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Short Communication

Experimintal Study of Peracute Fowl Cholera due to *Pasteurella multocida* Vaccinal Strain (serotype A1) in Chickens

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ABSTRACT

In order to show the type and severity of gross and histopathologic lesions induce by vaccinal strain (serotype A1) of *Pateurella mulocida*, ten four-week-old SPF chickens were inoculated intramuscularly with 75 cfu of (0.5 ml of 10^{-7} dilution) bacterium. All birds died in less than 16 hours. No prominent gross lesions were observed in different organs. In microscopic examination, the most common histopathological findings were as congestion and hemorrhage. Moreover, large amount of viscid mucus were observed in digestive tracts.

Keywords: Fowl cholera, histopathology, *pasteurella multocida*, chicken

INTRODUCTION

Fowl cholera (FC) (avian cholera, avian pasteurellosis, or avian hemorrhagic septicemia) is a contagious disease which can cause economic losses in poultry industry, has been recognized in domesticated and wild birds for more than two centuries(Davies *et al* 2003, Rimler and Rhoades 1989). It is usually appears as a septicemic disease associated with high morbidity and mortality (Glisson *et al* 2003). The causative agent of FC is *Pasteurella multocida*, a non-motile, small gram negative rod of the family Pasteurellacae. It has been also recognized as causative agent of hemorrhagic septicemia in cattle and buffaloes; atrophic rhinitis in pigs (Davies *et al* 2003). The virulence of *P. mutocida* in relation to FC is a highly complex entity. Attempts to understand the determinants of virulence factors have met with limited success. However, P. mutocida constituents such as toxins, capsule, lipopolysacchride, outer membrane proteins and plasmid have been considered as virulence factors (Pyone *et al* 1999). *P. mutocida* serotype A1 currently used for preparation of FC vaccine in Razi Vaccine and Serum Research Institute, Karaj-Iran, had already been isolated from an outbreak of FC in a group of household birds including ducks, turkey, geese and chickens in Astara, a city in Gilan province in north of Iran. High morbidity and nearly 70% mortality has been reported among different species of birds (Tavasoli *et al* 1984). It was found that the Astara strain was highly virulent in chickens and mice (Sototdehnia *et al* 2004).

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The range of lesions due to avian pasteurellosis differs from peracute to chronic. So, the objective of present study was to determine the type and severity of gross and histopathologic lesions in some of chicken's tissues following intramuscular injection of Astara vaccinal strain (serotype A1) of *P. mutocida*. These data was used for completing the phenotypic as well as molecular characteristic of the P. mutocida vaccinal strain which was needed in documentation. Other characteristic of this stain like as protein profile and restriction enzymes analysis of chromosomal DNA have been already determined by Jabbari *et al* (2002).

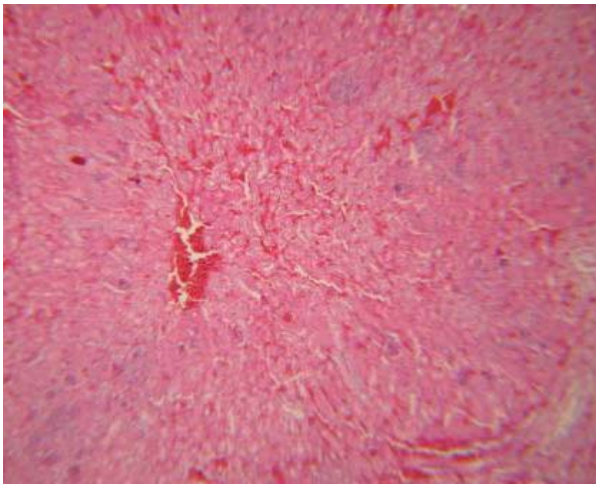
Materials and Methods

Bacteria. The *P. mutocida* serotype A1, isolated from FC outbreaks in Astara in 1984 (Tavasoli *et al* 1984) was used to cause experimental disease in chickens. The phenotype and molecular characteristic of this isolate have been determined previously (Jabbari *et al* 2002). A lyophilized ampule of the bacterium was resuspened in 10 ml of triptose phosphate broth (TPB) and incubated at 37° ^{C} for 18 hours .1 ml of overnight culture was subcultured in 5ml of TPB and incubated at 37° ^{C} for 6 hours with moderate shaking. Then, ten-fold serial dilutions of the culture were prepared in sterile phosphate buffer saline (pH 7.2).

Challenge. Ten four-weeks-old SPF chicken was inoculated by 75 cfu of *P. mutocida* (0.5 ml of dilution 10^{-7}) intramuscularly. The birds were fed ad libitum. 16 hours post inoculation all chickens found dead. Subsequently, necropsy was performed and different organs were sampled for both reisolation of bacteria and histopathologic examination.

Reisolation of bacteria. Samples of different organs of dead birds consisted of heart, liver and spleen was cultured for reisolation of the inoculated bacteria.

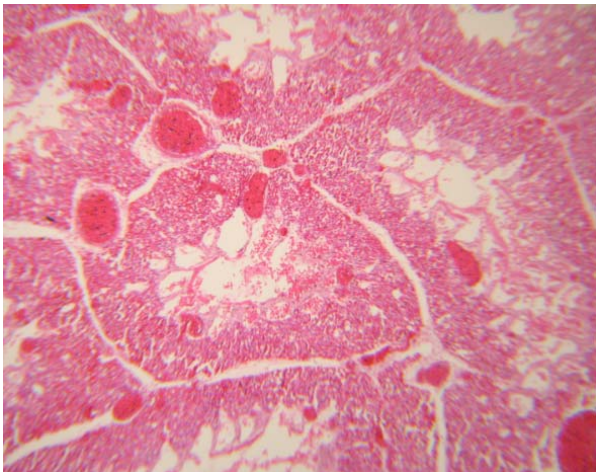
Histopathologic examination. samples of different organs were kept in 10% formalin until fixation. Then tissues were routinely processed to paraffin blocks, sectioned at 5µm, deparaffinized, stained with H&E and finally examined by a light microscope.



Figuer 1. Fowl cholera- Congestion and scattered hemorrhage in the kidney (H&E × 40).

RESULTS

Gross lesions. On necropsy of the all birds no obvious lesions were found in the carcases.



Figuer 2. Fowl cholera- Congestion and hemorrhage into the parabronchial lumina (H&E × 100).

Microscopic lesions. On histopathological examination of the lung, liver, spleen, heart,

kidneys and, mucosa of the, intestine congestion and hemorrhages were the most prominent features, which were relatively more distributed in the kidneys. One of the additional apparent findings of the present study was the presence of viscid mucosal casts in the lumen of the intestine. Moreover, mild depletion of the lymphocytes of the bursa of fabricius of some birds was noticed.

DISCUSSION

We performed this study for completing the phenotypic as well as molecular characteristic of the *P. mutocida* vaccinal strain which was needed in documentation. As it was mentioned 16 hours after inoculation, of the isolated bacterium, all chickens found dead without any apparent gross lesions. This revealed that inoculated bacterium have a high severity for chickens. However, severity of microscopic lesion could be classified as mild to moderate type. Reisolasion of inoculated bacterium (Astara vaccinal strain, serotype A1, of *P. mutocida*) from the tissues of all dead birds confirmed that earlier mentioned lesions was due to challenged bacterium.

Pathogenicity or virulence of *P. mutocida* in relation to FC is complex and variable depending on the strain, host species, and variations within the strain or host and condition of contact between the two (Glisson *et al* 2003). Mature chickens are more susceptible than young ones and turkeys are much more susceptible than chickens to infection with *P. mutocida*, and (Heddleston, K. L. 1972). So, more investigation for determining pathogenicity of isolated bacterium for turkeys is suggested.

The results of present study showed that the most prominent histopathologic lesions in dead chickens were as congestion and hemorrhage. Lesions of FC vary in type and severity depending to the course of the disease, whether acute or chronic. In acute form, as in present study, most of the postmortem lesions are associated with vascular disturbances (Glisson *et al* 2003). Rhoades (1964) observed severe general passive hyperemia in chickens that died from acute FC. This lesion was considered to be indicative of shock and was attributed to the action of endotoxin. Heddleston and rebers (1975) demonstrated that the sign of acute FC were induced in chickens by injection of fractional amounts of a, nitrogen-containing phosphorylated lipopolysaccharide, endotoxin. The endotoxin was present in the vascular system of turkeys with FC and could be detected with Limulus lysate test and antisrum in the gel diffusion precipitin test. More investigation on the endotoxins as well as heat-labile protein toxins of the isolated bacterium is suggested. Because, free endotoxin can induce active immunity (Heddleston and rebers 1975) and heat-labile protein toxins have been found in serogoup A and D strains islolated from different animal species (Nielsen *et al* 1986).

As we seen in present study, large amount of viscid mucus may be observed in the digestive tract, particularly in the pharynx, crop, and intestine (Glisson *et al* 2003). Mild depletion of the bursa of fabricius was an unusual observation of the present study. This finding may be a pathogenicity characteristic of the Astara vaccinal strain (serotype A1) of *P. mutocida* or as a result of other unknown causes. Therefore, more investigation is needed for confirmation of such findings.

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