AVIAN DISEASES 42:1–5, 1998

Necrotizing Enteritis as a Cause of Mortality in Laysan Albatross, Diomedea immutabilis, Chicks on Midway Atoll, Hawaii

T. M. Work,[A] M. R. Smith,[B] and R. Duncan[B]

[A]U.S. Geological Survey, Biological Resources Division, National Wildlife Health Center, Honolulu Field Station, P.O. Box 50167, Honolulu, HI 96750
[B]U.S. Geological Survey, Biological Resources Division, National Wildlife Health Center, Madison, WI 53711

Received 23 December 1996

SUMMARY. A necropsy survey of Laysan albatross, *Diomedea immutabilis*, chicks on Midway Atoll in June 1993, 1994, and 1995 revealed 54% (21/39), 67% (49/71), and 93% (15/16), respectively, to have enteritis as the most severe pathologic finding. The lesion was limited to the ileum, ceca, and large intestine. We were unable to attribute a single infectious etiology to this lesion. Many birds with enteritis also exhibited renal lesions similar to those encountered in chickens experimentally deprived of water. We propose that enteritis is a significant cause of mortality in Laysan albatross chicks on Midway and that it may be a sequela to dehydration. It is likely that the pathology of dehydration in Laysan albatross differs from that in chickens largely because of diet.

RESUMEN. Enteritis necrosante como causa de mortalidad en albatroses de Laysán jóvenes (Diomedea inmutabilis) en Atoll, Hawaii.

Una encuesta de las necropsias realizadas en albatros de Laysán (*Diomedea inmutabilis*) en Atoll, Hawaii, durante el mes de Junio de los años 1993, 1994 y 1995, reveló un 54% (21/39), 67% (49/71) y 93% (15/16) respectivamente, de enteritis como el hecho patológico más importante. La lesión estaba limitada al íleo, ciego e intestino grueso. No se pudo atribuir una sóla etiología a la lesión. Muchas aves con enteritis también mostraron lesiones renales similares a las encontradas en pollos deshidratados. Proponemos que la enteritis es una causa significante de mortalidad en albatros de Laysán y que puede ser secuela de deshidratación. Es posible que la patología de la deshidratación del albatros de Laysán sea diferente a la de los pollos, principalmente debido a la dieta.

Key words: Laysan albatross, pathology, epizootiology

Midway Atoll contains the largest breeding colony of Laysan albatross in the world with *ca.* 300,000 breeding pairs. Breeding in this species is highly synchronous (12). Adults arrive annually from open sea in October–November to lay eggs. Chicks hatch in January–February and depend solely on adults for food. Chicks begin fledging in June–July, whereupon morbidity can be substantial with 1000+ chicks dying per day.

Previous mortality surveys in albatross chicks on Midway Atoll implicated lead poisoning (21) and dehydration (22) as the major causes of chick morbidity and mortality. Sileo *et al.* (22) based their diagnosis of dehydration on gross findings, clinical response of birds to oral rehydration, and presence of microscopic renal lesions interpreted as sequelae to dehydration.

Yet, dehydration in birds is typically an antemortem diagnosis obtained through examination of hematology, clinical chemistry, weight loss, and clinical signs such as skin turgor, capillary refill time, and clinical demeanor (1). Of the 16 chicks that Sileo *et al.* (22) supplemented with fluids, five were suspected of having lead poisoning, and of an additional six that died, three were diagnosed with aspergillosis or lead poisoning and two of the remaining three had potentially fatal chigger infestations. Without further clues as to its pathophysiology, diagnosing dehydration in seabirds based on pathology alone may be problematic.

Dehydration is a symptom of either inadequate fluid intake or excessive fluid loss. Sileo *et al.* (22) suggested that reduced parental feeding led to albatross chicks becoming dehydrated but offered no data to confirm this. During necropsy surveys of near-fledging Laysan albatross chicks in 1994–95, we encountered a lesion that could partly explain why some Laysan albatross chicks appear dehydrated on Midway.

MATERIALS AND METHODS

Midway Atoll comprises three islands, the largest of which is Sand Island (22). Albatross chick carcasses were collected throughout Sand Island and necropsied during 1 wk each in June 1993, 1994, and 1995. At this time of year, chicks are approximately the same age because of the synchronous breeding history of the species (12). Thirty-nine chicks were necropsied in 1993, 71 in 1994, and 16 in 1995. Only freshly dead chicks were selected for necropsy and were judged as such based on appearance of eyes, mucous membranes, and lack of fly larvae on mucous membranes.

Necropsies comprised weighing birds to the nearest 0.1 kg using a spring scale and performing a systematic external and internal examination of organ systems and stomach contents. Tissues from all chicks were examined microscopically, including lung, kidney, gonad, adrenal, liver, heart, spleen, pancreas, cerebrum, cerebellum, esophagus, proventriculus, small and large intestine, cecum, bursa, thyroid, parathyroid, peripheral nerve, air sac, and skin. Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, cut into 5-μm sections, and stained with hematoxylin and eosin. Grocott's methenamine silver and periodic acid–Schiff were used to confirm fungi, Gram stain to classify bacteria as gram positive or negative, and alcian blue to detect sulfated mucosubstances (19).

Livers from all birds were frozen separately in sealed plastic bags at −20 C and analyzed for lead (μg/g [w/w]) at the National Wildlife Health Center in Madison, WI (9). Limit of detection for liver lead was 0.25 μg/g. We analyzed for lead because lead poisoning in albatross has been documented by others (21). In 1995, intestines from 10 birds were saved in selenite broth that was held refrigerated for 1 wk. The broth was then plated onto blood and XLT-4 agar and incubated at 37 C for 24 hr at the National Wildlife Health Center. Isolates of *Salmonella* were typed at National Veterinary Services Laboratory in Ames, IA (8). Small intestines and ceca from five chicks were stored in sealed plastic bags at −20 C for 2 wk prior to virus isolation in cell culture (6) and embryonated hens' eggs (20).

Table 1. Number of Laysan albatross chicks in each diagnostic category.

Category	Number of chicks		
	1993	1994	1995
Enteritis	21	49	15
Lead	8	6	1
Trauma	2	3	0
Undetermined	8	14	0

Animals were grouped into three diagnostic categories according to the most severe gross and/or microscopic lesion. Birds with liver lead levels ≥7 μg/g were considered lead poisoned (7). Birds with histologic evidence of moderate to severe necrotizing enteritis and typhlitis were classified as enteritis. Birds with gross evidence of trauma were classified as trauma. We had a final category of undetermined for birds for which we could not reach a diagnosis. Weights for birds in the lead, trauma, and enteritis categories were pooled for all years, data evaluated for normality, and the three categories compared using ANOVA (5). Alpha was 0.05.

RESULTS

The most common pathologic diagnosis for all years was enteritis (Table 1). Most birds with enteritis were in good to excellent flesh. The most consistent gross lesion was presence of moderate amounts of inspissated material in the distal small intestine and ceca and occasional urate calculi in the cloaca. On microscopy, all birds had moderate to severe necrotizing enteritis generally limited to the distal small intestine and ceca (Fig. 1A). The lesion was characterized by large intraluminal amorphous cores of eosinophilic cellular debris and ingesta mixed with clumps of short gram-negative rods and gram-positive coccoid bacteria. The superficial mucosa was diffusely necrotic with clumps of bacteria and often accompanied by a prominent lymphoplasmacytic infiltrate within the lamina propria that occasionally extended focally into the underlying muscularis (Fig. 1B). Inclusions were not noted and protozoa were rarely seen. No virus was isolated from intestines of five birds with this lesion. Of 10 birds tested, four were positive for *Salmonella*, one for *Salmonella ohio*, one for *Salmonella oranienburg*, and two for *Salmonella san-diego*.

Other significant microscopic lesions in birds with enteritis included splenic lymphoid deple-

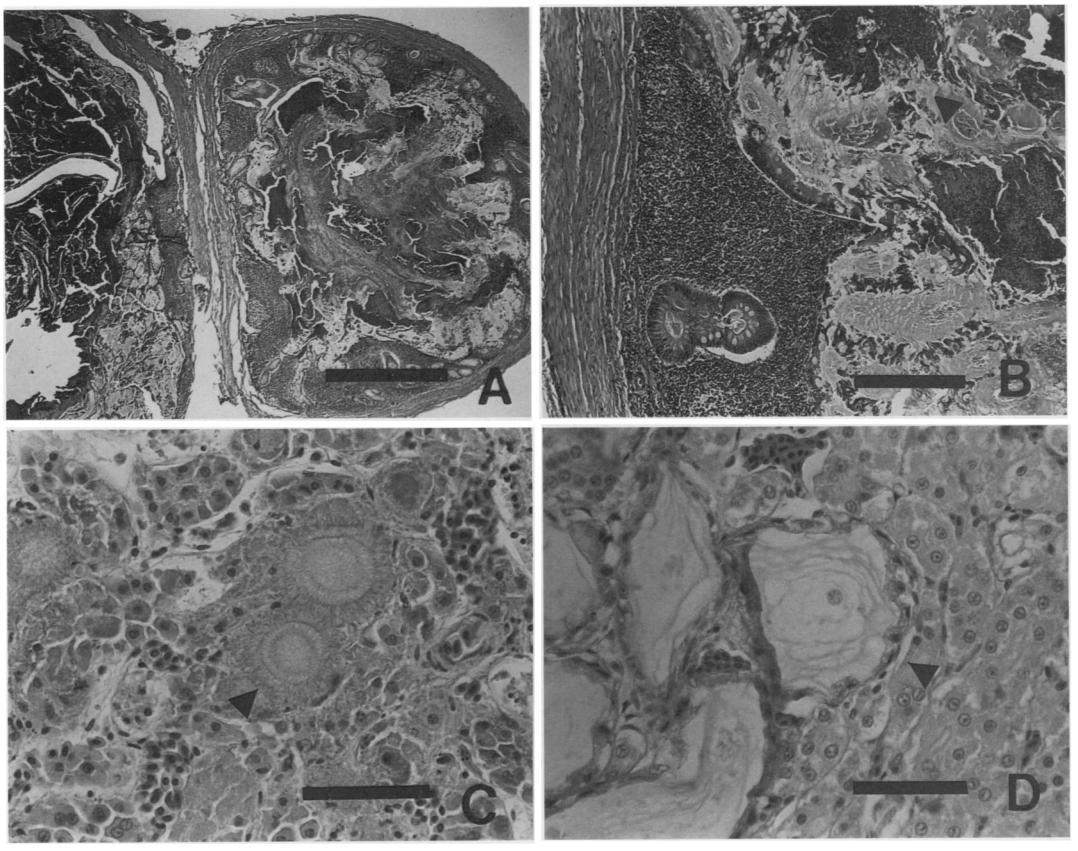

Fig. 1. (A) Small intestine and attached ceca—note diffuse necrosis of superficial mucosa on left. Bar = 1000 μm. (B) Lamina propria of small intestine—note diffuse lymphoplasmacytic infiltrate on left and necrosis of superficial mucosa on right. Bar = 200 μm. (C) Kidney—note stellate urate crystals within necrotic proximal tubules. Bar = 70 μm. (D) Kidney—note distal tubules distended with mucus concretions surrounded by flattened tubule cells. Bar = 50 μm.

tion and, less often, reticuloendothelial hyperplasia. The most common renal lesions were of two types: distention of proximal and distal tubule lumen with stellate crystals surrounded by necrotic cell debris and occasional granulocytes (Fig. 1C), and distention of distal tubules and collecting ducts with grey wispy material that stained blue with Alcian blue and was surrounded by flattened, hypereosinophilic tubule cells with pyknotic nuclei (Fig. 1D). Glomerulonephritis characterized by swollen hypercellular glomeruli was noted in five birds. Four birds with enteritis had bacteremias characterized by multiple foci of necrosis in the liver, heart, and lung accompanied by gram-negative bacteria. Cultures were not done. One bird had a granulomatous pneumonia associated with an unidentified fungus, and another had a severe diffuse suppurative encephalitis limited to the cerebellum and accompanied by mild pulmonary edema. Incidental lesions included unidentified gravid female trematodes distending distal collecting ducts of the kidneys, hepatocellular atrophy, and, rarely, mild focal necrosis of the liver, spleen, and adrenal. One bird had severe acute necrosis of the myocardium.

Most birds with lead poisoning appeared in good to excellent flesh and were grossly unremarkable except for two birds with petechiae on the dorsal lungs. On microscopy, we saw moderate to severe splenic lymphoid depletion and occasional reticuloendothelial hyperplasia. Six birds had mild to severe necrotizing enteritis and mild to severe nephrosis. Hepatocellular atrophy was less commonly seen. One animal had multifocal and perivascular hemorrhages ac-

companied by focal necrosis in the cerebrum and brain stem. Liver lead levels in this group ranged from 7.74 to 70.5 μg/g.

Traumatized birds had multiple fractures of the skull, synsacrum, or limbs accompanied by bruising of breast muscles and internal hemorrhage. No other lesion was noted. Birds in the trauma category (2.9 ± 0.6 kg) weighed significantly ($P < 0.05$) more than those in either the enteritis (1.8 ± 0.4 kg) or lead poisoning (1.8 ± 0.4 kg) categories.

DISCUSSION

The high prevalence of enteritis in albatross during all 3 yr was not documented in other studies (22). Like us, Sileo *et al.* (22) noted the gross presence of inspissated feces in the distal intestines of birds they classified as dehydrated; however, the gut lesions were not examined histologically. It is likely that our pathologic diagnosis of enteritis is analogous to Sileo *et al.*'s (22) diagnosis of dehydration. We suspected that necrotizing enteritis was a primary lesion that led to the birds' inability to reabsorb water through the gut, thereby providing a potential mechanism to explain Sileo *et al.*'s (22) suspicions of dehydration.

Failure to detect viral inclusions or protozoa on microscopy does not exclude the possibility that viruses could have initiated primary lesions subsequently invaded by bacteria, yet data in this study did not reveal such evidence. Tissue culture failed to reveal viruses. Although some viruses may not have persisted long enough in a hostile enteric environment during 2 wk storage at −20 C, we judge that the limited extent of the lesion (ileum, cecum, large intestine) was not compatible with a systemic enteric viral infection.

The morphology of the bacteria seen in enteritis lesions and pattern of mucosal necrosis make it unlikely that clostridia were responsible for the lesion (2,10). Small gram-negative rods associated with mucosal necrosis in many cases of enteritis implicated *Salmonella* or *Escherichia coli*. However, in poultry, colibacillosis generally causes multiorgan inflammation, such as airsacculitis, pericarditis, and perihepatitis (11), something not usually observed in albatross chicks with enteritis. Although the gross and microscopic lesions in enteritis birds were compatible with salmonellosis (15), our isolation of multiple types of *Salmonella* from only 4 of 10 birds makes this diagnosis suspect. Our inability to isolate *Salmonella* from all birds with enteritis may have been caused by the 1-wk storage in selenite broth. Logistics of travel to and from Midway prevented any earlier laboratory tests.

Although Bierer *et al.* (3) observed proventriculitis in chickens experimentally deprived of water, neither they nor other investigators (23) recorded intestinal lesions. Many albatross with enteritis had renal lesions suggestive of dehydration. Some of these (Fig. 1C) were similar to lesions seen by Sileo *et al.* (22), whereas others (Fig. 1D) were more typical of those noted by Onderka *et al.* (18), who described dilated collecting ducts with mucin concretions in dehydrated immature chickens. Renal lesions seen in Laysan albatross were somewhat more severe than those observed by Swayne and Radin (23) in kidneys of chickens that underwent water deprivation.

Because of the lack of evidence of a primary inciting cause of necrotizing enteritis in albatross, dehydration must be considered a possibility. Birds with necrotizing enteritis were significantly lighter than traumatized but otherwise healthy birds. Weight loss is a clinical sign of dehydration (1). A plausible scenario is that loss of water resulting from evaporative cooling or inadequate food intake in Laysan albatross chicks leads to accumulation of urates and urate concretions in the urodeum and kidney. Colonic reflux into the ceca and ileum (13) along with prolonged contact of acidic urates with intestinal mucosa lead to tissue necrosis, inability to reabsorb water, and exacerbation of dehydration. The difference in lesions between seabirds and chickens may lie with the protein-rich diet of seabirds, which would presumably lead to relatively higher urate production in this group relative to granivorous birds.

Our findings of lead as the second most common cause of death in albatross chicks were similar to those of Sileo *et al.* (22). Like others (21), we were unable to demonstrate some of the characteristic pathology of lead poisoning seen in other avian species, such as emaciation, myocardial degeneration and necrosis, fibrinoid necrosis of vessels of multiple organs, and cerebral and cerebellar hemorrhages accompanied by peripheral nerve demyelination and inflammation (4,14,16,17). Presence of necrotizing

enteritis in some lead poisoned birds suggests that these birds, too, may become dehydrated, a supposition supported by the almost identical mean weights between both lead poisoned and necrotizing enteritis birds.

REFERENCES

1. Abou-Madi, N., and G. V. Kollias. Avian fluid therapy. In: Current veterinary therapy, vol. 6, small animal practice. R. W. Kirk and J. D. Bonagura, eds. W. B. Saunders Company, Philadelphia, PA. pp. 1154–1159. 1992.

2. Berkhoff, H. A. Ulcerative enteritis (quail disease). In: Diseases of poultry. B. W. Calnek, H. J. Barnes, C. W. Beard, W. M. Reid, and H. W. Yoder, eds. Iowa State University Press, Ames, IA. pp. 257–264. 1991.

3. Bierer, B. W., W. T. Carll, and T. H. Eleazer. A comparison of the survival time and gross pathology in producing and nonproducing white leghorn hens deprived of water. Poult. Sci. 45:65–67. 1965.

4. Cook, R. S., and D. O. Trainer. Experimental lead poisoning of Canada geese. J. Wildl. Manag. 30: 1–7. 1966.

5. Daniel, W. W. Biostatistics: a foundation for analysis in the health sciences. John Wiley and Sons, New York. 1987.

6. Docherty, D. E., and P. G. Slota. Use of Muscovy duck embryo fibroblasts for the isolation of viruses from wild birds. J. Tissue Cult. Methods 11: 165–170. 1977.

7. Eisler, R. Lead hazards to fish, wildlife, and invertebrates: a synoptic review. United States Fish and Wildlife Service, Washington, DC. Biological Report 75(1.14). 1977.

8. Ewing, W. H., and P. R. Edwards. Identification of Enterobacteriaceae. Elsevier, New York. 1976.

9. Fernandez, F. J., and D. Hilligoss. An improved graphite furnace method for the determination of lead in blood using matrix modification and the L'vov platform. At. Spectrosc. 3:130–131. 1972.

10. Ficken, M. D. Necrotic enteritis. In: Diseases of poultry. B. W. Calnek, H. J. Barnes, C. W. Beard, W. M. Reid, and H. W. Yoder, eds. Iowa State University Press, Ames, IA. pp. 138–144. 1991.

11. Gross, W. B. Colibacillosis. In: Diseases of poultry. B. W. Calnek, H. J. Barnes, C. W. Beard, W. M. Reid, and H. W. Yoder, eds. Iowa State University Press, Ames, IA. pp. 264–267. 1991.

12. Harrison, G. S. Seabirds of Hawaii. Natural history and conservation. Cornell University Press, New York. 1990.

13. Hill, K. J. Physiology of the digestive tract. In: Physiology and biochemistry of the domestic fowl, vol. 4. B. M. Freeman, ed. Academic Press, New York. pp. 31–49. 1983.

14. Hunter, B., and G. Wobeser. Encephalopathy and peripheral neuropathy in lead-poisoned mallard ducks. Avian Dis. 24:169–177. 1979.

15. Nagaraja, K. V., B. S. Pomeroy, and J. E. Williams. Paratyphoid infections. In: Diseases of poultry. B. W. Calnek, H. J. Barnes, C. W. Beard, W. M. Reid, and H. W. Yoder, eds. Iowa State University Press, Ames, IA. pp. 99–129. 1991.

16. Ochiai, K., K. Jin, M. Goryo, T. Tsuzuki, and C. Itakura. Pathomorphologic findings of lead poisoning in white-fronted geese. Vet. Pathol. 30:522–527. 1992.

17. Ochiai, K., K. Jin, C. Itakura, M. Goryo, K. Yamashita, N. Mizuno, T. Fujinaga, and T. Tsuzuki. Pathological study of lead poisoning in whooper swans (Cygnus cygnus) in Japan. Avian Dis. 36:313–323. 1992.

18. Onderka, D. K., J. A. Hanson, F. L. Leggett, and L. D. Armstrong. Renal pathology in chicks following water deprivation. Avian Dis. 31:735–739. 1977.

19. Prophet, E. B., B. Mills, J. B. Arrington, and L. H. Sobin. Laboratory methods in histotechnology. Armed Forces Institute of Pathology, Washington, DC. 1992.

20. Senne, D. A. Virus propagation in embryonating eggs. In: A laboratory manual for the isolation and identification of avian pathogens, 3rd ed. H. G. Purchase, L. H. Arp, C. H. Domermuth, and J. E. Pearson, eds. American Association of Avian Pathologists, Kennett Square, PA. pp. 176–181. 1979.

21. Sileo, L., and S. I. Fefer. Paint chip poisoning of Laysan albatross at Midway Atoll. J. Wildl. Dis. 23:432–437. 1977.

22. Sileo, L., P. R. Sievert, and M. D. Samuel. 1990. Causes of mortality of albatross chicks at Midway Atoll. J. Wildl. Dis. 26:329–337. 1990.

23. Swayne, D. E., and M. J. Radin. The pathophysiological effects of water and feed restriction in chickens. Avian Pathol. 20:649–661. 1991.

ACKNOWLEDGMENTS

We are grateful to Mike Nishimoto, William Iko, Bob Dusek, Lawrence Ames, Bob Rameyer, and Allison Beale, who provided field assistance on Midway, and Ken McDermond for permission to work on the Northwestern Hawaiian Islands National Wildlife Refuge. Thanks also to Doug Docherty for virus isolation and Lou Sileo and Carol Meteyer for providing second opinions on some of the histology. David Swayne, H. L. Shivaprasad, J. Christian Franson, Lou Sileo, and anonymous reviewers kindly provided constructive comments on this manuscript. The State of Hawaii Department of Agriculture Veterinary Branch's gracious loan of laboratory space to the Honolulu Field Station made much of this work possible.