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The Pathogenesis of *Salmonella enteritidis* in Experimentally Infected Ducks: An Immunohistochemistry Study

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Abstract: To study the pathogenesis of *Salmonella enteritidis* in experimentally infected ducks. Researchers used the immunohistochemical localization and histopathological to examine various tissues of ducks following oral infection were investigated. The time course for the appearance of viral antigen and tissue lesions in various tissues was coincidental. The study suggested that the bacteria levels of SE in systemic organs have a close correlation with the progression of disease. The abundance of target lamina propria and lymphoid cells may contribute to the high levels of bacteria infection and replication in lymphoid and intestinal tissues.

Key words: *Salmonella enteritidis*, pathogenesis, immunohistochemical assay, ducks infection, SE, China

INTRODUCTION

Salmonella enteritidis (SE) is an enteric pathogen that colonizes the intestinal tract of a variety of animals, especially humans and poultry and accounts for millions of cases of gastroenteritis and food-borne illness each year have became a significant public health problem (Braden, 2006). Previous studies showed infection SE is initiated by estensive colonization of Salmonella in the intestine, it also can perisist for a long time. After the initial colonization at the intestinal epithelial surface, the SE invades and spreads among a wide range of tissues (Yan *et al*., 2008). As the most important that the lamina propria and lymphocytes are the principal sites of bacteria replication. The objectives of the present study were to determine the pathogenesis of SE in ducks by a time-course study using immunohistochemical to correlate these findings with histopathological examination of selected tissues which yield valuable insight into fully understanding the pathogenic steps of SE infection.

MATERIALS AND METHODS

Bacterial strains: A high-virulence strain of *S. enteritidis* (Phage type 4; No.: 50338) was purchased from National Center for Medical Culture Collection (He *et al*., 2010).

Animals and experimental design: The infection model was based on previous studies (Gast and Beard, 1990). About 7 days old specific-pathogen-free Peking ducks were purchased for the study. They were kept in individual cages under 12L:8D and provided with feed and water. All of the ducks were found to be negative for *S. enteritidis* by an ELISA. To follow the SE infection in young birds, the ducks were divided into 2 groups with 24 each. The 1st group of ducks were orally inoculated with PBS. Ducks in the 2nd group were orally inoculated with 0.2 mL (2×10^9 cfu) of SE. The two groups of ducks were housed in two different isolators to prevent cross contamination and observed twice daily for signs of illness and mortality. Two inoculated ducks were euthanatized at 8, 12 h, 1-3 days postinoculation (pi) and thereafter 3 days until 9 days. Sections of the intestine and kidney, heart, spleen, liver were collected. The tissue were quickly submerged in 4% formaldehyde for 24 h. Then embedded in paraffin.

Immunohistochemical localization of SE antigen: The tissue sections (at 4 μm thickness) were prepared and air-dried on slides. Then sections were deparaffinized and rehydrated in PBS. Followed for 10 min antigen retrieval by microwave with 0.01 mol L^{-1} citrate buffer solution (pH 6.0). Endogenous peroxidase activity was blocked with 3% H_2O_2 in methanol for 20 min, washed with PBS contain 0.05% tween. Non-specific binding was minimized by treating the sections with 10% Bovine Serum Albumin (BSA) at 37°C for 30 min. The sections were then blotted with diluted SE-specific IgG (1:25) for 40 min at 37°C washed with PBS. Then the sections were blotted with HRP-conjugated goat anti-rabbit IgG (1:100). For negative controls, 1% BSA were used to dilute the primary and secondary antibodies. The sections were analyzed under a light microscope and positively stained cells from at least three randomly selected fields.

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Histopathological examination: For histopathological examination, paraffin-embedded sections were cut at 4 μm thickness and stained with haematoxylin and eosin (Turnbull and Richmond, 1978).

RESULTS AND DISCUSSION

The distribution of *Salmonella enteritidis* in tissues of infected chickens was examined by immuno-histochemistry. The clusters of bacteria were seen adhering to the epithelium of the intestine at 4 HPI. By 8 HPI, they moved into the lamina propria and within cells of the spleen, fabricius. Also, they can be seen in some other tissues. No specific staining was observed in any gastrointestines of uninfected ducks that were orally inoculated with PBS. Even though, Salmonella-infected chickens generally looked healthy with normal weight gain, the pathology of the infection was readily detectable by histology including, lamina propia of the intestine and spleen, Fabricius and occasionally hemorrhagic enteritidis. These lesions were prominent at 4 HPI, moderate at 8 HPI and less noticeable at 6 DPI. In this study, the localization of the SE and histopathological examination during the acute stage of SE infection in ducks was investigated.

The study show that the SE were observed in the tissues and cells of intestine at 4 HPI. Thereafter at 8 HPI, researchers can detected the SE in large part of the tissues. The time course for the appearance of antigen and tissue lesions in various tissues was coincidental. These results may suggest that the levels of SE in systemic organs have a close correlation with the progression of disease. It is important to note that the lymphoid and intestinal organs examined contained relatively numbers of bacterias throughout the infection, especially in the spleen, bursa, jejunum, ileum and cecum tissues. Immunohistochemical detection revealed that SE antigen was found most abundantly in the lamina propria cells of intestine and lymphocytes of bursa as well as most of the macrophages and some lymphocytes in the spleen and fabris (He *et al*., 2011a, b).

The lesions of lymphoid organs including lymphoid depletion and necrosis were detected by histological examination and the epithelial hypertrophy was also showed in bursa. In the intestine, specific staining for SE antigen was seen mostly in the lamina propria cells and the cells of the crypt and also in the macrophages and fibroblasts of the lamina propria of bursa. Furthermore, vascular damage and hemorrhage were observed in all infected organs examined. These results may suggest that the lymphoid and intestinal organs are the major target organs of SE replication and the lamina propria cells of intestine and lymphocytes probably served as the principal site of bacteria replication (Mutinelli *et al*., 2003). Furthermore, the high levels of bacterias in these tissues most likely reflects the abundance of target lamina propria and lymphoid cells in these tissues and therefore, play a key role in the pathogenesis of acute SE manifest as severe tissue lesions on the lymphoid organs and small intestine.

IHC assay have a priority of detection and location cells and organs in comparison to traditional methods. IHC need only one liquid phase incubation step, a single tracer reagent. Specific antibodies are detected when they bind to the tracer to produce a labeled antibody-tracer. In recent study, IHC assay have some potential application for the diagnosis of SE infection in poultry (Andreasen *et al*., 2001).

CONCLUSION

The present study provides a detailed description of the patterns immunohistochemical localization of SE antigen and the histopathological examination in various organs. The bacteria levels of SE in systemic organs have a close correlation with the development of disease. The high numbers of bacterias infection and replication in lymphoid and small intestinal tissues most likely reflects the abundance of target epithelial and lymphoid cells in these tissues.

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