective tissue spaces (pulmonary edema). Three of 84 Laysan Ducks had mild infections with proventricular nematodes (suspected *Echinuria* sp.) characterized by presence of cross sections of nematodes within the lumen of proventricular glands occasionally associated with mild to moderate amounts of eosinophilic cellular debris (Work et al., 2004). One pintail also had a mild infestation of proventricular nematodes with little to no inflammatory response. Final diagnoses for necropsied Laysan Ducks from Midway were confirmed ($n=10$) or suspected ($n=51$) botulism type C, septicemia ($n=4$), and trauma ($n=1$). Final diagnosis for six necropsied Northern Pintails was confirmed ($n=3$) or suspected ($n=3$) botulism type C. Live collected fish (*Gambusia affinis*), midges, and dragonfly larvae all were negative for botulinum toxin type C.

DISCUSSION

Avian botulism is a well-known cause of waterfowl mortality worldwide and can cause morbidity and mortality among large numbers of birds (Wobeser, 1997). Although much is known about this disease on the continents (Rocke and Friend, 1999), there is much less information about botulism on small islands. Island ecosystems are characterized by small and isolated populations of flora and fauna, many of which may be endangered. Nevertheless, because of their small geographic areas, small islands provide an opportunity to document and manage botulism epidemics at a level of detail that may not be possible in larger ecosystems. The botulism event described in this paper resulted in the death of large numbers of ducks in a short period of time. Fifty-six of the 71 carcasses (78%) found on the first day were moderately to severely decomposed, indicating that mortality had been ongoing for 2–3 wk previous to the discovery. The isolated nature of the ecosystem, delayed detection of mortality, inability to exclude congregating ducks, and lack of ability to manage water levels hampered efforts to abate mortality. Prior to this epizootic, only a single case of avian botulism had been documented on Midway; a hatch-year male Laysan Duck in July 2007, three years after the species' reintroduction.

We suspect that the type C botulism epidemic originated at the Catchment pond on Sand Island because this pond is the largest body of water on Midway, was heavily used by waterfowl, contributed most of the oldest carcasses, and contributed the highest number of carcass recoveries. After drainage of this pond, workers removed ~180,000 l of sludge from the concrete-lined Catchment, including many decomposing seabird carcasses. Midway Atoll supports the world's largest population of Laysan Albatross (*Phoebastria immutabilis*), and abundance of albatross reaches 1.5 million. Such high densities of seabirds result in seasonal mortality, particularly of chicks (Sileo et al., 1990), and decomposing carcasses provide a protein source that can promote the production of botulinum toxin type C in carcass or wetland sediments (Bell et al., 1955; Soos and Wobeser, 2006). Midway's coastal and marine zones have numerous scavengers, but the terrestrial zone lacks large scavengers. Thus, carcasses tend to persist and host fly larvae initiate the carcass-maggot cycle characteristic of avian botulism. Additionally, Midway is subtropical, and ambient temperatures can exceed 25 C in July and August, when this epidemic occurred. When temperatures in carcasses and anaerobic substrates are warm (~25 C or greater), *Clostridium botulinum* is more likely to produce type C botulinum toxin (Rocke and Bollinger, 2007), and toxin-laden fly maggots may develop more rapidly (Soos and Wobeser, 2006).

As the epidemic progressed, carcass recoveries were dispersed among 12 wetlands. Postrelease monitoring data using mark-resight methods and radiotelemetry showed that Laysan Ducks move freely between the islands of the atoll and among wetlands. Recently fledged juveniles make larger movements and have larger home ranges than breeding adults. The epidemic occurred just after the peak of juvenile fledging, when mobility was high, and carcass recovery on Eastern Islands was highest at the Monument wetland, which harbored duck densities of up to 5 ducks/m^2.

For most botulism outbreaks, the site of botulism ingestion is unknown (Rocke and Bollinger, 2007), and our data do not permit us to identify the substrate source of the initial proliferation of botulinum toxin or to disentangle movement of ducks affected by botulism from actual toxin production at a particular wetland. However, given the acute onset of mortality and the debilitating nature of botulism intoxication, we suspect that many dead ducks were intoxicated at or near the wetland of carcass recovery.

The measures recommended to mitigate the effects of avian botulism in wetlands include carcass removal, water management (draining or flooding), and hazing waterfowl away from suspected sources of toxin (Wobeser, 1997). On Midway, the Catchment was flooded with approximately five times its volume (1.5 million l) of freshwater after the discovery of the epidemic, but there were only two wetlands from which water could be drained (Catchment and Tarmac). After carcass collection and subsequent drainage of the Catchment and Tarmac, mortalities abated. Hazing waterfowl was problematic because shallow, partially drained wetlands are a foraging attraction to dabbling ducks, and there were no materials to exclude birds from high-risk wetlands (i.e., netting and exclusion fences). Botulism was apparently present at many sites, and thus dispersing birds from drained wetlands were at risk of intoxication at nearby wetlands. Island ducks have limited botulism-free refugia to which they can potentially retreat and thus are at higher risk compared to birds of larger landscapes or wetland ecosystems. This left carcass removal as the primary management option at most wetlands, and the difficulty in locating all duck carcasses in large wetlands is well known (Cliplef and Wobeser. 1993). However, carcass collection may remain an important and useful response to botulism on small wetlands that are under intensive surveillance.

We cannot explain why a large-scale botulism epidemic suddenly occurred on Midway in August 2008, when Laysan Ducks had been present on the island since October 2004. Furthermore, this disease on Laysan Island had been uncommon for the past 18 yr in the well-monitored Laysan Duck population. Rocke and Samuel (1999) found pH, redox potential, and temperature to be predictive factors favoring the production of botulinum toxin in wetlands. The epidemic was initiated during the warmest month of the year on Midway, and this finding is consistent with other cases of botulism C, which have also occurred during the warmest times of the year (Rocke and Bollinger, 2007). In the future, some of these parameters could be monitored on Midway to generate explanatory variables. Given the behavior of botulism in other wetlands, future outbreaks on Midway are likely (Wobeser et al., 1987). Analyses of various potential live wetland prey items failed to identify botulinum toxin, so the route of exposure remains unknown.

Botulism does not present with pathognomonic lesions (Wobeser, 1997), and lesions that are present are usually associated with drowning (Rocke and Friend, 1999). This study confirmed those findings in that the most noticeable lesion in affected ducks was mild to severe pulmonary edema, probably secondary to inhalation of water. A few ducks were septic, and, although the causative bacteria were not identified, the route of exposure may have been inhalation.

This epidemic clearly points to the need for wetland management and long-term monitoring for future translocations of Laysan Ducks, given their vulnerability to catastrophic disease epidemics (Work et al., 2004; Seavy et al., 2009). Frequent and systematic population monitoring should allow early detection of botulismm and immediate carcass collection should reduce the duration, severity, and magnitude of epidemics and allow for timely water management when possible. Removal of seabird carcasses from wetlands (a rich protein source favoring botulinum toxin production) and type C botulinum toxoid vaccination of rehabilitated, or demographically and genetically valuable birds may also be beneficial (Martinez and Wobeser, 1999; Rocke et al., 2000; Rocke and Bollinger, 2007).

As of this writing, Midway has implemented management strategies that may reduce future avian botulism mortalities. These include: 1) annual flooding of the concrete-lined catchment during the hot summer months; 2) drainage and cleaning of the catchment in the cool fall or winter months to remove accumulated debris and seabird remains; 3) vegetation removal around wetlands to facilitate carcass detection; 4) weekly Laysan Duck population monitoring that includes carcass removal and searches for sick birds; 5) wetland carcass searches daily during the hot summer months; and 6) usage of heavy equipment and portable pumps to annually remove “sludge” from wetlands to improve water quality.

ACKNOWLEDGMENTS

Staff from various agencies volunteered countless hours to help with this epidemic, and their efforts are greatly appreciated. We are particularly grateful to the following people for their help in carcass retrieval and in rehabilitation of sick ducks: Renee Eismueller and Jimmy Breeden (US Geological Survey), Matthew Brown and Leona Laniawe (US Fish & Wildlife Service), and Linda Elliott (Hawaii Wildlife Center). USFWS volunteers and Chugach contractors graciously donated many hours during the emergency response. We also thank Brenda Berlowski-Zier for her technical assistance with the botulism assays. Jeff Walters and Tonie Rocke kindly provided constructive comments.

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Submitted for publication 8 July 2009.