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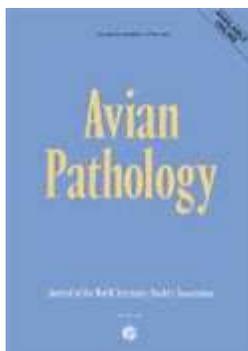
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Enzootic outbreak of necrotic gastritis associated with *Clostridium perfringens*  
in broiler chickens

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## Summary

Clinical morphological investigations were carried out in a flock of 22,000 Ross 308 broiler chickens at the age of 38 days, that experienced a sudden increase in mortality rates. The morbidity and mortality rates were followed out. A gross anatomical examination of 150 bodies (7%) of all 1541 dead chickens was performed. In all necropsied birds, without exception, the typical macroscopic lesions were observed only in the gizzard. Focal or diffuse pseudomembranous deposits were found subcuticularly and on gizzard mucous coat. Microscopically, hyalinization, desquamated epithelial cells and single foci of microorganisms were present among the formed pseudomembranes. Among the fibrin networks of coagulated exudate, single bacilli were detected. *Clostridium perfringens* was isolated from all studied gastric samples. The PCR tests were positive for  $\alpha$  toxin and negative for  $\beta$  and  $\beta_2$  toxin.

## Introduction

Clostridial infections in domestic and wild birds are associated with more than four disease states. *Clostridium colinum* causes ulcerative enteritis; *Clostridium perfringens* and *Clostridium septicum* are isolated in cases of necrotic enteritis (NE) or gangrenous dermatitis and *Clostridium botulinum* is a cause of botulism. Recently, *Cl. perfringens* was found to be involved in the etiology of cholangiohepatitis, generally detected during slaughterhouse inspections (Kaldhusdal *et al.*, 2001; Lovland & Kaldhusdal, 2001; Sasaki *et al.*, 2000). This agent is also associated with cellulitis affecting the tail in turkeys (Carr *et al.*, 1996), gizzard erosions in White Leghorn pullets (Fossum *et al.*, 1988) and umbilical infections in newly hatched chicks (Jordan, 1996).

A condition termed “gastric erosions” and/or “gastric ulcerations” has been reported in commercial broiler chickens in association with avian adenovirus infection (Ono *et al.*, 2001; 2003 a b), mycotoxin-contaminated feed (Hedman *et al.*, 1995; Hoerr, 2003), vitamin B<sub>6</sub> deficiency (Daghir & Haddad, 1981), suboptimal vitamin E concentrations (Janssen & Germs, 1973), inadequate levels of sulphur-containing amino acids (Miller *et al.*, 1975), high dietary copper concentrations (Poupolis & Jencen, 1976), pelleted feed (Ross, 1979), as well as with the inclusion of some fish meals in feeds with subsequent release of histamine and gizzerosine (Harry & Tucker, 1976; Okazaki *et al.*, 1983; Sugahara *et al.*, 1988; Sharma & Pandey, 1980; Tisljar *et al.*, 2002). In these instances, the cuticle of the gizzard in affected birds appeared fissured, thickened and with altered colour (Fossum *et al.*, 1988).

Novoa-Carido *et al.* (2006) determined association between *Cl. perfringens* induced NE and a number of immunological, dietary and environmental factors in chickens. In some cases apart the NE lesions, there were ulcerative necrotic lesions of gizzard cuticle and mucous coat. In addition, the authors evidenced a positive correlation between the severity of detected

gizzard lesions and the number of caecal *Cl. perfringens* counts. Although *Cl. perfringens* was recovered from gastric lesions, the etiological role of the agent remained unclear. In another report cases of gastric necrotic lesions were detected in capercaillies reared in captivity after a necrotic enteritis outbreak associated with *Cl. perfringens* biotype A (Stuve *et al.*, 1992). In all examined birds, liver necroses were also present.

The purpose of the present study was to describe the pathomorphology of a spontaneous outbreak of necrotic gastritis associated with *Cl. perfringens* in broiler chickens.

## Material and methods

The morbidity and mortality rates were followed in a flock of 22,000 *Ross* 308 broiler chickens at the age of 38 days that experienced a sudden increase in mortality. Gross anatomy examinations of 150 out of 1541 dead chickens were performed. Specimens were obtained from the visceral organs of 20 examined cadavres (liver, spleen, heart, small intestine, proventriculus and gizzard) for histological examination. The materials were fixed in 10% neutral formalin, processed by routine techniques and embedded in paraffin. Cross sections of approx. 5 µm, were stained with haematoxylin/eosin (H/E).

The same number of specimens from the same organs were submitted to microbiological examination. The swabs of the transport medium with active charcoal were inoculated on TSC (Tryptose Sulfite Cycloserine) agar (Merck®). Petri dishes were flooded with 5-10 ml TSC-agar, melted and cooled to 45-47°C. The incubation was done anaerobically at 37°C for 20±2 h. Typical colonies, chosen for identification, were inoculated in fluid thioglycolate medium (Merck®). After incubation at 37°C for up to 18-24 h in anaerobic conditions, 5 drops of the

thioglycolate medium were inoculated in lactose sulfite broth (Merck®) in inverted Durham tubes. They were incubated at 46°C for up to 18-24 h in anaerobic conditions.

The number of colony-forming units per g (cfu/g) was determined after preparation of a 10-fold dilution of the stock suspension in buffered peptone water (BPW) as a diluent. Then serial dilutions were performed with 1 ml suspension and 9 ml BPW. The number of dilutions was made according to the expected number of clostridia in two replicates. Results from quantitative bacteriological examinations were analyzed with a Wilcoxon two-sample test (Dean *et al.*, 1990).

The outbreak isolates were investigated for presence of toxins by PCR test according to the method described by Garmory *et al.* (2000).

## Results

The outbreak of disease occurred at the age of 38 days in a flock of 22,000 Ross 308 broiler chickens. Chickens were usually found suddenly dead during the night. Daily mortality rates are presented on Figure 1. The disease lasted for 7 days and during that time, 1,541 chickens (7%) died. The peak in morbidity and mortality rates was by the 3<sup>rd</sup>-4<sup>th</sup> day after the disease outbreak. After the 3<sup>rd</sup> day of the outbreak, the involvement of clostridial infection was suspected and birds were orally given amoxicillin (Vetrimoxin®50) at 20 mg/kg for 5 days. One day after, a sudden reduction in mortality was observed ( $\approx 70\%$ ), as seen from the graph. Forty-eight hours after the intake of the antibiotic, the mortality rates were already within the acceptable limits.

The gross anatomy examinations showed a good condition of dead chickens. Sometimes, after removal of the skin, petechiae or echymoses in the thigh and pectoral

muscles were occasionally observed. In some instances, the serous coats of the proventriculus and the gizzard were affected by extensive suffusions. In all necropsied birds, without exceptions, characteristic macroscopic lesions were observed only in the gizzard. After removal of gizzard content, the cuticle surface was exposed and underneath, focal or diffusely necrotic areas of lighter yellowish colour could be seen. In some cases, the necrosis embedded the cuticle itself, and it appeared as boiled or powdered with a limestone-like substance (Figure 2). In other cases, various-sized areas of the necrotic cuticle had dropped or were lacking. In some regions, the cuticle was raised and detached from the underlying mucous coat. After removal of the cuticle, that was separated very easily, focal or diffuse masses of amorphous, cheese-like matter covering the gastric mucosa, were detected. These masses were pseudomembranes of various thickness with a spongy appearance and grey-yellowish colour. Most commonly, they were detached together with the necrotic cuticle layer, but sometimes remained partially or completely on the mucous layer of the gizzard (Figure 3). Beneath the pseudomembranous deposits, the mucous surface was usually hyperemic and sometimes eroded. Rarely, the mucosa on the transition between the proventriculus and the gizzard was eroded or ulcerated. In single cases, the cuticle was enveloped by necrotic-ulcerative lesions (Figure 4). In some instances, the same processes were found to have affected the underlying mucous coat (Figure 5). In the other parts of the gastrointestinal tract there were no pathological alterations.

Microscopically, a layer of serous fibrinous exudate of various thickness was observed on the necrotic mucous surface of all studied gizzard specimens. Among the formed pseudomembranes, there were hyalinization, desquamated epithelial cells and separate foci of blue-stained microorganisms. Among the fibrin network and coagulated exudate, single or foci of bacilli were observed (Figure 6). In one gizzard specimen, mucosal haemorrhages were

observed, the liver was congested, however, in the other organs, there were no microscopical lesions.

Microbiologically, growth of typical black colonies on culture media were detected in all 20 gizzard specimens. The bacteria, forming specific (black) colonies in sulfite-cycloserine agar and in lactose-sulfite broth (blackening of the medium and over ¼ of the Durham tube' volume filled with gas) were identified as *Cl. perfringens*. The average number was higher than  $3.5 \times 10^6$  cfu/g for the gizzard samples. The PCR tests were positive for  $\alpha$  toxin and negative for  $\beta$  and  $\beta_2$  toxins. The culture media of other microbiologically studied organs were sterile.

## Discussion

The morphological traits of the lesions observed and the etiology of this outbreak allowed description of an unusual clinicomorphological manifestation of a *Cl. perfringens* infection in broiler chickens. Gastric alterations were the only lesion observed and no NE-specific changes were noted. *Cl. perfringens* was isolated only from the gizzard. The lesions observed could be related to the described association between the caecal counts of *Cl. perfringens* and detected ulcerative necrotic lesions in broilers (Novoa-Carido *et al.*, 2006). In our case gizzard lesions could be referred to as severe. The observed average mortality rate was 7.0% compared to 2.7% in Novoa-Carido *et al.* (2006). The present findings could be compared also to gastric necrotic lesions in captive capercaillies, associated with *Cl. perfringens* biotype A (Stuve *et al.*, 1992). In this case however, lesions were observed after a NE outbreak and some of birds had also liver necrotic changes.

The case differed from reported adenovirus gastric erosions (Ono *et al.*, 2003 a) by a number of traits. The erosions described by Ono *et al.* (2003 a) were not accompanied by overt clinical signs or increased mortality rates, however, lesions were based on samples obtained from the slaughterhouse. Furthermore, the histological examination of the gastric mucous epithelium of affected chickens revealed intranuclear inclusion bodies typical for adenovirus infection (Ono *et al.*, 2003 b) that were not observed in any of samples examined by us.

The lesions in this study appeared as typical necrotic pseudomembranous inflammation of the gizzard mucosa, but not as erosions. Thus, our lesions were morphologically different from cuticle erosions observed in cases of mycotoxin-contaminated feed (Hedman *et al.*, 1995; Hoerr 2003), vitamin B<sub>6</sub> deficiency (Daghir & Haddad, 1981), suboptimal vitamin E concentrations (Janssen & Germs, 1973), inadequate levels of sulphur-containing amino acids (Miller *et al.*, 1975), high dietary copper concentrations (Poupolis & Jencen, 1976), pelleted feed (Ross, 1979), as well as with the inclusion of some fish meals in feeds with subsequent release of histamine and gizzerosine (Harry & Tucker, 1976; Okazaki *et al.*, 1983; Sugahara *et al.*, 1988; Sharma & Pandey, 1990; Tisljar *et al.*, 2002).

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## Figure Legends

**Figure 1.** *Daily mortality rates after outbreak of disease.*

**Figure 2.** *The cuticle of the gizzard is imbibed, with coarse surface and looks like boiled (a), and peripherally is raised and detached from the underlying mucous coat by an amorphous cheese-like matter (arrow).*

**Figure 3.** *Detection of pseudomembranes (arrow) with spongy appearance and creamy yellowish colour after removal of the cuticle.*

**Figure 4.** *Severe necrotic ulcerative lesion among the necrotic cuticle (arrow).*

**Figure 5.** *The object from Figure 4 after removal of the cuticle. Only the cuticle (arrow) was affected, there were no alterations in the underlying mucous coat.*

**Figure 6.** *Diffuse pseudomembranous deposits on the gizzard mucous coat, among which hyalinization (arrow 1) and focal clusters of bacilli (arrow 2), H/E, bar = 40  $\mu$ m .*

### Daily mortality rates

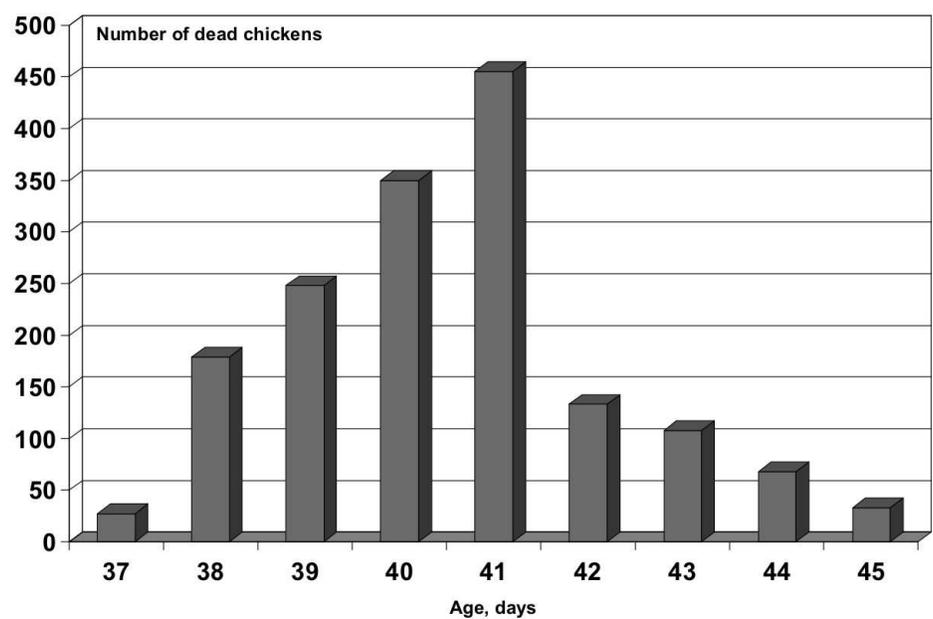


Fig. 1. Daily mortality rates after outbreak of disease  
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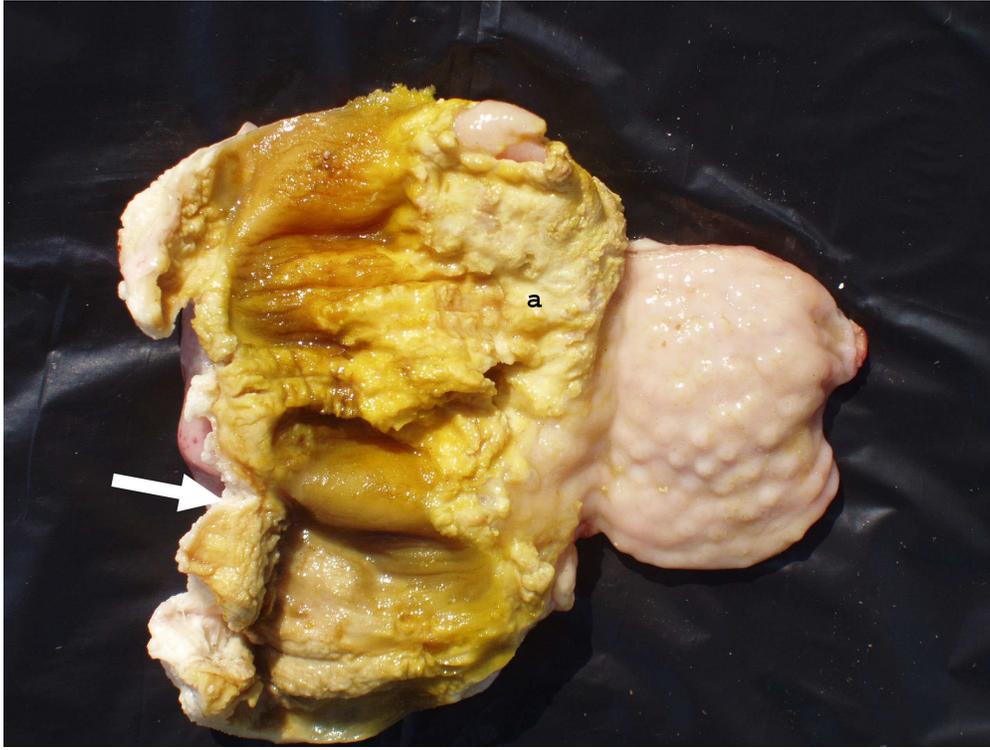


Fig. 2. The cuticle of the gizzard is imbibed, with coarse surface and looks like boiled (a), and peripherally is raised and detached from the underlying mucous coat by an amorphous cheese-like matter (arrow).

451x338mm (144 x 144 DPI)

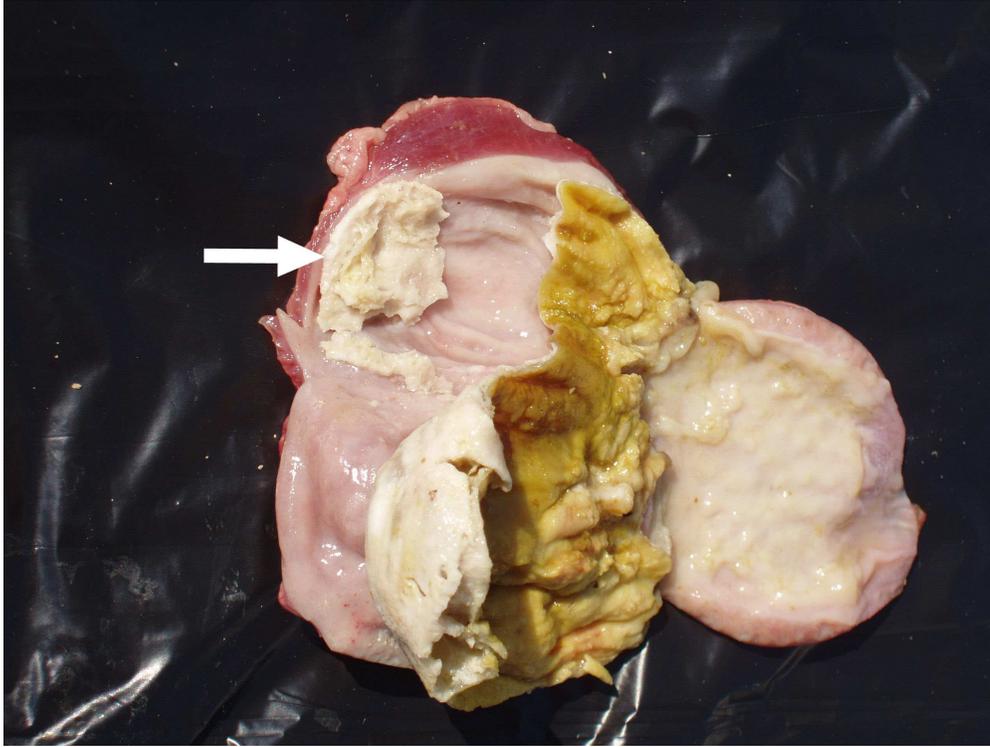


Fig. 3. Detection of pseudomembranes (arrow) with spongy appearance and creamy yellowish colour after removal of the cuticle  
451x338mm (144 x 144 DPI)

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Fig. 4. Severe necrotic ulcerative lesion among the necrotic cuticle (arrow).  
451x338mm (144 x 144 DPI)



Fig. 5. The object from Fig. 4 after removal of the cuticle. Only the cuticle (arrow) was affected, there were no alterations in the underlying mucous coat.  
451x338mm (144 x 144 DPI)

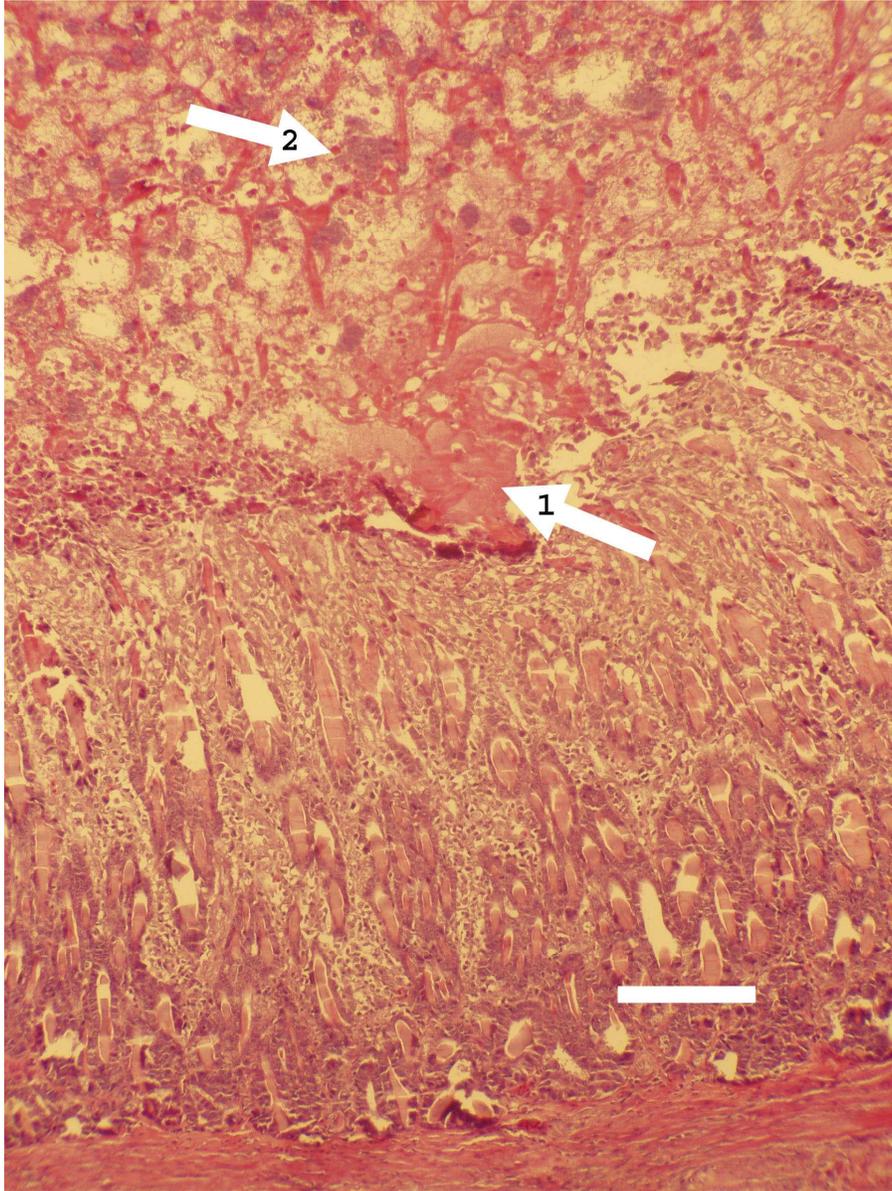


Fig. 6. Diffuse pseudomembranous deposits on the gizzard mucous coat, among which hyalinization (arrow 1) and focal clusters of bacilli (arrow 2), H/E, bar = 40  $\mu$ m .  
338x451mm (144 x 144 DPI)