INCLUSION-BODY HEPATITIS
IN BROILER CHICKENS

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SUMMARY

Eighty-six outbreaks of inclusion-body hepatitis were identified in broiler chickens in Ontario. The disease occurred mainly in birds 5 weeks old, and mortality (ranging from 0.1 to 7\%) was most common in the spring. Grossly, the common lesions were stellate or punctiform hemorrhagic areas in extremely fatty livers, enlarged pale kidneys, hemorrhagic lesions in the visceral fat and muscles, pale bone marrow, and an extremely small firm bursa of Fabricius. Histological examination of tissues revealed intranuclear inclusion bodies in the hepatocytes with massive necrosis of liver parenchyma, regression of the follicles and proliferation of interstitial connective tissue in the bursa, aplastic bone marrow with little hemopoietic tissue functioning, and a moderate to severe nephrosis involving the proximal and distal convoluted tubules. Bacteriological examination was negative. Electron-microscope examination of the livers revealed abnormal intranuclear structures; however, no distinct virus particles were seen. A virus was isolated and produced cytopathic changes in chick and duck embryo cell cultures.

INTRODUCTION

In 1963, Helmboldt and Frazier (4) identified an inclusion-body hepatitis in broiler chicken flocks resulting in what they described as an “acute hepatic catastrophe.” The major pathology in those cases was a severe hepatic degeneration with fatty metamorphosis and necrosis of the hepatic parenchyma. Those workers described the intranuclear inclusion bodies as mauve-coloured Cowdry Type-A inclusions and suggested that there was a viral etiology to the disease. The mortality in both flocks was less than 0.1\%. They attempted a virus isolation in embryos 7 and 10 days old, and disease passage was also attempted in 4-day-old chicks, but

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858
neither procedure was successful in identifying an etiological agent. A similar condition was reported from Western Canada in 1970 (6), with hepatic lesions and intranuclear inclusions identical to those reported by Helmboldt and Frazier. The Canadian workers reported mortality as high as 8% in broiler chickens 5 weeks old. They suggested that the inclusions were similar to those caused by virus infections. Their electron-microscope examination of affected livers did not reveal virus particles in the nuclei, but they observed what they called Rous-sarcoma-like particles budding from the plasma membrane. Attempts at virus isolation failed on several media. The condition has been reported from the United Kingdom (9) and Pennsylvania (11). It was also recognized by the present authors in material submitted from the Maritime Provinces and Quebec.

Similar conditions, all attributed to virus infections, have been reported in other birds, such as the owl (1), falcon (16), pigeon (2,10,15), parrot (3), and pheasant (14). This paper summarizes findings in 86 outbreaks of a condition producing an inclusion-body hepatitis in Ontario between January 1969 and July 1971.

FIELD INVESTIGATION

The syndrome was first recognized early in 1969, and since that time it has become endemic, causing severe economic loss in many flocks. Outbreaks on 49 premises were investigated in the first year, with total mortality ranging from 0.1 to 7%, occasionally reaching 1% per day. In all cases the birds were chicken broilers, and in most the disease ran its course in 7 to 10 days and disappeared abruptly. Sudden mortality was a characteristic of the disease, with moribund birds very rarely found. The age incidence was usually around 5 weeks (Chart I). No other relationship could be found between affected flocks as to strain of bird, hatchery, feed, management, or medicinal compounds. In the fall of 1969 and throughout most of 1970 and 1971, the disease was less common; it was found most often in the spring (Chart II).

MATERIALS AND METHODS

Bacteriological examination was made of the heart, lungs, muscle, intestines, gizzard, proventriculus, pancreas, and liver on bovine blood agar and MacConkey plates under both aerobic and anaerobic conditions. Material from infected birds was prepared and inoculated into the yolk sac or on the chorioallantoic membrane of embryonated hen’s eggs and on chick and duck embryo cell cultures.
Brain, skin, heart, lungs, muscle, intestine, gizzard, proventriculus, pancreas, liver, bursa of Fabricius, spleen, bone marrow, kidney, and thymus tissues were fixed in 10% buffered neutral formalin solution, processed routinely, and embedded in paraffin. The sections were stained with hematoxylin and eosin, periodic acid-Schiff, and other selected stains.

Tissues were fixed in gluteraldehyde and 1% buffered osmium (12). They were then embedded in epon, and thin sections were cut and stained with uranyl acetate and lead citrate (13). They were then viewed under a Philips 200 electron microscope.

Fig. 1. Liver from 5-week-old broiler chicken affected with inclusion-body hepatitis.

Fig. 2. Hepatocyte with intranuclear inclusions (H & E). ×800.

Fig. 3. Atrophic follicles of bursa of Fabricius showing proliferation of interfollicular connective tissue and loss of lymphocytes in 5-week-old bird affected with inclusion-body hepatitis (H & E). ×280.
RESULTS AND DISCUSSION

The most common lesions observed grossly were stellate or punctiform hemorrhagic areas on extremely fatty livers (Fig. 1). Noted in a few cases were areas of frank hemorrhages under the capsule. The kidneys were greatly enlarged and pale in the majority of cases and contained petechial hemorrhages and urate-like crystals in abundance. Also commonly seen were hemorrhagic lesions on the breast and leg muscles and throughout the fat of the visceral organs. The bursa of Fabricius was usually much smaller than normal and often extremely firm. The bone marrow was occasionally fatty and yellow, some birds had extremely thin blood with packed cell volumes of 20 to 25.

Examination of the livers by microscope revealed the typical intranuclear inclusions (Fig. 2). There was a tendency for the hepatic necrosis to start in a periacinar arrangement and work toward the portal triads, eventually destroying the entire acinus and causing massive necrosis. These necrotic areas were commonly filled with red blood cells, sometimes macrophages, and often many small lymphocytes. Found in a few specimens were areas of bile duct proliferation suggestive of hepatic repair. Occasionally, livers from birds from affected flocks were found to have inclusion bodies

Chart I. Graph showing the age incidence for the three years of this investigation.

Chart II. Graph showing the seasonal incidence of the disease over the three-year period covered by this survey.
in the hepatocytes even though no major damage in the liver was seen. Kidneys often showed a moderate to severe nephritis, with pyknotic nuclei and swelling of the epithelial cells of the distal convoluted tubules. Damage to the glomeruli and collecting tubules was usually minimal. Some areas of the kidneys were so degenerate that the blood cells escaped freely from their vessels. The bone marrow was often aplastic, with little or no hemopoietic tissue functioning and both the erythrocyte and granulocyte series seeming to be affected. Large vacuolated cells were common in the bone marrow, indicative of the presence of fat. The bursa of Fabricius was always in a very reactive stage, the medullary and cortical areas of the follicles were devoid of lymphocytes, while the interfollicular connective tissue was proliferating and partially or completely obliterating the remaining follicular sites (Fig. 3). A few cystic structures formed from the epithelium had also developed in the interfollicular areas close to the surface. The spleen was occasionally found to be devoid of lymphocytes and on occasion to have small necrotic areas in the germinal follicles. Histological examination of the remaining tissue revealed no consistent abnormalities. No bacterial agents were isolated. No virus was isolated on chick embryo inoculation, though a virus-like agent which produced cytopathic changes in chick and duck embryo cultures was obtained from the livers. Further studies are being carried out to characterize this agent.

Abnormal nuclear structures resembling inclusion bodies were seen under the electron microscope, but no distinct virus particles could be found (Figs. 4, 5).

Fig. 4. Electron micrograph of abnormal structures in the nucleus of affected liver cells. ×10,000.

Fig. 5. Higher magnification of an intranuclear structure showing the fibrillar nature of the amorphous matrix. ×33,600.
The histological lesions in the bursa of Fabricius (Fig. 3) and the appearance of the Cowdry Type-A inclusion bodies (Fig. 2) in the hepatocytes of the liver suggested an infectious viral etiology, possibly a herpesvirus. The histological lesions in the bursa of Fabricius in birds with inclusion-body hepatitis are similar to those described in Marek's disease (8). A reactive stage of the bursa of Fabricius, devoid of lymphocytes with extensive connective tissue proliferation and small cystic structures, has been reported in this disease (5,8). The hemopoietic destruction and aplastic anemia have also been reported (7). The histological appearance of the bursa of Fabricius, the appearance of the typical intranuclear inclusion bodies, aplastic anemia, and the apparent infectious nature of the disease suggest a viral etiology. The possibility that Marek's disease virus is the cause of this condition warrants further examination.

REFERENCES


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