NOTE Pathology

Necrotic Hepatitis due to *Clostridium perfringens* Infection in Newly Hatched Broiler Chicks

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(Received 7 March 2003/Accepted 22 July 2003)

**ABSTRACT.** Multiple necrotic hepatitis lesions of 5 newly hatched broiler chicks in three flocks derived from two hatcheries were examined pathologically. The livers were brittle, and multiple yellowish or green foci were scattered on the surface and cut surface. The main histological finding was well-demarcated multi-focal necrosis in the liver. Many Gram-positive large bacilli that reacted positively with polyclonal anti-*Clostridium perfringens* serum were observed in necrotic areas.

**KEY WORDS:** *Clostridium perfringens*, necrotic hepatitis, newly hatched chick.

*Clostridium perfringens* infection has been reported in various animal species, especially, necrotic enteritis of young chickens infected with *C. perfringens* has been well described [1,7,8]. However, no naturally occurring outbreaks of necrotic hepatitis due to *C. perfringens* infection in newly hatched chicks have been reported in the literature. The purpose of the present report is to describe increased mortality in the three flocks of newly hatched broiler chicks caused by necrotic hepatitis in which *C. perfringens* infection was suspected.

Three flocks (A, B, C) of 18,849 broiler chicks derived from two hatcheries experienced higher than normal mortality. The outbreaks were noted between 2 days and 7 days of age. The total mortality per affected flock at 7 days of age was 1.7% in flock A, 1.9% in flock B and 2% in flock C. Tissue specimens were collected from 2 cases each of flocks A and B, and three cases from flock C. In all of these cases death occurred at 6 days of age. The liver, spleen, kidneys, heart, lungs, bursa of Fabricius, thymus and all parts of the intestine from affected chicks were collected. Tissue samples were fixed in 10% formalin using routine procedures and embedded in paraffin wax. Sections were cut at approximately 4-µm thickness and stained with hematoxylin and eosin (HE). Selected sections were stained with Gram’s stain.

The bacteria in the necrotic areas of affected chicks was diagnosed by the immunofluorescence staining technique. Paraffin-embedded sections were deparaffinized and rehydrated in xylene followed by a grade series of ethanol, and finally rinsed with phosphate buffered saline (PBS) pH 7.2. The specimens were incubated overnight at 4°C with a chicken polyclonal anti-*C. perfringens* type A serum prepared against *C. perfringens* [9]. Fluorescein isothiocyanate-conjugated anti-chicken IgG rabbit IgG (ICN Pharmaceuticals, U.S.A.) was used as secondary antibody and was incubated with the sections for 30 min at 37°C. Unbound conjugate was removed by rinsing with three changes of PBS. Finally, the sections were examined by UV microscopy.

Gross pathological change was restricted to the liver. All livers were brittle and had well-demarcated, yellowish, multiple necrotic foci of variable sizes (Fig. 1). There were no significant gross lesions in the other organs. Histologically, the main lesion was multi-focal necrosis of the liver (Fig. 2). Large and small coagulative necrotic areas consisted of necrotic hepatocytes and fibrin exudates. There were few inflammatory cells in the necrotic areas. Many Gram-positive large bacilli were observed in the necrotic areas, and they reacted positively with polyclonal anti-*C. perfringens* serum. Gram-positive bacilli were also seen in the gall bladder and extrahepatic bile ducts (Figs. 3 and 4). Bile pigments were seen in the lumen of bile ducts. Necrotic areas with infiltration of heterophilis and macrophages were seen in the thymus and bursa of Fabricius. Many Gram-positive bacilli were observed in the blood vessels of the spleen, kidneys, heart, lungs, small intestine, pancreas, thymus and bursa of Fabricius. No significant lesions were seen in other organs.

Necrotic hepatitis due to *C. perfringens* infection in newly hatched chicks has not been well documented. There are a few reports of necrotizing hepatitis in newborn chicks due to experimental reovirus infection [5]. In the present study, many Gram-positive bacilli were observed in the affected chicks. In addition, bacilli in the affected liver and gall bladder reacted positively in immunofluorescence staining using polyclonal anti-*C. perfringens* serum. Therefore, *C. perfringens* might have played an important role in the present disease.

*C. perfringens* can be found in feces, soil, dust, intestinal contents, contaminated feed and litter. In particular, contaminated feed and litter have been incriminated as sources of infection [1, 4, 6]. *C. perfringens* has been isolated from cases of necrotic enteritis of chickens. Necrotic enteritis is
a major disease of broiler chickens aged between 2 and 5 weeks and of layer chickens aged between 3 and 6 months [1]. Gross lesions in the outbreaks are usually confined to the small intestine. Histopathological changes are characterized by severe hemorrhagic necrosis of the intestinal mucosa with pseudomembrane. Focal necrosis of the liver has been reported in cases of necrotic enteritis [7, 8]. *C. perfringens* was isolated from the liver of chickens in which necrotic enteritis was induced experimentally [3]. The route of infection by *C. perfringens* has been considered to be hematogenous or lymphogenous [8]. We could not specify the route in the present study. Because the outbreaks were noted between 2 days and 7 days of age and occurred as synchronized outbreaks, it was considered that bacterial infection of the newly hatched chicks occurred in the hatchery.

*Escherichia coli*, *Streptococcus fecalis* and *Clostridium* sp. are the most common bacterial isolates associated with yolk-related problems of the bird [2]. Yolk sac infection of chicks that are newly hatched or a few days old may be caused by *E. coli*, *Staphylococcus aureus*, or *Salmonella* spp. [8]. Gross lesions in the naturally occurring cases are usually confined to Meckel's diverticulum. The umbilical cord might have been infected with *C. perfringens* in the present cases, but it was impossible to determine whether the yolk sac had been infected.

REFERENCES