ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 3 (3): 206-210, 2004

An *Escherichia Coli* Epizootic in Captive Mallards (*Anas platyrhynchos*)

Debra L. Miller[1]*, Josh Hatkin[2], Zaher A. Radi[1], Michael J. Mauel[3]
[1]Veterinary Diagnostic and Investigational Laboratory, University of Georgia, Tifton, Georgia 31793, USA
[2]B1600 Westfield Ct. Albany, Georgia 31721, USA
[3]Aquatic Diagnostic Laboratory, Mississippi State University, Stoneville, Mississippi 38776, USA
E-mail: dmiller@tifton.uga.edu

Abstract: Captive mallard (*Anas platyrhynchos*) facilities are a common source of birds for game preserves. An outbreak of *Escherichia coli* septicemia in 2 and 8-wk-old ducklings was diagnosed in a captive mallard facility in South Georgia. Additionally, fungal hyphae were observed in the 2-wk-old birds but not the 8-wk-old birds. Ducklings were overcrowded in both the brooding facility (2-wk-old birds) and within the flight enclosures. The single small pond within the flight enclosure was contaminated with *E. coli*. Although the stress of captivity may have contributed to the ducklings' deaths, poor husbandry was the primary factor in these birds' deaths.

Key words: Aspergillosis, captive mallards, husbandry, *E. coli*, water quality

Introduction

Game preserves often raise their own game species or obtain them from captive facilities. Mallard preserves are common in the Southeast and captive mallard facilities are often used for stocking preserves. Congregating wild species can result in disease problems that are often associated with poor husbandry.

Escherichia coli and *Aspergillus* spp. are opportunistic organisms that most often affect individuals with compromised immune systems. Both organisms are potentially deadly to avian species and tend to occur secondary to other agents (i.e., viral or *Mycoplasma* spp.) or poor environmental conditions (i.e., poor air quality or overstocking) (Carrasco *et al*., 2001; Cork *et al*., 1999; Ditrich *et al*., 1979; Dyar *et al*., 1984; Fallacara *et al*., 2001; Ficken, 1996; Burr, 1981; Zinkl *et al*., 1974). *E. coli* is a common cause of septicemia in many avian species (Barnes and Gross, 1997; Gerlach, 1994). Multiple organ systems may be affected including the respiratory, gastrointestinal and nervous systems. The predominant target organs for *Aspergillus* spp. are the lung, brain, and eye (Dyar *et al*., 1984). Clinical signs and gross lesions are dependent on the organ system(s) affected.

Materials and Methods

A mallard producer in South Georgia operated an "all in-all out" captive mallard facility. In brief, ducklings (N = 2000) were purchased from a commercial supplier at 1-day of age and confined to an approximately 256 m^2 (0.13 m^2 per duckling) poorly ventilated metal building until 2 wks of age. Wheat straw was used for litter. All ducklings were fed a commercial medicated (Amprolium) starter duck food (Southern States, Richmond, VA) until 4 wks of age. At 4 wks, the feed was changed to a mallard conditioner (Southern States, Richmond, VA) containing direct fed microbials (Primalac, Star Labs, St. Joseph, MO). Feed was provided *ad libitum*.

At 2 wks of age, ducklings were allowed free access between the building and the outdoor enclosure (approximately 0.20 ha). Another facility normally housed the older (8-wk-old) ducklings but, in September 2002, this other facility was under repair and all birds were housed at one facility (negating the "all in-all out" system). Therefore, the outdoor enclosure contained at least 1000 8-wk-old ducklings at the time that the 2-wk old ducklings emerged from the building. The enclosure included a pond that was approximately 3m^2 with a maximum depth of about 15 cm (Fig. 1). This pond served as the sole source of drinking water for all the ducks. A small earthen dam was used to control the water level. Filtration or aeration systems were not used. Mortality began to increase shortly after the 2-wk old ducklings were allowed outside. Deaths rose from 2-3 ducklings per wk to about 5 ducklings per day. Both age groups were affected. As a precaution, the producer moved approximately 700 of the 8-wk-old ducklings to the site that was under repair.

Clinically, the birds were lethargic with opisthotonos, quivering, "wing-walking", vocalizing and convulsing. Convulsions lasted about 30-40 seconds and recurred at irregular intervals. Complete necropsies were performed on a total of 18 birds collected from both age-groups.

Gross examination of the 2-wk-old ducklings revealed lesions that were primarily confined to the air sacs, spleen, lungs, liver and heart. The air sacs and the serosal surface of the livers were generally cloudy to opaque. Fibrinous exudates and white/yellow plaques (2-4 mm diameter) were present throughout (Fig. 2). The lungs of all birds were turgid and wet and, in the 2-wk-

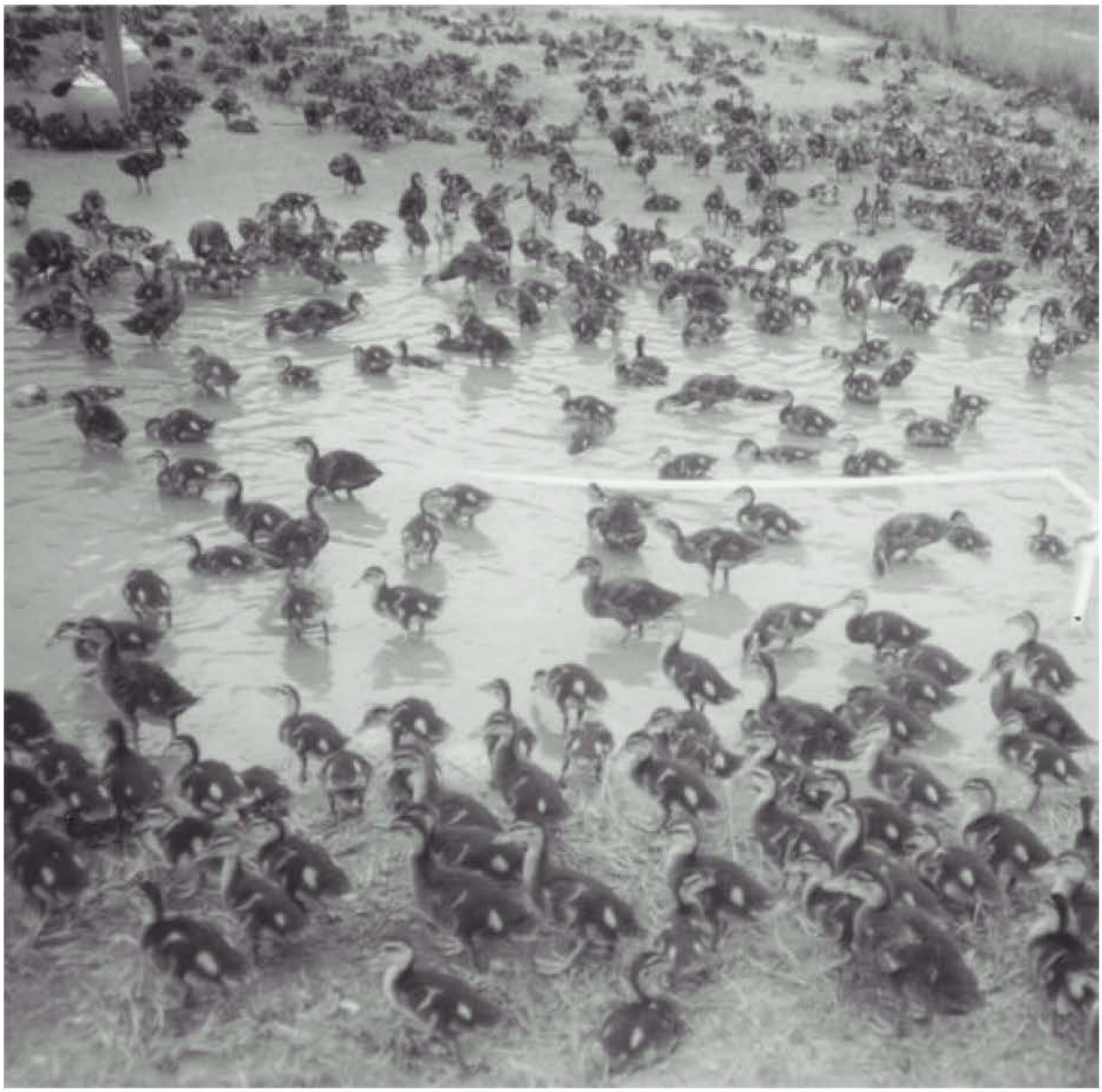

Fig. 1: Captive mallard facility in South Georgia. The enclosure at this facility contained approximately 1000 8-wk-old ducklings and approximately 2000 2-wk-old ducklings at the time of the *Escherichia coli* epizootic. This enclosure included a pond that was approximately 3m^2 with a maximum depth of about 15 cm. This pond served as the sole source of drinking water for all the ducks

old ducklings, contained occasional plaques similar to those found on the air sacs. The 8-wk-old birds had approximately 10 ml of ascitic fluid within the body cavity. In both age groups, heart base fat was absent and scattered irregular white foci were present at the apex of the heart and in the ventricular and atrial walls. The blood was watery. Two 8-wk-old birds had enlarged and mottled red/tan spleens. No lesions were present on the intestinal mucosa. Sections of all tissues were collected, fixed in 10% formalin, paraffin-embedded, sectioned at approximately 5 µm and stained with hematoxylin and eosin for examination with light microscopy. Selected tissues were also stained with Brown and Brenn tissue gram stain, Periodic Acid -Schiff and for Acid Fast organisms. Air sacs, lungs and myocardium were cultured aerobically on Tripticase-Soy Agar with 5% added sheep blood and MacConkey agar. Bacteria were identified with the bioMerieux API 20E

system (bioMerieux, Hazelwood, MO). Finally, cloacal and pharyngeal swabs were collected from 20 additional birds (five pools of four birds each) for viral testing by Directagen Flu A (Becton-Dickinson, Sparks, MD).

Results

Light microscopic examination of the birds lungs revealed numerous intra and extracellular Gram negative rod-shaped bacteria (Fig. 3). Multi focal to coalescing granulomas with central cores of fungal hyphae and conidiophores (Fig. 4) were present in the lungs from the 2-wk-old ducklings The segmented fungal hyphae were 3-5 µm in diameter and had dichotomous branching. Cell walls were parallel. Such features are consistent with *Aspergillus* spp. There were also severe zonal fibrinoheterophilic and peribronchial lymphocytic infiltrates, both of which appeared unrelated to the fungal granulomatous pneumonia. Acid-fast stains were negative.

There was severe zonal to diffuse fibrinoid hepatic necrosis in all birds. Some of the necrotic areas contained densely packed colonies of Gram-negative rods, degenerate and necrotic hepatocytes and scattered inflammatory (granulocytic) cells. Multiple hepatic sinusoids contained micro thrombi and granulocytes (many of which were degranulated). The Disse's space was widened in Multi focal areas. There were Multi focal hepatic perivascular infiltrates containing lymphocytes, plasma cells and granulocytes. Within all birds, the myocardium contained severe Multi focal to zonal lymphoplasmacytic infiltration and moderate to severe focal to zonal fragmentation of myocardial fibers. Gram-negative bacterial rods were observed within numerous capillaries. Densely packed colonies of these bacteria extended perivascularly into the myocardium.

Large numbers of Gram-negative bacterial rods were in the spleens of all birds. Severe diffuse lymphoid depletion was present throughout the spleens. Similarly, severe diffuse fibrinoid necrosis and large numbers of Gram-negative bacterial rods were seen within the intestinal lamina propria and capillaries of the intestinal muscularis of all birds. Distal renal tubules of all birds were degenerate and necrotic. Many contained a few bacterial rods. Multiple collecting tubules were metaplastic. Mild granulocytic infiltration in the cerebellar meningeal membranes of all birds was noted, particularly between the foliae, and small numbers of Gram-negative bacterial rods were invading the submeningeal hemispheric neuropile. Few brain capillaries had micro thrombi.

Laboratory test results revealed multiple etiologic agents. All bacterial plates yielded pure cultures of *E. coli*. Colonies were too numerous to count. No influenza virus was detected from pharyngeal or cloacal swabs.

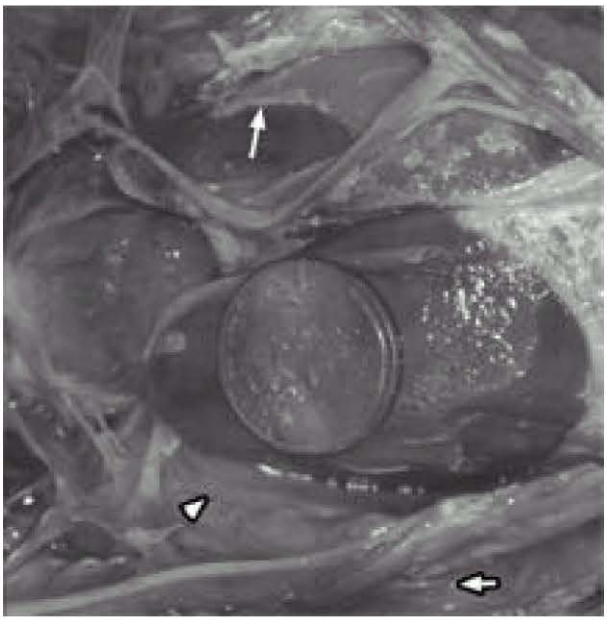

Fig. 2: Gross view of 2 and 8-wk-old ducklings from the *Escherichia coli* epizootic in South Georgia. The air sacs and the serosal surface of the livers were generally cloudy to opaque (long arrow). Fibrinous exudates and white/yellow plaques (short arrow) and neovascularization (arrowhead) were present throughout

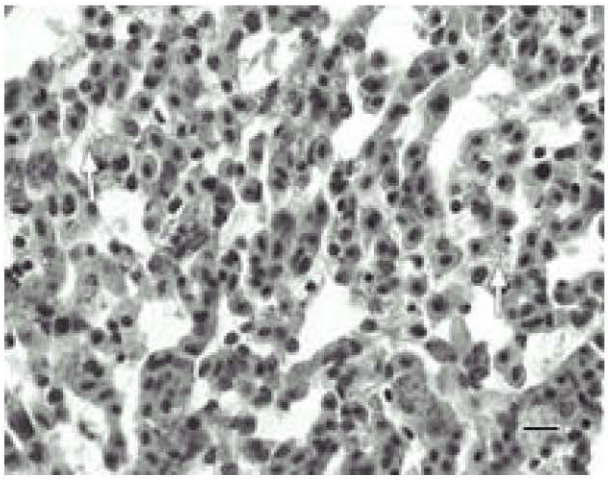

Fig. 3: Light microscopic view of the lungs from the 2 and 8-wk-old duckling from the *Escherichia coli* epizootic in South Georgia illustrating the numerous intra and extracellular Gram negative rod-shaped bacteria (arrows). Bar = 10 µm

Discussion

Death of these birds was due to septicemia/bacteremia. *Escherichia coli*, type 1 was the etiologic agent. The ducks were overcrowded in the small pond in which

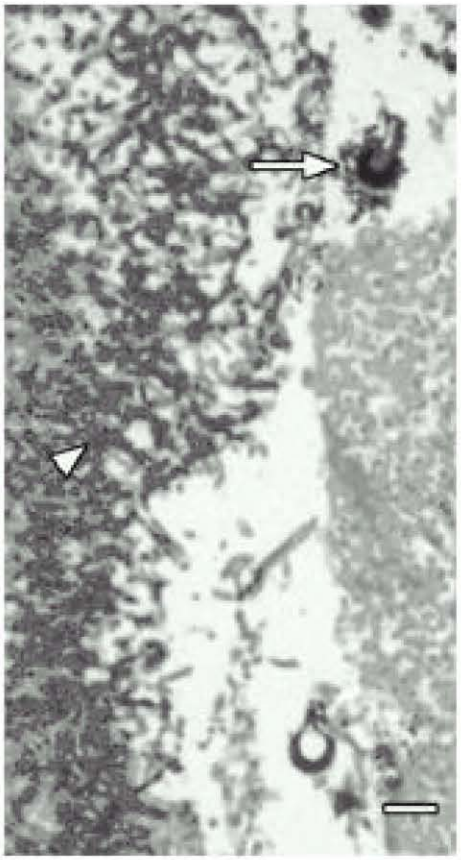

Fig. 4: Light microscopic view of the lungs from the 2-wk-old duckling from the *Escherichia coli* epizootic in South Georgia illustrating fungal hyphae (arrowhead) and conidiophores (arrow). Bar = 15 µm

drainage and fresh water were limited. *E. coli* has been reported as the most common bacterial agent isolated from free-living waterfowl (composing 78% of the bacteria isolated in mallards) and water contamination from dense congregations of waterfowl species has been suspected to be a source for human exposure (Fallacara *et al.*, 2001; Samadpoour *et al.*, 2002).

For wild species, captivity often serves as a stressor that results in susceptibility of the species to opportunistic agents (Fatunmbi and Bankole, 1984; Kocan and Perry, 1976). *E. coli* was reported by as one of several bacterial agents isolated from wild-trapped canvasback ducks that died while in captivity (Kocan and Perry, 1976). The primary factor in the deaths of the canvasbacks in Kocan and Perry's study was determined to be the stress of captivity. Although the stress of captivity may have contributed to the susceptibility of the mallard ducklings to disease, poor husbandry was most likely the major factor given the high levels of water contamination with *E. coli* and because the ducklings were hatched in captivity.

The fungal hyphea observed in the 2-wk-old birds was probably a secondary (and compounding) factor due to poor air quality in the building they inhabited prior to being released into the flight cage. Aspergillosis has been sporadically reported in multiple free-ranging avian species and, although it may be attributed to an initial debilitating/immunocompromizing insult, it is often the only infectious agent identified (Burr, 1981; Carrasco *et* al., 2001; Cork *et al.*, 1999; Ditrich *et al.*, 1979; Locke *et al.*, 1969; Redig *et al.*, 1980; Zinkl *et al.*, 1974). For the 2-wk-old mallard ducklings, the straw litter may have been a contributing factor as the dust created a medium upon which *E. coli* and *Aspergillus* spores could attach. Inhalation of the aerosolized dust, created by the ducklings' movements, likely resulted in the introduction of the bacteria and fungal spores into the air sacs.

In summary, good husbandry is a key factor in managing captive-born mallards. Overcrowding, with subsequent gross fecal contamination and poor ventilation were the primary factors in these birds deaths. Further, water contamination of *E. coli* can pose a potential threat of human exposure to the facility caretakers and possibly to humans and animals downstream from the facility. Additionally, the potential use of subclinically infected birds as game could potentially affect consumers (hunters and their families) either during the cleaning and cooking process or if the meat is undercooked. This case study highlights the importance of good husbandry in facilities that raise captive mallards for game preserves.

Acknowledgements

We wish to thank the staff of the Georgia Poultry Laboratory in Camilla, Georgia and the Tifton Veterinary Diagnostic and Investigational Laboratory for their help in analyzing the samples.

References

Barnes, H.J. and W.B. Gross, 1997. Colibacillosis. In: Calnek, B.W., Barnes, H.J., Beard, C.W., McDougald, L.R. and Saif, Y.M. (EDs.), Diseases of poultry, 10th ed. Iowa State University Press, Ames, Iowa, pp: 131-142.

Burr, E.W., 1981. Enzootic aspergillosis in wild red vented cockatoos in the Philippines. Mycopathologia, 73: 21-28.

Carrasco, L., J.S. Lima, Jr., D.C. Halfen, F.J. Salguero, P. Sanchez-Cordon and G. Becker, 2001. Systemic aspergillosis in an oiled magellanic penguin (Spheniscus magellanicus). J. Vet. Med. B Infect. Dis. Vet. Public Health, 48: 551-554.

Cork, S.C., M.R. Alley, A.C. Johnstone and P.H. Stockdale, 1999. Aspergillosis and other causes of mortality in the stitchbird in New Zealand. J. Wildl. Dis., 35: 481-486.

Ditrich, O., M. Otcenasek, M. Velesky and P. Koubek, 1979. Disseminated aspergillosis in a loon. Vet. Med. (Praha), 24: 633-639.

Dyar, P.M., O.J. Fletcher and R.K. Page, 1984. Aspergillosis in turkeys associated with use of contaminated litter. Avian Dis., 28: 250-255.

Fallacara, D.M., C. M. Nonahan, T.Y. Morishita and R.F. Wack, 2001. Fecal shedding and antimicrobial susceptibility of selected bacterial pathogens and a survey of intestinal parasites in free-living waterfowl. Avian Dis., 45: 128-135.

Fatunmbi, O.O. and A. Bankole, 1984. Severes disseminated aspergillosis in a captive Abyssinian tawny eagle (Aquila rapax raptor). J. Wildl. Dis., 20: 52-54.

Ficken, M.D., 1996. Respiratory System. In: Riddell, C. (ED), Avian Histopathology, 2nd ed. American Assoc. Avian Pathologists, Kennett Square, Pennsylvania, pp: 89-109.

Gerlach, H.B., 1994. In: Ritchie, B.W., Harrison, G.J. and Harrison, L.R. (EDs). Avian Medicine: Principles and Application. Wingers Publishing Co., Lake Worth, Florida, pp: 943-983.

Kocan, R.M. and M.C. Perry, 1976. Infection and mortality in captive wild-trapped canvasback ducks. J. Wildl. Dis., 12: 30-33.

Locke, L.N., G.E. Bagley, D.N. Frickie and L.T. Young, 1969. Lead poisoning and aspergillosis in an Andean Condor. J. Am. Vet. Med. Assoc., 155: 1052-1056.

Redig, P.T, M.R. Fuller and D.L. Evans, 1980. Prevalence of Aspergillus fumigatus in free living goshauks (Accipiter gentiles atricapillus). J. Wildl. Dis., 16: 169-174.

Samadpoour, M., J. Stewart, K. Steingart, C. Addy, J. Louderback, M. McGinn, J. Ellington and T. Newman, 2002. Laboratory investigation of an *E.coli* O157:H7 outbreak associated with swimming in Battle Ground Lake, Vancouver, Washington. J. Environ. Health, 64: 16-20.

Zinkl, J.G., J.M. Hyland and J.J. Hurt, 1974. Aspergillosis in common crows in Nebraska. J. Wildl. Dis., 13: 191-193.