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Short Communication

Non-*Clostridium perfringens* cholangiohepatitis in Broiler Chicken Livers Condemned During Veterinary Inspection at a Slaughterhouse of Uruguay

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Abstract

Cholangiohepatitis in chickens causes economic losses by liver condemnations at the slaughterhouse, as well as, poor productive performance in commercial farms. The purpose of this study was to describe the histopathologic changes due to cholangiohepatitis in slaughtered broiler chickens from 39 to 45 days of age, referred by the veterinary inspector from a commercial abattoir of southern Uruguay. In all the studied livers, the epithelium of the intrahepatic duct showed degeneration and necrosis with various inflammatory cells and fibrosis. The bacterial culture of *Clostridium perfringens* from fresh samples of these livers was negative and Gram-positive bacteria and PAS-positive fungi were not detected in the affected bile ducts.

Key words: Chicken, cholangiohepatitis, liver, pathology, veterinary inspection

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cholangiohepatitis in broiler chicken mainly affects the intrahepatic bile ducts and the outbreak of this disease causes significant economic losses in the poultry industry due to condemnations at the slaughterhouse and poor productive performance¹⁻³. This disease was first described in Scotland⁴ and then was detected in Canada⁵⁻⁷, Japan^{8,9} and Brazil¹⁰. *Clostridium perfringens* infection has been suspected as the cause of the disease but other factors have also been discussed^{8,10-12}. In Brazil, a study of 100 condemned chicken livers, reported a high incidence (21%) of multifocal heterophilic cholangiohepatitis¹⁰. In other South American countries, the main histologic lesions and the incidence of chicken liver condemnation of commercial abattoirs are not yet characterized. The purpose of this study was to clarify the histological changes caused by this disease in the livers condemned at a broiler chicken slaughterhouse of Southern Uruguay. According to the best of our knowledge, this is the first diagnosis of cholangiohepatitis in broiler chickens in Uruguay.

Four fresh condemned livers of broiler chickens from commercial farms were referred by the veterinary inspector of a commercial abattoir of Canelones Department (Uruguay) to the Diagnostic Laboratory of Pathology Unit, Faculty of Veterinary-Universidad de la República (Montevideo, Uruguay) for histopathological study. Two of the livers belonged to 39-days-old broiler chickens and the remaining two to 45-days-old broiler chickens. According to the veterinary inspector, the cause of condemnation was hepatomegaly, with enhanced lobular pattern, rounded borders and focally extensive pale discoloration of the hepatic surface in all the cases. The liver specimens were fixed in a 10% buffered formalin solution for 7 days, processed, sectioned at 4 µm and subsequently stained with Hematoxylin and Eosin (H&E). They were also stained with Periodic acid Schiff (PAS) and Gram stains. The histopathological study was carried out by four veterinary pathologists (CL, BL, KY, JMV). Simultaneously, the bacterial culture to isolate *Clostridium perfringens* was done on fresh samples from the same livers following standard methodologies for anaerobes (VI). Also, the same samples were cultured aerobically on trypticase soy agar supplemented with 5% sheep blood and trypticase soy agar incubated at 37°C for 72 h.

Grossly, the formalin-fixed livers were enlarged and pale. The histopathological changes were characteristic in the intrahepatic bile ducts and were similar in all four chickens. The epithelial cells of intrahepatic bile ducts showed hyperplastic and necrotic changes with aggregation

of inflammatory cells such as heterophils, lymphocytes, plasma cells and histiocytic cells (Fig. 1a-c). Hepatocytes around these changes often showed necrosis and in the hepatic parenchyma multifocal severe periportal inflammatory infiltrates and fibrosis, typical lesions of cholangiohepatitis were diagnosed (Fig. 1d). In the Glisson's capsules of the four livers, the intralobular bile ducts also showed the similar changes described above and normal structures of the Glisson's capsules were destroyed. The histopathological changes were more severe in chickens of 45 days than in those of 39 days. The bacterial culture for *C. perfringens* and aerobic culture from all the livers examined was negative. Gram-positive bacteria and PAS-positive fungi were not detected in the affected lesions. In one of the four chickens, aggregates of inflammatory cells, necrosis and Gram-positive bacteria were observed in the connective tissue surrounding the perihilar bile duct but these changes were not detected in the other three cases.

Cholangiohepatitis has been previously reported in companion and farm animals associated with different bacteria species^{10,13-16}. The histopathological lesions found in this study were similar to those seen in previously reported cases of cholangiohepatitis detected in broiler chickens caused by *C. perfringens*⁶. *Clostridium perfringens* is a common environmental bacterium where broiler chickens are raised, so its presence in the intestine is expected¹⁷. Its route of infection, as are occurred in other enteric bacteria such as *Escherichia coli* and *Salmonella* spp., is characterized by ascending from the biliary tract and associated with biliary stasis^{17,18}. There are reports of experimental infections through ligation and/or inoculation of this bacterium^{6,19}. Severe fibrosis has been reported by experimental inoculation of *C. perfringens*^{6,19}. On the other hand, Handharyani *et al.*⁸ demonstrated that the presence of this microorganism does not appear to be essential in the pathogenesis of changes in the liver dysfunction. Microscopically, we observed typical lesions of cholangiohepatitis, with similar changes described in hepatic parenchyma and the Glisson's capsules (perihepatitis) but no bacteria were detected in the bile ducts of all chickens in this study. In addition, the culture of *C. perfringens* in samples from livers was negative. The hepatic changes in the present study were similar to those caused by *C. perfringens* but it was not possible to conclude that these lesions were caused by this bacterial species. Similar findings were reported by Barcelos *et al.*¹⁰. Further studies are needed to elucidate possible causes of these hepatic lesions. In this study, inflammation and necrosis with a bacterial mass were observed in the connective tissue surrounding the perihilar bile duct of the liver of one chicken. Unfortunately,

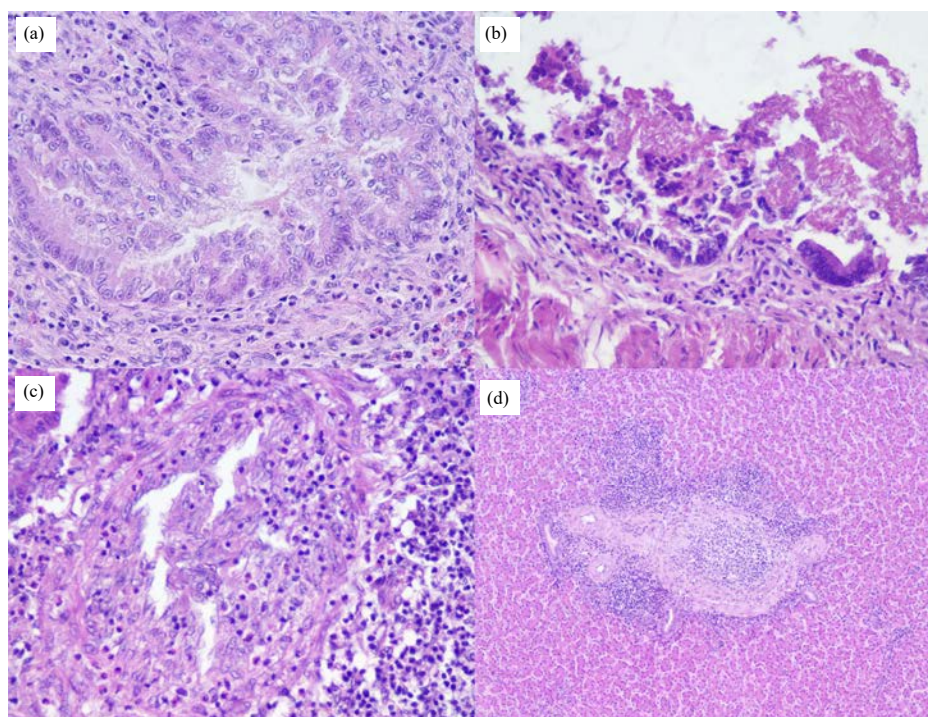


Fig. 1(a-d): Liver, broiler chickens. a, b, c-Intrahepatic bile ducts. H&E. $\times 400$

(a) Proliferation of the bile ductal epithelial cells with reaction of inflammatory cells and proliferation of connective tissue, (b) Focal necrosis of the ductal epithelial cells, (c) Disappearance of normal bile duct structure and reaction of various inflammatory cells, (d) Hepatic parenchyma with severe periportal inflammatory infiltrate and fibrosis. H&E. $\times 100$

the pathogenesis of this lesion was not clarified up to now. The facts that no Gram-positive bacteria were found in the bile ducts around the lesion and no changes were detected in the other three studied chickens suggested no association with *C. perfringens* infection.

Conclusion

The main changes in four condemned livers of clinically normal broiler chickens were the proliferation and necrosis on the intrahepatic bile ductal epithelium accompanied with inflammation and fibrosis. No bacteria were detected in the affected bile ducts and the bacterial culture of *C. perfringens* was negative.

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