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A Comparative Study on the Virulence of *Pasteurella multocida* Local Sudanese Vaccine Strains

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Abstract: The purpose of this study, was to compare the virulence between the two local Sudanese strains of *Pasteurella multocida* B and E. The virulence experiment has been conducted in calves and rabbits. The calves and rabbits were divided into three groups; group (I) was infected with strain B, group (II) was infected with strain E and group (III) was determined as control. The finding of this study, showed that *P. multocida* local Sudanese vaccine strain B is more virulent than strain E.

Key words: Virulence, *Pasteurella multocida*, rabbits, calves, Sudanese strains

INTRODUCTION

Pasteurella multocida (P. multocida) is the etiological agent of Hemorrhagic Septicemia (HS) in cattle, fowl cholera in birds, atrophic rhinitis in pigs rhinitis, pneumonia, otitis media septicemia, meritis and death in rabbits (Mannheim, 1984; Jarvinen *et al*., 1998). In man *P. multocida* infection is usually associated with close contact with pets, such as dogs and cats and it frequently causes localized wound infections, cellulites, meningitis and septic arthritis, endocarditis and peritonitis associated with peritoneal dialysis (Vasques *et al*., 1998; Musio and Tiu, 1998; Layton, 1999).

HS is one of the threatening contagious diseases to the great livestock in Sudan. It is an acute septicemic diseases caused by *P. multocida* serotype B and E. It is endemic disease in Sudan and was reported from all part of the country with a seasonal nature (Shigidi and Mustafa, 1979). The control of this disease is depending on the vaccination with a bivalent bacterin. The vaccine is produced at the Central Veterinary Research Laboratories (CVRL) from these two strains.

MATERIALS AND METHODS

Bacterial strains and growth conditions: The 2 strains of *P. multocida* (B and E) which were used in this study were obtained from the department of bacterial vaccines at CVRL. They had been isolated from different outbreaks of HS in Sudan (Shigidi and Mustafa, 1979). Then they were propagated and kept lyophilized at 20°C for the use in vaccine production. Some phenotypic and genotypic characterization of these two strains had been done in a previous study (Sarah *et al*., 2005).

One ampoule of each strain was aseptically opened and reconstituted in tubes containing 4.5 mL brain heart infusion broth and incubated overnight at 37°C, then checked for purity.

Experimental animals: All the animals which used in this study were local breed and were obtained from the local market. The calves were 9-12 months old and rabbits 4-6 month old. For the virulence experiment 9 calves and 18 rabbits were used. The calves were divided into three groups three of each. The rabbits divided into three groups each of six. Group (I) infected with strain B, Group (II) infected with strain E and group (III) was injected with placebo as control. Animals in all groups were observed after injection at beginning of every hour to record the time of death of the animals.

Rout of administration: All the experimental animals were injected s/c.

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Virulence in calves: The infected dose was 1 mL containing 7.8×10^6 CFU mL^{-1} organisms. This dose represents the LD_{50} (Nagy and Penn, 1976). Calves of group I were infected strain B. Calves of group II were infected with strain E. Group III injected with 1 mL normal saline as placebo. The animals were kept under observation for clinical signs, then after death post-mortem for gross examination was done to examine the changes. Samples were taken aseptically from the lung, heart, liver and tissue fluids and then cultured onto blood agar and then incubated at 37°C for 18 h to recover the organisms.

Virulence in rabbits: The infected dose was 2 mL (OD = 0.15). Rabbits of group (I) were infected with strain B. Rabbits of group (II) were infected with strain E. Rabbits of group (III) were injected with 2 mL of brain heart infusion broth as placebo.

Histopathological examination: Tissue samples were taken from the dead rabbits of group (I) and (II). Samples of lung, liver, kidney and spleen were collected in 10% formalin. They were prepared then stained with hematoxylin-eosin.

Statically analysis: Statically analysis was done by Student's t- Test (www.graphpad.com/quickcalcs/ttest1).

RESULTS

In the infected calves, the following clinical signs were observed, dyspnea, respiratory distress, depression, lameness, swelling at the site of injection and edematous swelling in the head-throat-brisket region and fore limbs followed by recumbence at later stages then death. The post-mortem findings included fluids in the thoracic cavity and the pericardial sac, petechial hemorrhage in many tissues, congested lungs and pneumonic changes, congested lymph nodes. The time of death in the infected calves in case of strain B was: 30, 36 and 40 h, while incase of strain E time of death after infection was: 40, 72 and 96 h (Table 1).

In the infected rabbits, the following clinical signs were observed, dyspnea, respiratory distress, depression, recumbence at later stages then death. In case of strain B the time of death in the infected rabbits was: 10, 11, 11, 12, 13 and 15 h, while incase of strain E the time was: 14, 15, 18, 18, 22 and 25 h (Table 2).

The histopathological lesions in rabbits were as follows; in lung: There was interstitial pneumonia, generalized alveolitis with some hemorrhage and infiltration of leukocytes. The alveolar walls were thickened by proliferation of the epithelial cells. The alveolar septa were distended and filled by extra vasated red blood cells and degenerated erythrocytes and inflammatory cells (neutriophil, lymphocytes). The capillaries (vein)

Table 1: Time of death in calves after infection with *P. multocida* strain B and E (Time h^{-1})

The calves	Time of death in group I (B)	Time of death in group II (E)
Calf No. 1	30 h	40 h
Calf No. 2	36 h	72 h
Calf No. 3	40 h	96 h
Mean	35.3 h	69.3 h

Table 2: Time of death in rabbits after infection with *P. multocida* strain B and E (Time h^{-1})

The rabbits	Time of death in group I (B)	Time of death in group II (E)
Rabbit No. 1	10 h	14 h
Rabbit No. 2	11 h	15 h
Rabbit No. 3	11 h	18 h
Rabbit No. 4	12 h	18 h
Rabbit No. 5	13 h	22 h
Rabbit No. 6	15 h	25 h
Mean	12 h	18.6 h

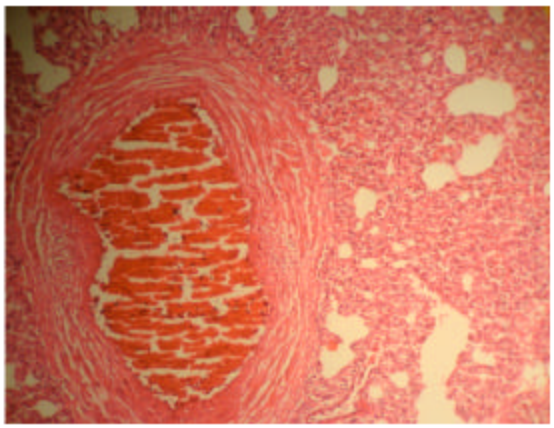

Fig. 1a: Lung of rabbit infected with *P. multocida* srain B

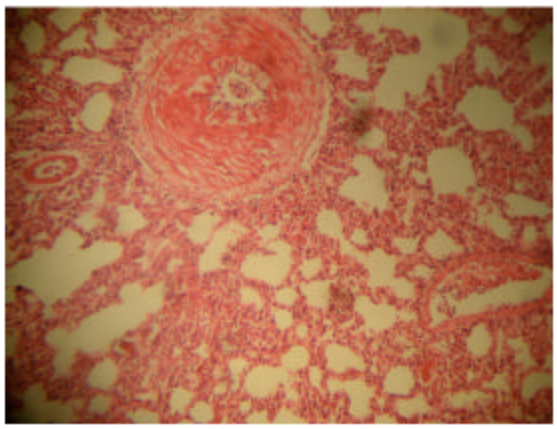

Fig. 1b: Lung of rabbit infected with *P. multocida* srain E

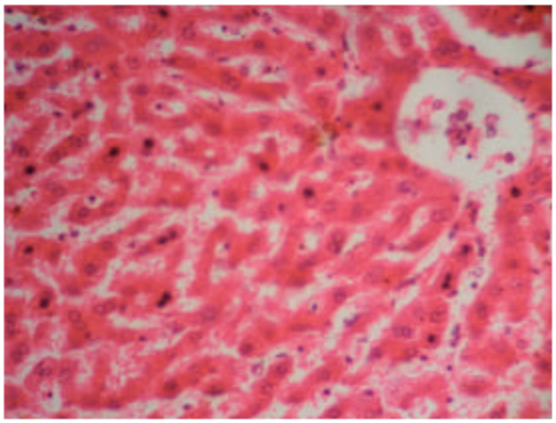

Fig. 2a: Liver of rabbit infected with *P. multocida* strain B

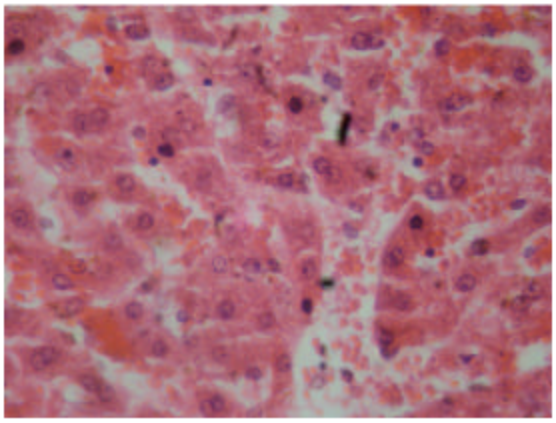

Fig. 2b: Liver of rabbit infected with *P. multocida* srain E

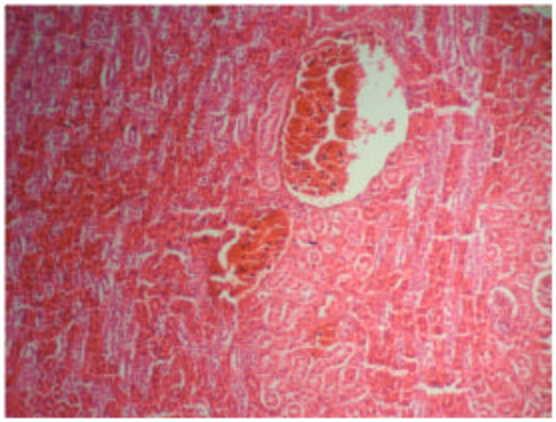

Fig. 3a: Kidney of rabbit infected with *P. multocida* strain B

were dilated and engorged with red blood cells. The artery wall was thick (Fig. 1a and b). In liver: congestion with hemorrhagic necrosis of hepatocytes (Fig. 2a and b).

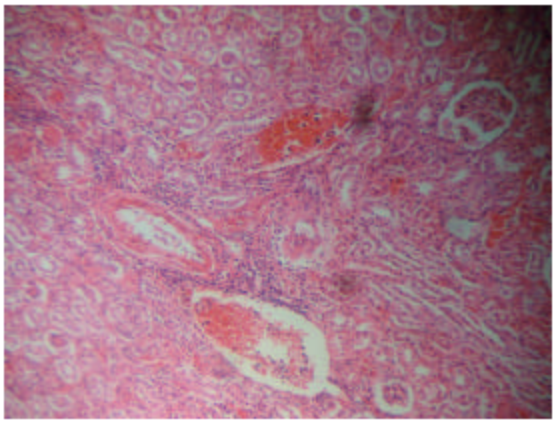

Fig. 3b: Kidney of rabbit infected with *P. multocida* srain E

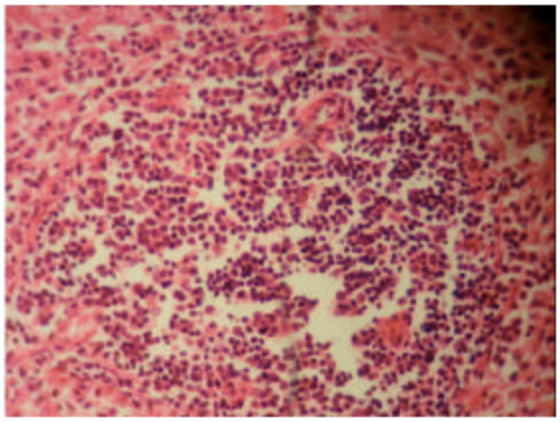

Fig. 4a: Spleen of rabbit infected with *P. multocida* strain B

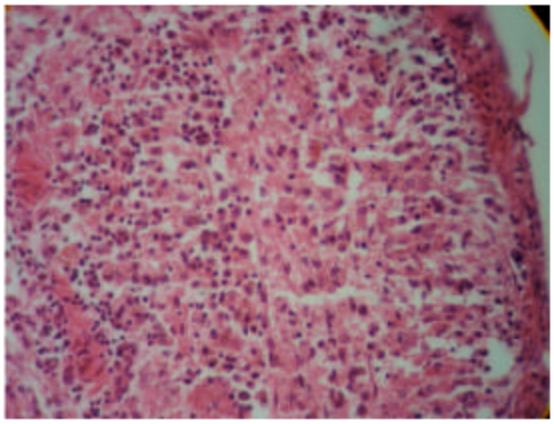

Fig. 4b: Spleen of rabit infected with *P. multocida* srain E

In kidney: Severe hemorrhage, necrosis of tubules with cellular infiltration (Fig. 3a and b). In spleen: Depletion of lymphoid follicles with inflammatory cells (Fig. 4a and b).

All the animals in control groups showed no clinical signs till the 10th day after injection.

DISCUSSION

A bivalent HS vaccine made from the two strains (B and E) of *P. multocida* is usually produced in Sudan. While preparing the seed in rabbits, it was observed that rabbits which infected with strain B always died in shorter time than the rabbits which infected with strain E. To verify this observation this experiment was conducted to determine whether strain B is more virulent than strain E and this was carried out in calves and rabbits.

In the infected calves it was observed that the there were variations in the incubation period and the duration of illness between the two strains. In case of strain B the onset of the clinical signs and the time from infection up to death is shorter (Mean = 35.3 h) than the time of strain E (Mean = 69.3 h) (Table 1). The probability of this results ($p = 0.1081$), this difference is considered to be not statistically significant.

As for infected rabbits, there were clear variations in the time of death between the two groups (Table 2). In case of strain B the time of death after infection is shorter (Mean = 12 h) than the time of death in case of strain E (Mean = 18.6 h). According to this result this difference is considered to be very statistically significant ($p = 0.0049$).

It was observed that the difference between strain B and E in the virulence is not significant in the case of the calves ($p = 0.1081$), while it is very statistically significant in the case of the rabbits ($p = 0.0049$). That may be due to the few number of the used calves in the experiment which referred to the high cost of calves.

The same histopathological lesions were detected in the infected rabbits by strain B and E but the lesions which caused by strain B in group (I) was more severe than that caused by strain E in group (II) in the lung, liver, kidney and spleen (Fig. 1a, b, 2a, b, 3a, b, 4a and b).

From all these findings, we concluded that the local Sudanese *P. multocida* strain B is more virulent than strain E. These observations need more investigations for determination and study of the virulent gene of these strains. The virulence of *P. multocida* strains is depends on the capsule and the toxins (Boyce and Adler, 2000; Adlam and Rutter, 1989) so further research should be done about these components at the molecular level. Also the findings of this study, will help in increasing the knowledge about the epidemiology of HS disease in Sudan.

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