CASE REPORT

Ulcerative Enteritis Caused by *Clostridium colinum* in Chickens

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Introduction

Ulcerative enteritis or "Quail Disease", a fatal enteric disease primarily of captive quail birds (1, 2, 9, 13), was also recorded in other avian species such as pheasants, grouse, pigeons and Californian quails (3, 9, 11, 18, 25).

Ulcerative enteritis was reported in chickens (9, 10, 12, 19, 22, 23, 24) and it was reproduced experimentally in quail and chickens (4, 7, 15, 16, 17, 21). Both morbidity and mortality due to this disease were highly variable and were affected by factors such as coccidiosis, overcrowding, withdrawal of food and water, medication and management. Most of the authors cited agree that the small intestine was the consistent site of gross lesions; followed in order of frequency of pathological changes: the liver and the spleen. Various microorganisms were isolated during the investigation of the disease in quail chickens and turkeys (2, 19, 20, 24) but *Clostridium colinum* is now generally accepted to be the causative agent of ulcerative enteritis (4, 5, 6, 25).

This report describes an outbreak of ulcerative enteritis which occurred in a large layer operation of pullets and is the first report of the disease in chickens in Canada.

Case History

The disease occurred in a flock of 15,000 nine week old pullets. The birds were being reared in battery cages, there was no history of overcrowding and they were not being medicated. The average mortality for the seven days previous to the outbreak had been three per day and on the first day of the outbreak this rose to 21. Thirty percent of the birds were affected. Streptomycin, administered at the level of one gram per gallon of drinking water, controlled this disease and no further losses were reported.

Materials and Methods

Twenty-one dead birds and four live birds were submitted for necropsy. Pieces of liver, small intestine and spleen showing gross changes were fixed in 10% formalin and were processed by routine paraffin embedding methods, sectioned at 6 μm and stained with haematoxylin and eosin and Gram's stain. Intestinal scrapings of duodenum, jejunum and cecum of all the birds were examined microscopically for coccidial oocysts. Samples of liver from three affected birds were separately homogenized and inoculated onto Columbia agar[1] plates which had been prereduced by storage under anaerobic conditions.[2] Plates were incubated at 37°C for 48–72 hours. Individual colonies were subcultured onto prereduced Columbia agar plates and incubated anaerobically.

Standard aerobic broth (phenol red base)[3] containing different carbohydrates at 0.5% or 1.0% concentrations were stored anaerobically for 24 hours before inoculating with pure culture of a 48-hour growth of the bacterium isolated. Test carbohydrate broths were incubated anaerobically for 48 hours and acid production was tested by the ability to produce a yellow colour change following the addition of three drops of bromcresol purple (pH 5.2). Motility was observed by phase contrast microscopy of a 24-hour growth in broth culture. One drop of a 24-hour broth culture was placed on a copper grid coated with formvar-carbon and stained with 1% phosphotungstic acid before examining for the presence of flagellae in the electron microscope. Lecithinase and lipase activity was tested on egg yolk agar (14). Fermentation products from glucose were analyzed in peptone-yeast-glucose broth, incubated for 48 hours (14). Aesculin hydrolysis, nitrate reduction and gelatin hydrolysis were tested (8).

Four four week old chickens were inoculated intravenously with 2×10^8 viable organisms which had been cultured anaerobically on Columbia agar before washing off and suspending in peptone-yeast glucose broth.

Pathology

The live birds showed droopiness, ruffled feathers, weakness, inappetence and watery diarrhea. At necropsy all the birds showed similar lesions which consisted of small shallow to deep-seated intestinal ulcers in the lower third of

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[1]Oxoid Ltd., London, England.

[2]Gaspak, Baltimore Biological Laboratories, Cockeysville, Maryland.

[3]Difco Laboratories, Detroit, Michigan.

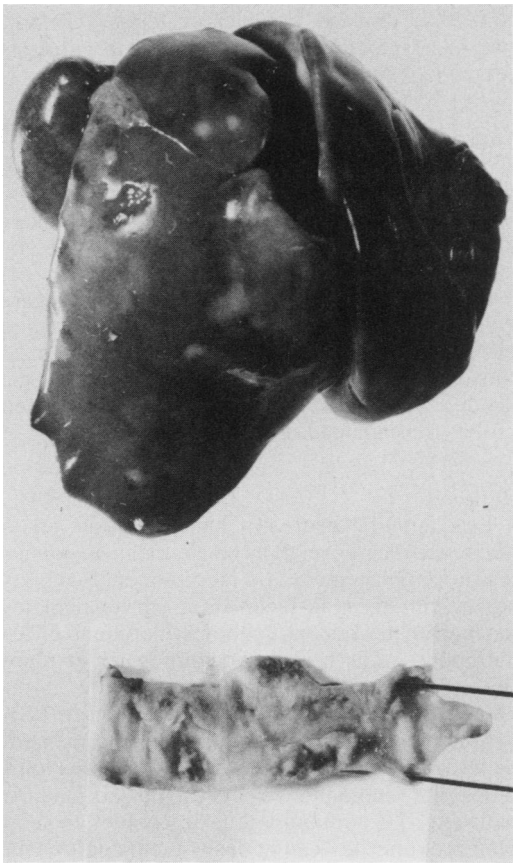

FIGURE 1. Focal to widespread areas of hepatic necrosis distributed uniformly on the serosal surface (top).

Circumscribed intestinal ulcerations covered by necrotic pseudomembrane and surrounded by hemorrhagic line of demarcation. The black lines on the right are forceps (bottom).

the small intestine. The ulcerated areas were usually visible from the serosal surface of the intestine as pale gray areas of necrosis which were often circular but sometimes irregular in outline. The average diameter was about 1 cm but larger ulcerated areas were also evident, particularly where adjacent ulcers coalesced.

On the mucosal surface these areas of ulceration were covered by a necrotic pseudomembrane which, on removal, revealed a hemorrhagic line of demarcation which separated the devitalized area from healthy tissue (Figure 1). The liver of each bird showed widespread pale, localized areas of infarction which were randomly scattered over the serosal surface (Figure 1). Splenomegaly was a consistent finding, as were splenic hemorrhage and pale focal areas of necrosis. Intravenous inoculation of the organism resulted in death of the four birds approximately 16 hours after inoculation; there was marked and severe swelling at the site of inoculation. At necropsy there were numerous pinpoint necrotic areas in the liver and a hemorrhagic duodenitis but no ulcers were seen in the small intestine. The spleens were enlarged about twice the normal size and were hemorrhagic.

The ulcerated areas showed necrosis involving the villous epithelium, lamina propria and submucosa. Some caseous, intensely eosinophilic, proteinaceous exudate with scattered clumps of bacterial colonies occupied the necrotic areas and seemed in places to spread out and form plaque-like pseudomembranes. Large numbers of heterophils and other mononuclear leukocytes infiltrated the periphery of the ulcers. Some of the ulcerated areas appeared almost to perforate the serosal surface of the small intestine. Casts of fibrin, bacterial colonies, desquamated intestinal epithelium and infiltrating leukocytes were found at intervals in the intestinal lumen. There was slight edema of the muscular coat of the small intestine. Numerous Gram-positive and Gram-negative rods of a uniform size were seen, particularly in ulcerated and necrotic areas.

The microscopic lesions observed in the liver in the natural disease were predominantly focal, localized areas of coagulation necrosis with bacterial colonies in the centre. Moderate infiltration of heterophilic and other mononuclear leukocytes was seen in the necrotic areas. Massive areas of necrosis involving a large amount of hepatic tissue were, however, also evident. Only Gram-positive bacilli were seen in liver tissue.

In experimentally inoculated chickens, small focal areas of coagulation necrosis were seen in the liver and large numbers of bacterial colonies occupied the centres of these areas. The spleen showed severe depletion of lymphocytes, massive hemorrhage and scattered clumps of bacterial colonies. The duodena of these four inoculated birds were characterized by severely engorged blood capillaries, necrosis of villous tips, desquamation of villous epithelium and hemorrhage.

Bacteriology

Gram-stained smears of the liver homogenates revealed large numbers of 4 μm long, 1.0 μm thick, Gram-negative rods, some of which showed subterminal enlargements; a small proportion stained Gram-positive. Several hundred nonhemolytic 3 mm diameter, round, grey, semitranslucent colonies were recovered from the livers of all birds when the plates were examined 48 hours after inoculation. Bacteria identified as *Clostridium perfringens* on the basis of morphology, Gram-stain and characteristic double hemolytic zones were also present in variable but lower numbers. Isolated colonies of the predominant organism 72 hours after inoculation were flatter and showed more irregular, flowing margins. Gram-stained smears of colonies on both

primary culture and on subculture revealed Gram-negative rods, some of which showed slight subterminal enlargement; a small proportion of bacteria in the colony stained Gram-positive. Rods were commonly 3–4 × 1.0 μm, single or paired, but chains were occasionally present. Obvious subterminal spore formation rather than slight enlargement was rarely seen and free spores were never found. The bacterium did not grow aerobically.

A light turbid growth with small amounts of gas was observed in peptone-yeast-broth 48 hours after inoculation. Acid was produced from dextrose, maltose, mannose, raffinose, salicin, sucrose and trehalose but not from arabinose, inositol, lactose, rhamnose, sorbitol or xylose. Aesculin was hydrolyzed; no growth was observed in nitrate broth, gelatin was not liquefied, and lipase and lecithinase activity was not evident. Fermentation products from glucose were acetic acid in major amounts with minor amounts of formic acid. The organism was motile and possessed peritrichious flagellae. The bacterium was recovered in large numbers from livers of experimental birds which died after intravenous injection. An extremely severe inflammatory reaction occurred at the site of inoculation.

Discussion

The pathological changes present in the birds bear superficial resemblance to coccidiosis, histomoniasis and salmonellosis. Gross and histopathological changes are similar to those described in outbreaks of ulcerative enteritis (3, 9, 10, 11, 12, 13, 18, 22, 24) and the isolation of *Clostridium colinum* leaves no doubt that the disease described is ulcerative enteritis. No difficulty was experienced in the isolation of the organism nor in the testing of its metabolic properties; immunofluoresence has been suggested as a diagnostic aid (7) but seems unnecessary since the organism is readily isolated. The only feature which appears to differ from the original description (4, 6) is that the majority of the organisms stain Gram-negative rather than Gram-positive in both tissue homogenate and in pure culture. Using the Brown and Brenn modification of Gram's stain, all organisms in the liver stain Gram-positive. Severe swelling of the inoculation site suggests that the organism produces a potent toxin of some type. The recent report of the disease in robins in Florida (25) suggests that the natural habitat of the organism is in the soil, and that only when large numbers of birds are kept together can an outbreak of the disease occur due to a build-up in numbers of the organism. The disease has been previously reported in pigeons in Canada (11) but this is the first description in chickens. This outbreak in replacement pullets that are not in contact with soil nor litter material seems to be unique and is reported to draw awareness to the fact that possible contamination of feed or drinking water can cause severe outbreaks of ulcerative enteritis even in chickens reared in wire cages.

Summary

An acute outbreak of ulcerative enteritis in replacement pullets is described. The clinical signs, mortality pattern and the pathognomonic lesions are presented as well as methods used in the isolation and identification of *Clostridium colinum*. Four four week old chickens which were given the isolate intravenously died and gross and histopathological lesions seen were similar to those present in the natural disease. The etiologic agent was thought to have reached the caged pullets through feed or water.

Résumé

Les auteurs décrivent une éruption aiguë d'entérite ulcéreuse, dans un troupeau de poulettes de remplacement. Ils mentionnent aussi les signes cliniques, la façon dont survenaient les mortalités, les lésions pathognomoniques et les méthodes qu'ils utilisèrent pour isoler et identifier *Clostridium colinum*. L'injection intraveineuse de cette bactérie à quatre poulets âgés de quatre semaines provoqua le développement de lésions macroscopiques et histologiques mortelles et semblables à celles de la maladie naturelle. La contamination des poulettes semblait provenir de l'eau et des aliments qu'on leur donnait.

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