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NON-PULMONARY LESIONS ASSOCIATED WITH A DEMONECROTIC TOXIN-PRODUCING *PASTEURELLA MULTOCIDA* D:3 INFECTION IN RABBITS

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SUMMARY

Six New Zealand White rabbits inoculated intranasally with *Pasteurella multocida* D:3 died acutely as early as day 3 post-infection and developed lesions in the liver, kidney, heart and brain apart from the nasal cavity, trachea and lungs. *Pasteurella multocida* D:3 was isolated from these organs. All affected organs were severely congested. There were diffused hepatocyte degeneration with cytoplasmic vacuolation, particularly those around the portal triad and central veins. The hepatocyte enlargements resulted in narrowing of the sinusoids. Several Kupffer cells that lined the affected sinusoids appeared enlarged. The heart was haemorrhagic and the myocardial fibres were necrotic, atrophied and separated by oedema. Heterophils and mononuclear cells infiltrated the myocardium. The pericardium was thickened with fibrin deposition and heterophil infiltration. Suppurative meningoencephalitis and axonal demyelination were observed in two rabbits that died at day 4 post-infection. Rabbits that survived until day 21 post-infection did not develop the non–pulmonary lesions and had no *P. multocida* in the non-pulmonary sites .

Keywords: Non-pulmonary lesions, toxin, *Pasteurella multocida*, rabbits

INTRODUCTION

Pasteurella multocida not only causes rhinitis and pneumonia in rabbits but has been associated with otitis media, orchitis, metritis, pyometra, abscesses and conjunctivitis. Otitis externa, tympanitis, meningoencephalomyelitis and suppurative mandibular osteomyelitis have also been observed in naturally infected rabbits (Murray *et al.*, 1985). However, the presence of lesions with concurrent isolation of known serotypes of *P. multocida* from the non-pulmonary sites has not been widely studied in rabbits. On the other hand, the presence of lesion at non-respiratory organs has not been consistent with the isolation of *P. multocida* from these organs (Lu *et al.*, 1987; Dillehay *et al.*, 1990). Rabbits infected with *P. multocida* D:3 developed only pulmonary congestion and bronchoalveolitis (Percy *et al.*, 1986) with no evidence of lesions and isolation of *P multocida* in other organs.

This paper reports the pathologic changes in the non-respiratory organs of rabbits infected with a dermonecrotic toxin-producing *P. multocida* serotype D:3.

MATERIALS AND METHODS

Experimental animals

Sixteen *P. multocida*-free rabbits were obtained from the Animal Resource Centre, Universiti Putra Malaysia. The rabbits were housed individually in stainless steel cages and provided with water and rabbit pellet *ad libitum*. These rabbits were negative for *P. multocida* following three attempts of isolation from the nasal cavity.

Pasteurella multocida *inoculum*

P. multocida D:3 isolated from a rabbit with pneumonia was used to infect rabbits in group1. The isolate was maintained on nutrient agar slant (Oxoid) at 25^0 C and was subcultured on 5% horse blood agar at 37^{0}C overnight before one colony was selected and grown in brain-heart infusion (BHI) broth (Oxoid) at 37^{0}C for 18 hours. A mouse was inoculated intraperitoneally with 0.1mL of the cultured broth before the microorganism was re-isolated from the heart blood of the mouse after 24 hours. The isolate was identified as type D by the acriflavin flocculation test (Carter and Subronto, 1973). Serotyping was carried out by gel diffusion and precipitin test (GDPT) as described by Heddleston *et al.* (1972). Sera were raised in 12-16 week old chicken by the method of Brogden and Rebers (1978). The bacterial concentration was determined using the standard plate count method.

Experimental design

The rabbits were divided into 2 equal groups. Rabbits in group 1 were inoculated intranasally with *P. multocida* at a dose rate of 2 x 10^8 cfu/mL of bacteria. Rabbits in group 2 were not infected but were inoculated intranasally with phosphate buffered saline solutions, pH7.4 as control. Surviving rabbits were killed by severing the jugular veins following anaesthesia with 0.5 mL Ketavet (Ketamine hydrochloride; 100mg/mL; Delta Veterinary Laboratories Pty. Ltd., Australia) and 0.2 mL

Romazine (Xylazine; 20mg/mL; Jurox Pty. Ltd., Australia).

Bacteriology

Nasal swabs were collected at three-day intervals for bacterial isolation throughout the experimental period. Swabs were also taken from the nasal cavity, trachea and lung at post-mortem.

Pathology

The rabbits were examined for gross and microscopic lesions. Sections of the nasal mucosa, trachea, lung and various organs were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5um, stained with haematoxylin and eosin and examined for microscopic lesions.

Detection of dermonecrotic toxin

Dermonecrotic toxin was demonstrated according to the procedure described by Carter (1990). An isolate of *P. multocida* D:3 was inoculated into 5mL heart infusion broth containing 0.3% yeast extract and incubated at 37^{0}C for 6 hours. The culture was then sonicated in an ice bath for 5 minutes at an intensity of 55 (Vibra cell, Sonic & Material Inco, USA) and centrifuged at 2000g for 20 minutes at 4^{0}C. The supernatant was filtered through a 0.22 um membrane and a Guinea pig was injected intradermally with 100uL of the filtrate. The necrotic zone that developed was measured after 48 hours. Filtrate of a strain of *P. multocida* known to be negative for the toxin was used as control.

RESULTS

A total of six animals died. Deaths occurred on days 3 (n=1), 4 (n=4) and 9 (n=1) while two surviving rabbits were killed on days 14 (n=1) and 21 (n=1). *Pasteurella multocida* D:3 was isolated, mainly in pure culture, from the liver, kidney, heart and brain of five rabbits that died on day 3, 4 and 9. *Pasteurella multocida* D:3 was also isolated from the nasal cavity, trachea and lungs of these rabbits. The rabbit that was killed on day 14 had *P. multocida* D:3 in the nasal mucosa and brain whereas the rabbit that was killed on day 21 had *P. multocida* D:3 only in the nasal cavity and trachea. *Pasteurella multocida* D:3 was not isolated from any of the organs of the control uninfected rabbits.

Severe congestion was observed in the organs of rabbits with *P. multocida.* There were diffuse hepatocyte degeneration with cytoplasmic vacuolation and dilation of the sinusoids. Some of the degenerated hepatocyes, particularly those adjacent to the central veins, were necrotic and atrophied (Fig. 1). The enlargement of hepatocytes around some portal triads and central veins resulted in the narrowing of the sinusoids. Some of these enlarged hepatocytes were binucleated. Distinct margination of the chromatin of some hepatocytes resulted in a crescent-shaped halo in the nucleoplasm. Kupffer cells lining the sinusoids adjacent to the megahepatocytes were enlarged (Fig. 2).

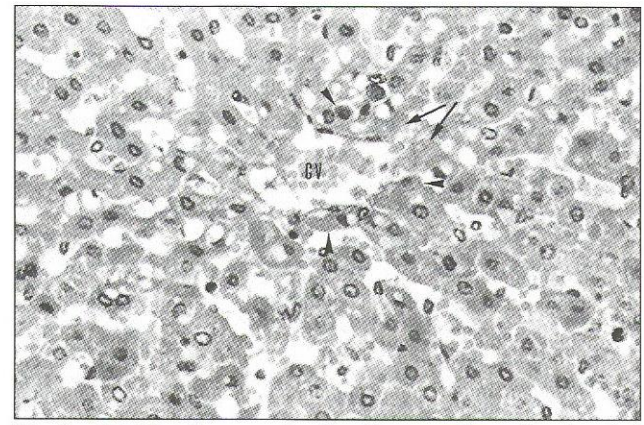

Fig 1. Light micrograph of a liver section of a treated rabbit in group 1 showing hepatocytes degeneration with hepatocytes containing vacuoles in the cytoplasm. The sinusoids adjacent to the degenerated hepatocytes are dilated. Some hepatocytes adjacent to the central vein (CV) are atrophied (arrowheads) and some are karyolytic (arrows). HE x250.

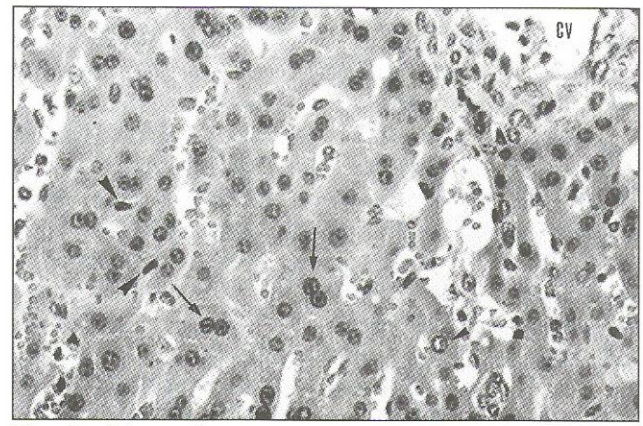

Fig. 2. Light micrograph of a liver section of a treated rabbit in group 1 showing enlarged hepatocytes (megahepatocytes) adjacent to a central vein (CV) which results in narrowing of the sinusoids. Some megahepatocytes are binucleated (arrows) and some have nuclei with distinct margination of chromatin forming crescent-shaped halo in the nucleoplasm (small arrowheads). Note enlargement of several kupffer cells lining the sinusoid of megahepatocytes (large arrrowheads). HE x250.

The heart of all rabbits in group 1 were severely congested and haemorrhagic. The pericardium of two rabbits was thickened with fibrin deposition and heterophils infiltration of which some were necrotic.

The myocardium was infiltrated by heterophils while oedema fluid separated the muscle fibres. Some muscle bundles were atrophied and necrotic with occasional vacuolation of the sarcoplasm (Fig. 3). Cells of the renal tubules of the infected rabbits had swollen cytoplasmic vacuolation. Some of the cells were necrotic and sloughed into the lumen. Infected rabbits had meningeal congestion and two rabbits that died on day 4 p.i. had heterophil infiltrations in the meninges and brain matrix (Fig. 4). Demyelination of the cerebral cortex was also observed.

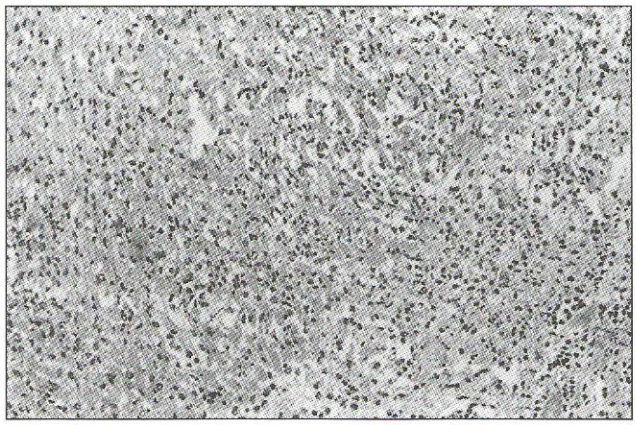

Fig. 3. Light micrograph of a heart section of a treated rabbit showing oedematous separation of the muscle bundles and heterophils infiltration of the myocardium. Atrophy of some muscle bundles are evident. HE x250.

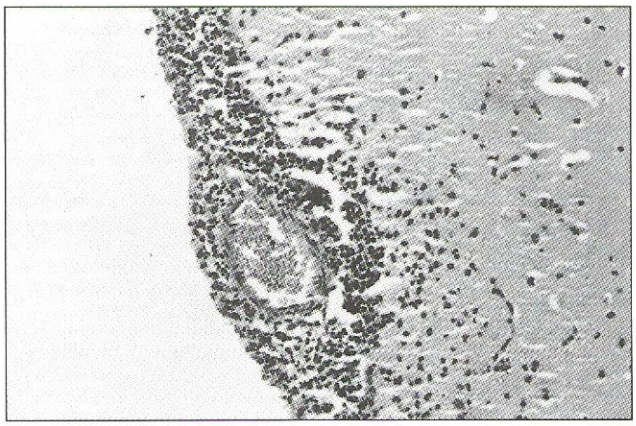

Fig. 4. Light micrograph of a brain section of a treated rabbits showing thickened meninges as a result of severe heterophils infiltration and slight oedema. Note a congested meningeal vessel and heteophils infiltration of the cerebral matrix. HE x150.

The nasal mucosa, trachea and lungs of the infected rabbits were severely congested. Various types of rhinitis and tracheitis were observed in the infected rabbits. Those that died on days 3 and 4 showed acute rhinitis and tracheitis, those that died on days 9 and 14 showed subacute while the rabbits that were killed on day 21 had chronic rhinitis and tracheitis. The two infected rabbits that died on day 4 had fibrinous pneumonia while the rabbits that were sacrificed at days 14 and 21 had chronic suppurative bronchopneumonia.

DISCUSSION

The results of this study shows that *P. multocida* type D:3, administered intranasally, can simultaneously colonise the liver, kidney, heart and brain other than the nasal cavity, trachea and lungs. This was probably due to the septicaemia that developed following the administration leading to acute deaths. This, however, is in contrast with Percy *et al.* (1986) who observed only the pneumonic lesions following infection by *P. multocida* D:3. In another study, rabbits infected with *P. multocida* A:3 developed rhinitis and pneumonia while the bacteria was successfully isolated from the spleen and liver (Dillehay *et al.*, 1990).

Infection to the non-respiratory organs is believed to occur via the circulatory system in the nasal cavity. The cocco-bacillus bacterial cells were observed in the blood vessels of the trachea and lungs (M.H. Al-Haddawi; personal communication). Following the entrance into the circulatory system, the bacteria were then grew into overwhelming numbers and colonised the various organs resulting in lesions and deaths (Collin, 1973; Collin and Woolcock, 1976).

The lesion development and death were the result of toxin production by *P. multocida* D:3, which has been confirmed in guinea pigs (Chrisp and Foged, 1991). In fact, the toxin of *P. multocida* alone can cause lesions in various organs without the presence of the micro-organism at the site of action (Cheville *et al.*, 1988; Cheville and Rimler, 1989; Chrisp and Foged, 1991). The toxin, produced either in the nasal cavity or lung, can be readily absorbed to produce a systemic effect in distant organs (Chrisp and Foged. 1991). However, colonisation of the bacteria in distant organs may occur before the production and absorption of the toxin into the circulation.

The toxin isolated from *P. multocida* D:3 has been shown to cause hepatic necrosis, hepatocyte vacuolation (Chrisp and Foged, 1991), turbinate atrophy (DiGiacomo *et al.* 1991) and splenic lymphoid atrophy leading to lymphoid depletion in rabbits (Chrisp and Foged, 1991). The finding of hepatocyte enlargement is consistent with that reported in pigs exposed to the toxin of *P. multocida* of a non-specific type D derived from pigs with natural atropic rhinitis (Cheville and Rimler, 1989).

Rabbits that survived showed *P. multocida* colonisation and lesions only in the nasal cavity and lungs. This suggests individual susceptibility of the

host to growth and colonisation of the bacteria (Lu *et al.*, 1987; DeLong *et al.*, 1992) and to the effects of the toxin (Chrisp and Foged, 1991). The effects of toxin on various organs depend on the virulence of *P. multocida* serotypes (Jasni, 1998), the availability and affinity of specific receptors to the toxin produced (Rietschel and Brade, 1992). Thus, *P. multocida* D:3 used in this study was a virulent serotype that were able to colonise, cause septicaemia and lesions in various organs resulting in deaths.

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RINGKASAN

LESI BUKAN PULMONARI TERKAIT DENGAN JANGKITAN PASTEURELLA MULTOCIDA *D:3 PENGHASIL TOKSIN DERMATONEKROSIS PADA ARNAB*

Enam ekor arnab New Zealand White diinokulatkan secara intranasum dengan Pasteurella multocida *D:3 mati akut seawal hari 3 pasca-jangkitan dan mengembangkan lesi dalam hati, ginjal, jantung dan otak selain daripada rongga nasum, trakea dan paru-paru.* P. multocida *D:3 telah dipencil daripada organ tersebut. Kesemua organ yang terlibat sebak teruk. Penyahjanaan hepatosit tersebar dengan pemvakuolan sitoplasma berlaku khususnya di sekeliling triad portal dan vena pusat. Pembesaran hepatosit mengakibatkan penyempitan sinusoid. Beberapa sel Kupffer yang terderet pada sinusoid terlibat kelihatan besar. Jantung berhemoraj dan gentian miokardium nekrosis, atrofi dan terasing kerana edema. Heterofil dan sel mononukleus penyusupi miokardium. Perikardium tebal dengan pengenapan fibrin dan penyusupan heterofil. Menginoensefalitis bernanah dan penhaymielinan akson dicerap dalam dua ekor arnab yang mati pada hari 4 pasca-jangkitan. Arnab yang hidup sehingga hari 21 pasca-jangkitan tidak megembangkan lesi bukan pulmonari dan tiada* P. multocida *pada tapak bukan pulmonari*