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Necrotic enteritis in a captive mute swan (*Cygnus olor*) in the breeding season

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Abstract

A captive mute swan (*Cygnus olor*) in the brooding period was found dead without any specific signs. On necropsy, the entire digestive tract was severely necrotized with loosely adherent pseudomembranes, and *Clostridium perfringens* (CP) colonies isolated from the intestinal contents were identified as CP type A by polymerase chain reaction. Microscopically, the intestine and the liver were necrotized and intralesional bacterial clusters were observed. In this case, there were no other mortalities, so the condition of a single bird, in particular, stress from incubation effort, could act as the predisposing factor for necrotic enteritis. In this study, we report a unique case of necrotic enteritis associated with incubation in a captive mute swan.

Key words : *Cygnus olor*, Incubation effort, Mute swan, Necrotic enteritis, Stress factor

INTRODUCTION

Necrotic enteritis (NE) is a clinically dramatic enteric disease afflicting birds which is characterized by an intestinal necrotic fibrinous pseudomembrane with a "Turkish towel" appearance (Opengart and Songer, 2013). NE occurs in the presence of predisposing factors, such as feed ingredients, stress or intestinal parasites (Timbermont et al, 2011) that cause a microbial imbalance favorable for the overgrowth of the causal agent, *Clostridium perfringens* (CP).

There have been several reported outbreaks of NE in various wild waterfowl species including ducks, geese, and swans (Neimanis and Speck, 2012). In swans, NE is usually reported in winter when food shortages occur, and starvation or changes in diet are known to be predispose to NE factors (Duff et al, 2011; Neimanis and Speck, 2012). Diseases occurring in winter can also in-

fluence bird populations, in that health status over winter can affect breeding success the following spring (Drent et al, 2006), as well as through direct mortality effects.

In terms of breeding and management, various diseases afflicting mute swans have been reported (Batt et al, 1992). In particular, reproduction-related behavior, including incubation effort, can induce immune suppression and stress, which can make birds vulnerable to disease (Hanssen et al, 2005). In this study, NE occurrence associated with brooding behavior is reported in a captive mute swan. This report has implications for the management of many known diseases because successful reproduction is critical for population stability in both wild and captive mute swans.

CASE REPORT

In Cheong-ju zoo, a female mute swan (*Cygnus olor*)

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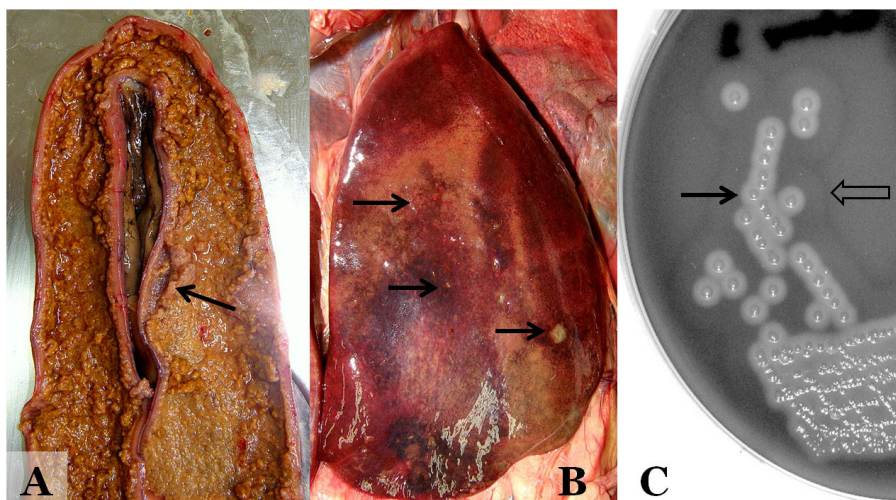


Fig. 1. Gross lesions found in the intestine and liver of a mute swan that died from necrotic enteritis, and the resulting cultures of *Clostridium perfringens*. (A) The mucosa of the intestinal tract was severely necrotized and lined by loosely adherent pseudomembranes (arrow). (B) The liver was enlarged, tan-colored, and had necrotic foci on the surface of the hepatic lobes (arrows). (C) *Clostridium perfringens* colonies were isolated from the intestinal contents on blood agar plate and exhibited double hemolysis with inner complete hemolysis (arrow outline) and outer incomplete hemolysis (arrow).

died in the late April 2011. The bird was kept with many waterfowl (127 birds of 17 species) in an outdoor space designed to resemble their natural habitat. This mute swan had been brooding a clutch of eggs before she was found dead in the pond without specific clinical signs observed. No further mortalities were reported in this habitat area.

Necropsy was performed following the standard procedures and organs with lesions were fixed in 10% neutral buffered formalin. The fixed tissues were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E), Gram-staining kit and Prussian blue solution according to the manufacturer's instructions. The contents of the duodenum and jejunum were cultured on sheep blood agar plates (BAP) (Hanil Komed Co, Kyunggi, Korea) under anaerobic conditions in a resealable pouch (BD GasPak™ EZ Pouch System, BD Diagnostics, Sparks, Maryland 21152, USA) overnight at 37°C. Polymerase chain reaction (PCR) was performed on the resulting colonies to detect the genes of CP toxins for patho-typing as described by Yoo et al. (1997). Intestinal mucosa was scraped for parasitological tests including direct smear and floatation using zinc sulfate solution, of which specific gravity was approximately 1.23. Screening for avian influenza was performed by egg inoculation of tracheal and cloacal swab

samples (Swayne et al, 2006).

Based on an external examination, the plumage of the dead mute swan was in good condition, there were no obvious signs of trauma, and it was slightly underweight at 7.32 kg. On necropsy, the entire intestinal tract, from duodenal loop to the cloaca, was covered in thick yellowish to brownish necrotic fibrinous layers (Fig. 1A) and no food was found in the gastrointestinal system. The liver was dull-edged and tan-colored with multifocal necrosis (Fig. 1B).

Microscopically, the intestinal mucosa was covered in the demarcated lines of necrotizing membranes (Fig. 2A) composed of abundant fibrins admixed with detached epithelial cells, necrotized cellular debris, inflammatory cells, erythrocytes and Gram-positive rods. The necrotic lesions found in the villi and lamina propria with lymphocytic infiltration were extended to the crypt part. In addition, some villi were separated from the submucosa of the intestine and the crypts were dilated. However, there was no evidence of coccidial infestation throughout the intestinal tract. The liver showed multiple areas of coagulative necrosis with intralesional bacteria clusters (Fig. 2B) which were stained Gram positive (Fig. 2C), and some of the necrotic zones had deeply basophilic pigments that stained blue exposed to Prussian blue solution (Fig. 2D).

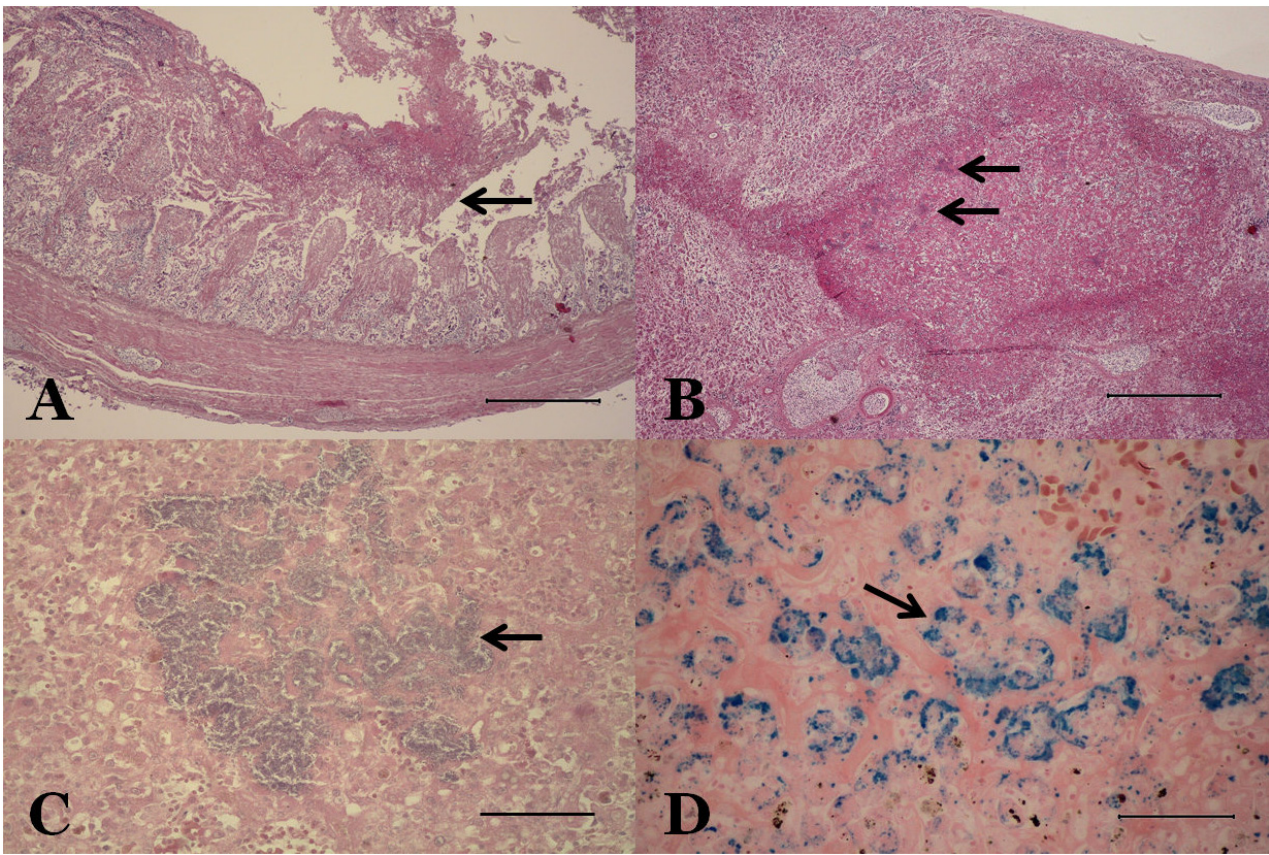


Fig. 2. Histopathology of the intestine (A) and the liver (B, C and D) of a mute swan that died from necrotic enteritis. (A) The intestine was necrotized and covered with a demarcated layer of abundant fibrin (arrow) admixed with necrotic cellular debris, inflammatory cells, erythrocytes and bacterial clusters. H&E stain. Bar=500 μ m. (B) The liver showed coagulative necrosis with intralosomal bacterial clusters (arrows). H&E stain. Bar=500 μ m. (C) Intralosomal bacteria (arrow) were stained blue to violet. Gram stain. Bar=50 μ m. (D) Hemosiderin pigments (arrow) were found within Kupffer cells as well as hepatocytes in the coagulative necrotized area. Prussian blue stain. Bar=50 μ m.

Microbiologically, circular, transparent colonies grew abundantly on the BAP and exhibited double hemolysis, an inner zone of complete hemolysis and an outer zone of partial hemolysis, which was initially identified as CP (Fig. 1C). PCR results for these isolated colonies were positive only for α -toxin, which demonstrated that this was a case of CP type A infection. Intestinal samples were negative for parasite infection including coccidiosis. Routine screening of tracheal and cloacal samples for avian influenza was negative.

DISCUSSION

In the present case, the bird suffered from severe chronic NE. According to the lesion scoring system suggested by Cooper and Songer (2009), this case was

scored at the highest possible level, suggesting that this bird died from severe and extensive necrotizing enteritis throughout the intestinal tract. As the disease progressed, CP extended from the jejunum and ileum, the starting points of NE (Cooper and Songer, 2009; Timbermont et al, 2011), to the entire digestive tract, which resulted in extensive necrotic enteritis throughout the intestine. CP eventually reached the portal blood stream and the hepatic triad areas, resulting in hepatic necrosis with intralosomal bacteria, which was evidence of NE-associated hepatic lesions (Løvland and Kaldhusdal, 1999). An apparent coagulative necrosis and a clear margin between necrotic and living tissue of the intestinal villi are typical enteric lesions found in the later stages of NE (Timbermont et al, 2011).

Iron accumulation in liver cells usually occurs in secondary hemosiderosis associated with underlying patho-

logic conditions including starvation and septicemia (Cork, 2000). In this case, the bird suffered from chronic disease, which could increase the retention of iron inside the cells of the reticuloendothelial system, leading to iron storage within Kupffer cells as well as hepatocytes (Løvland and Kaldhusdal, 1999; Weiss and Goodnough, 2005).

NE was only observed in one bird, despite the living area, feed and water shared between many waterfowl, which could have led to NE occurrence in other birds in that area. NE has various causes, such as feed composition, coccidiosis and stress (Timbermont et al, 2011). In this case, feed and water were shared among all birds and the affected bird did not have coccidiosis, so stress was thought to be the cause of NE progression. Various forms of physical and mental stress can influence the intestinal immune system, such as intestinal barrier defects (Söderholm and Perdue, 2001), which make the intestinal epithelia vulnerable to diseases like NE.

This bird was in her incubation period in a captive environment. For mute swans, brooding is a stressful period, because they showed highly territorial behaviors (Atlantic Flyway Council, 2003). Moreover, in captive surroundings, a primary cause of stress can be the high-density population itself (Timbermont et al, 2011), and overcrowding can lead to a failure to breed for various pathophysiological reasons (Nummi and Saari, 2003). In the breeding season, zoos need to provide sufficient space for nesting territories and pay close attention to breeding birds to prevent possible mortalities.

In terms of the management of waterfowl populations, various diseases associated with reproduction have been reported to cause mortality (Batt et al, 1992). In this study, a unique case of necrotic enteritis was reported as the cause of death of a brooding mute swan. As breeding is an important behavior for both wild and captive mute swans, further investigation of NE as a disease affecting breeding success could be useful.

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