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Experimental Respiratory Infection of Goats with *Mycoplasma arginini* and *Pasteurella haemolytica* A2

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ABSTRAK

Dua puluh satu ekor kambing baka tempatan yang berumur kira-kira lapan bulan telah dibahagikan kepada empat kumpulan yang terdiri daripada enam ekor dalam setiap kumpulan 1, 2 dan 3, dan tiga ekor dalam kumpulan 4. Kambing dalam kumpulan 1 dan 2 telah dijangkiti dengan Mycoplasma arginini *secara intra-trakea sebelum kambing dalam kumpulan 2 disuntik* Pasteurella haemolytica *A2 enam hari kemudian. Kambing dalam kumpulan 3 disuntik dengan* P. haemolytica *sahaja secara intra-trakea sementara kambing dalam kumpulan 4 disuntik dengan PBS. Kambing dibunuh pada hari ke 1, 3 dan 7 selepas suntikan* P. haemolytica. *Empat ekor kambing dalam kumpulan 1 dan tiga ekor kambing dalam kumpulan 3 menunjukkan tompok-tompok lesi pneumonia yang kurang teruk. Kambing dalam kumpulan 2 menunjukkan lesi pneumonia pasteurelosis yang teruk di bahagian antero-ventral peparu. Tidak seekor pun kambing dalam kumpulan 4 menunjukkan lesi pulmonari.* P. haemolytica *telah berjaya diasingkan daripada kesemua kambing dalam kumpulan 2 dan daripada tiga ekor kambing dalam kumpulan 3. Walau bagaimanapun,* M. arginini *gagal diasingkan.*

ABSTRACT

Twenty-one healthy local goats of about eight months old were divided into four groups consisting of six animals in groups 1, 2 and 3 and three animals in group 4. Goats in groups 1 and 2 were inoculated intratracheally with Mycoplasma arginini. *Goats in group 2 were inoculated again with* Pasteurella haemolytica *A2 six days later. Goats in group 3 were inoculated intratracheally with* P. haemolytica *A2 alone while goats in group 4 received intratracheal inoculation of PBS. The goats were euthanised at day 1, 3 and 7 post inoculation with* P. haemolytica. *Four goats in group 1 and three goats in group 3 had small patches of mild pneumonic lesions. Goats in group 2 had severe lung lesions typical of pneumonic pasteurellosis at the anteroventral region of the lungs. None of the goats in group 4 had pulmonary lesions.* P. haemolytica *was reisolated from all goats in group 2 and from three goats in group 3 but* M. arginini *was not reisolated.*

INTRODUCTION

Pneumonic pasteurellosis caused by *Pasteurella haemolytica* has been recognised as one of the most common diseases of sheep and goats (Gilmour 1980). The disease develops following various stresses on animals, and viral infection in the respiratory tract prior to the infection by *P. haemolytica* has been shown as one of the important causal factors (Davies *et al.* 1981; Buddle *et al.* 1990). While some species of *mycoplasma* have been recognised as primary pathogens of pneumonia in animals, the role of other species in causing lung disease of animals is still unknown (Jones 1983).

Mycoplasma arginini has been isolated from goats that died of pneumonic pasteurellosis in Malaysia, but the significance of this organism in this disease is uncertain (Sheikh-Omar and

Mutalib 1985). The present investigation was designed to study the role of *M. arginini* in the development of pneumonia either alone or in combination with *P. haemolytica* in healthy goats.

MATERIALS AND METHODS

Animals

Twenty-one clinically healthy local goats of about 8 months old were selected for the study. They were divided into four groups; six goats each in groups 1, 2 and 3 and three goats in group 4. The groups were kept in separate rooms and fed daily with cut grass. Drinking water was available *ad libitum*. All goats were healthy; neither *P. haemolytica* nor *M. arginini* were isolated from the nasal cavities for a period of 4 weeks prior to the experimental infection.

Inocula

The *M. arginini* strain used was isolated earlier from the lungs of a goat that had died of severe fibrinous pneumonia (Sheikh-Omar and Mutalib 1985). The organism had been cloned five times. To prepare the inoculum for each goat, 10 colonies of *M. arginini* were cultured in 1 ml mycoplasma broth before they were incubated in carbon dioxide for 7 d.

The *P. haemolytica* used for the experimental inoculation in this study had been isolated from the pneumonic lung of a goat, and was identified as *P. haemolytica* serotype A2. Prior to the inoculation, the organism had been grown in infusion broth and later diluted to 108 colony forming unit (cfu) per ml.

Experimental Design

All goats in groups 1 and 2 were inoculated with 1 ml of the prepared suspension of *M. arginini* intratracheally by inserting a hypodermic needle between the tracheal rings and injecting the inoculum with a syringe. Six days later, all six goats in group 2 were reinoculated intratracheally with 1 ml suspension of *P. haemolytica* A2 according to the method described earlier for *M. arginini*. The goats in group 3 were inoculated intratracheally with *P. haemolytica* alone while goats in group 4 were the control receiving phosphate-buffered saline (PBS).

The body temperature and clinical signs attributable to pneumonia were recorded daily. Two goats each from groups 1, 2 and 3 and one goat from group 4 were euthanised with saturated magnesium sulphate overdose at days 1, 3 and 7 post inoculation (p.i.) with *P. haemolytica*. Post mortem examination was carried out immediately with a detailed examination on the respiratory tract. Samples of the trachea and the lungs were collected for bacterial and mycoplasmal isolation and for histological examination.

Sample Processing

Isolations of *P. haemolytica* from tissue samples were made by inoculation onto 5 per cent blood and McConkey agars, incubated at 37° C for 24 h before the colonies suspected of being *P. haemolytica* were reinoculated onto blood agar and later identified (Lenette *et al.* 1974).

Mycoplasmal isolations were performed essentially as described by Sheikh-Omar and Mutalib (1985). Samples were cultured on PPLO agar base plus mycoplasma supplement-G (Oxoid). The agar plates were incubated in carbon dioxide and examined daily for the typical 'fried egg' colonies. Samples for histological examination were fixed in 10% buffered formalin for at least 24 h embedded in paraffin wax, sectioned at between 4 and 6 um and stained with haematoxylin and eosin.

RESULTS

Clinical Observations

All goats were healthy at the time of inoculation, but six goats were coughing for a few seconds during the intratracheal administration of the inoculum. None of the goats in groups 1, 3 and 4 had high body temperature or clinical signs of respiratory tract infection. Four goats in group 2 were found to be depressed and inactive as early as 24 h following inoculation of *P. haemolytica* A2. After day 3 p.i., the goats were found to have mucoid nasal discharges. The body temperature of all goats, however, remained normal throughout the study period.

Pathology

Group 1

The lungs of goats killed at day 1 p.i. were slightly congested and moderately oedematous. Four to five patches of dark red atelectatic and pneumonic areas of about 0.5 to 2 cm were observed particularly in the lung parenchyma near the terminal bronchi and major bronchioles. These lesions were observed mostly at the anteroventral region of the lungs. Histologically, there was moderate

bronchiolitis consisting of accumulation of a mixture of mononuclear cells and neutrophils in the subepithelial layer. The bronchiolar associated lymphoid tissue (BALT) was slightly hyperplastic and oedema fluid was found in most alveoli. The interalveolar septa were thickened due to hyperaemia and the presence of neutrophils and mononuclear cells.

Grossly, the distribution and size of pulmonary lesions observed in goats killed at day 3 p.i. were similar to those observed earlier at day 1 p.i. but the histological changes were less severe. There was bronchiolitis with marked BALT hyperplasia but the oedema fluid was not obvious and the interalveolar septa were not markedly thickened. Neutrophils were absent but alveolar macrophages were obvious in several areas.

At day 7 p.i. the gross pulmonary lesions were less extensive. Bronchiolitis and BALT hyperplasia were absent in most areas. Changes in the interalveolar septa were similar but milder than those observed at day 3 p.i.

Group 2

The lungs of goats killed at day 1 p.i. had lesions typical of pneumonic pasteurellosis, mostly involving similar lung areas as goats in group 1 (the anteroventral part of the right lung). The lesions that appeared dark red and firm were found to vary in size between 1 and 3 cm. The lungs were moderately oedematous and the distal trachea, bronchi and major bronchioles were markedly congested. Histologically, there was moderate bronchiolitis with mononuclear cells infiltration in the subepithelial layer. Interalveolar septa surrounding the affected bronchioles were slightly thickened due to congestion and presence of neutrophils and oedema fluid.

The ventral two-thirds of the right apical and intermediate lobes of the lungs of both goats killed at day 3 p.i. were dark red and firm. Mucoid exudate was observed on the cut surface of the affected lung parenchyma and from the lumen of bronchioles. The distal trachea, bronchi and major bronchioles were moderately congested. Most alveoli of the affected areas were congested and their spaces filled with oedema fluid and numerous neutrophils. Most bronchioles showed moderate bronchiolitis with similar neutrophilic exudate in the lumen.

The gross pulmonary lesions were most extensive in goats killed at day 7 p.i. The lesions involved the whole anterior one-third of the right lung and the ventral two-thirds of the apical lobes of the left lung; one goat had the right lung partially adhered to the thoracic wall. The cut surface of the lesions showed patches of pale necrotic areas of about 1 to 3 mm. Most affected alveoli were filled with a mixture of numerous alveolar macrophages, neutrophils and fibrin. Some interlobular septa were thickened due to the presence of the exudate. A similar exudate was observed in the lumen of many bronchioles.

Group 3

The lungs of goats in group 3 showed gross lesions similar to those of goats in group 1. There were patches of dark red discolouration in the anteroventral areas of the lungs. Histologically, these lesions consisted of slightly thickened inter-alveolar septa with moderate accumulations of neutrophils and macrophages in some alveoli.

Group 4

No obvious histological lesions were observed in the lungs of goats in this group except several foci of slightly congested areas.

Microbiology

M. arginini was not reisolated from the lungs of any of the goats. Reisolations of *P. haemolytica* were successfully made from the lungs of all goats in group 2 and of three goats in group 3.

DISCUSSION

Most goats developed pneumonic lesions affecting the same lung area, but the extent of the lesions was different. Some of the goats infected with *M. arginini* alone and *P. haemolytica* alone had mild, localised pulmonary lesions which remained relatively similar in extent throughout the duration of the infection. Combined *M. arginini* and *P. haemolytica* infection, however, produced severe and extensive lesions typical of pneumonic pasteurellosis, and the extent of the lesions increased with time of infection. Similar synergestic effects had been described for viruses used in experimental pneumonic pasteurellosis of goats and sheep (Davies *et al.* 1981; Davies *et al.* 1982; Buddle *et al.* 1990).

This study also demonstrated that *M. arginini* can produce only mild lesions in the lung of goats, and thus this may not lead to a serious disease. The organism appeared to be eliminated early as

a result of the inflammatory response following the infection. A similar inflammatory response in pneumonic lungs was observed to eliminate caprine herpesvirus infection in experimental pneumonic pasteurellosis of goats (Buddle *et al.* 1990). Although the mycoplasma was eliminated early, the infection was thought to have induced initial lung lesions for later establishment of *P. haemolytica* in lungs. Most intratracheal infections in goats with *P. haemolytica* alone resulted in either mild pneumonia or the infection being eliminated quickly with no obvious pneumonia (Buddle *et al.* 1990).

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