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THE GOAT AS A MODEL FOR STUDIES OF PNEUMONIC PASTEURELLOSIS CAUSED BY *PASTEURELLA MULTOCIDA*

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SUMMARY

A model of pneumonic pasteurellosis has been established in goats using *Pasteurella multocida* harvested from pneumonic lungs of goats (types A and D), rabbits (type A) and sheep (type D). The resultant infections were acute, subacute or chronic. The gross and histological lesions of the subacute and chronic infections were typical of pneumonic pasteurellosis. *P. multocida* type D produced significantly ($P<0.01$) more severe lesions when compared with other isolates. There were strong correlations between the clinical signs and the severity of lesions.

KEYWORDS: *Pasteurella multocida*; lung infection; goats.

INTRODUCTION

Pneumonic pasteurellosis is caused by either *Pasteurella multocida* or *Pasteurella haemolytica* and affects various species of animals. It is one of the most common diseases of cattle, goats and sheep throughout the world where outbreaks usually lead to high mortality and great economic loss to the ruminant industry (Gilmour *et al.*, 1991; Links *et al.*, 1992). Because *P. haemolytica* is frequently isolated from the lungs of affected animals, most investigations have focused on this species. *P. multocida* types A and D, however, have occasionally been associated with the disease in ruminants (Schiefer *et al.*, 1978; Links *et al.*, 1992).

Infection by *P. multocida* alone and mixed infection involving both *P. multocida* and *P. haemolytica* have been observed to occur naturally in goats (Loganathan & Chandrasekaran, 1992) but little work has been carried out to assess the significance of *P. multocida* in this species. One comparative study of the effects of *P. multocida* type B and *P. haemolytica* serotype A7 infection in goats indicated that more severe lesions were produced by *P. multocida* (Loganathan & Chandrasekaran, 1992). Because infection by *P. multocida* has been reported to occur naturally in

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goats (Loganathan & Chandrasekaran, 1992), and goats have been identified as a suitable animal model for pneumonic pasteurellosis study caused by *P. haemolytica* (Debey *et al.*, 1992), the present investigation was undertaken to assess the value of the goat as animal model for *P. multocida* infection.

MATERIALS AND METHODS

Animals

Twenty clinically healthy goats of about 7 months of age were selected from a farm. Nasal swabs were taken immediately after selection and every 3 days for at least 2 weeks to ensure that the goats were free of *P. haemolytica* and *P. multocida.* The animals were then transported by road to the experimental station where they were divided equally into four groups, kept in separate rooms and fed with cut grass and supplemented feed at the rate of 0.5 kg day^{-1}.

Inoculum

A total of four isolates of *P. multocida* were used: (1) *P. multocida* type A isolated from pneumonic lungs of a goat; (2) *P. multocida* type D isolated from pneumonic lungs of a goat; (3) *P. multocida* type D isolated from pneumonic lungs of a sheep and (4) *P. multocida* type A isolated from pneumonic lungs of a rabbit (Table I). All isolates were collected from freeze-dried stock cultures and grown on blood agar for 24 h. Six uniform colonies were selected and further grown for 18 h at 37°C in brain-heart infusion broths and used as the inoculum.

Experimental procedures

The animals were infected intratracheally with 4 ml of inoculum and observed daily for clinical signs of pneumonia. Daily scores were recorded for each animal as follows: nasal discharge=1; body temperature=2; coughing or dyspnoea=3; death=4. A cumulative score was obtained for each goat before the average daily scores for each goat and group were calculated. Moribund animals or those in

Table I
The average clinical and *post mortem* scores for goats with different inocula of *Pasteurella multocida*

Group	*Inoculum*	*Dose (cfu)*	*Average clinical score*	*Average* post mortem *score*
1	*Pasteurella multocida* type D (goat)	1.6×10^8	3.2 ± 1.1^a	5.0 ± 7.1^c
2	*Pasteurella multocida* type A (goat)	1.5 ± 10^8	2.5 ± 1.2^b	22.0 ± 19.4^d
3	*Pasteurella multocida* type D (sheep)	2.1 ± 10^8	3.0 ± 1.2^a	43.0 ± 9.8^e
4	*Pasteurella multocida* type A (rabbit)	2.3×10^8	2.2 ± 1.0^b	18.0 ± 19.3^d

Values with the same superscript do not differ significantly ($P>0.05$).

Table II
Scoring system for the lesions in respiratory tract

Respiratory lesions	*Points scored*
Pulmonary oedema	10
Pulmonary congestion	10
Hydrothorax	25
Pneumonia:	
5–10% lung lesions	5
11–25% lung lesions	10
>above 25% lung lesions	25

obvious distress within the study period were euthanased. Two weeks post infection (p.i.), surviving goats were killed, and lesions in the lungs measured and scored (Table II) according to the modified method of Gilmour *et al.* (1982), and an average point calculated for individual goats. Samples of lungs, submandibular lymph nodes and heart blood swabs were collected for the recovery of *P. multocida* and biotyping (Carter, 1955).

Lung tissue and tracheal samples were fixed in 10% formalin and 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer at 0°C for 4 h for histological and ultrastructural examinations, respectively. Samples of histological examination were embedded in paraffin, sectioned at 4 μm thick and stained with haematoxylin and eosin (H&E). For ultrastructural study, samples were washed overnight in a cacodylate buffer, post-fixed in 1% osmium tetraoxide, dehydrated in acetone, sectioned and post-stained with lead citrate and uranyl acetate before being examined using a Philip HMG 400 transmission electron microscope. The Duncan multiple range test was used to analyse the data statistically.

RESULTS

Clinical observations

Nineteen of the 20 infected goats exhibited clinical signs of respiratory tract infection. Nasal discharge, which appeared as early as day 3 p.i., was observed only in subacute and chronic cases whereas a rectal temperature >40°C was observed in only nine goats. No goats infected with the caprine *P. multocida* type D survived until the end of the 14 days study period. Three died <8 h p.i., while the remaining two died on days 7 and 8, respectively. Two goats infected with the caprine *P. multocida* type A died <8 h p.i. but the other goats in this group survived. Similarly, two goats infected with the ovine *P. multocida* type D died <8 h p.i., one on day 11 p.i. but the remaining two survived to the end of the study. One goat infected with the *P. multocida* type A rabbit isolate died within 8 h p.i., a second on day 6 p.i. and the remaining goats in this group survived. Goats infected with *P. multocida* type D showed a significantly ($P<0.05$) higher overall mean clinical score (Table I). The differences in the clinical scores of *P. multocida* type A isolated from different animal species were not significant ($P>0.05$).

Gross lesions

All goats, except one infected with caprine *P. multocida* type A, and two goats infected with rabbit *P. multocida* type A, developed various degrees of pneumonia. The goats that died within 8 h developed severe pulmonary oedema and hydrothorax with patches of dark red lesions of acute pneumonia. Pneumonic lesions, 3–18 mm in diameter, were distributed particularly at the anterior portion of the lungs, affecting 5–45% of the lung area. Pulmonary and tracheal congestion were frequently observed in such cases.

Forty percent of the infected goats died within 7 days of infection. They showed signs of either fibrinous pneumonia or fibrinous pleuropneumonia typical of pneumonic pasteurellosis. The lesions affected the anterior portion of the lungs. Survivors showed mild to moderate lesions typical of pneumonic pasteurellosis confined to the anterior lungs. The affected parts appeared firm.

The severity of the lesions produced in infected goats is summarized in Table I. Infection by *P. multocida* type D was found to produce significantly ($P<0.01$) more severe lesions compared with the infections by *P. multocida* type A. Goats infected with caprine *P. multocida* type D were most severely affected, followed by those infected with the ovine *P. multocida* type D, caprine *P. multocida* type A and the rabbit *P. multocida* type A (Table I). There was no significant difference ($P>0.05$) between the severity of lesions produced by either caprine or ovine *P. multocida* type D, and between *P. multocida* type A isolated from either goats or rabbits. In general, there were moderate to strong correlations between the clinical sign scores and the severity of lung lesions for all types of *P. multocida.*

Histopathological lesions

The histopathological lesions could be divided into three types: acute, subacute and chronic. The acute lesions showed marked thickening of the interalveolar septa with prominent and congested capillaries. There was also oedema fluid in the septa and many alveoli. An on-going inflammatory reaction was evidenced by the presence of numerous neutrophils in the congested blood vessels. The subacute lesions were in the form of severe bronchopneumonia with moderate congestion of the interalveolar septa and blood vessels. Most alveoli and bronchioles were filled with a mixture of neutrophils and macrophages whereas some other alveoli were filled with oedema fluid. The chronic lesions were typical of pneumonic pasteurellosis. The lesions consisted of bronchopneumonia with mainly macrophages and some fibrin in the alveoli. Mild to moderate tracheitis was observed only in animals that died within the 8 h period. The lesions consisted of accumulations of neutrophils in the mucosal layer beneath the epithelial cells with occasional focal necrosis of the epithelium and the presence of necrotic debris in the lumen.

Ultrastructural observations

Ultrastructurally an intense hyperaemia with congestion of red blood cells in the blood vessels of the interalveolar septa were seen in acute lesions with evidence of haemorrhages, protein-rich fluid and traces of fibrin in many alveoli. Much of the endothelium was seen to be slightly damaged, the capillaries thrombosed and bacteria were observed in the lumen of many blood vessels. Bacteria

were also readily apparent in the interstitial tissue of the alveolar septa and in the alveolar spaces, particularly in those adjacent to the affected blood vessels. There were numerous neutrophils and an abundance of protein-rich fluid and fibrin in the alveoli of goats that died subacutely <3 days p.i.. Blood vessels of the interalveolar septa were markedly congested. In the chronic lesions, numerous activated macrophages were observed in the alveoli often adjacent to bacteria. Some alveoli were filled with numerous fibrin bundles.

Microbiological observations

The organism used in the inoculum was recovered from lesions in pneumonic lungs in all instances and additionally from the submandibular lymph nodes and heart blood in animals that died <8 h of infection. No isolations were made from the three goats that did not develop lung lesions.

DISCUSSION

The goat has been recognized as a suitable animal model for studies of pneumonic pasteurellosis caused by *P. haemolytica* (Debey *et al.*, 1992) but a suitable animal model for pneumonic pasteurellosis caused by *P. multocida* has not been identified. Gourlay *et al.* (1989) used calves but this species proved uneconomic. The present study suggests that the goat may be a suitable model for the study of pneumonic pasteurellosis caused by both *P. haemolytica* and *P. multocida.*

Inoculation of goats with *P. multocida* isolated from lungs with typical pneumonic pasteurellosis resulted in acute, subacute and chronic infections. Similar infections have also been reported in sheep infected with *P. haemolytica* biotype A, as well as goats infected with *P. multocida* type B (Gilmour *et al.*, 1991; Loganathan & Chandrasekaran, 1992). However, a similar study in calves using *P. multocida* of unknown serotypes isolated from pneumonic lungs of calves failed to produce the acute infection (Gourlay *et al.*, 1989) although acute infection is typical for haemorrhagic septicaemia caused by *P. multocida* type B2 infection in cattle and buffaloes (Graydon *et al.*; 1992).

The design of the present study enabled us to identify clearly the effects of a major field pathogen of ruminant lungs under carefully controlled conditions. Infection with *P. multocida* type D generally produces much more severe lesions compared with infection by either stains of *P. multocida* type A. This is in agreement with a similar study that compared infections caused by *P. multocida* type B and *P. haemolytica* serotype A2 (Loganathan & Chandrasekaran, 1992). The severity of lesions produced by *P. multocida* has been associated with the ability to produce toxins (Erler & Schimmel, 1992), thus *P. multocida* type D is believed to be able to produce toxins more readily.

This study revealed that goats can be used to investigate the various aspects of pneumonic pasteurellosis, particularly in the pathogenesis and vaccination trials that eventually will lead to a better means of treatment and control of the disease.

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