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EXPERIMENTAL PNEUMONIA INDUCED BY A **BORDETELLA PARAPERTUSSIS**-LIKE ORGANISM IN THE OVINE AND MURINE LUNG

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ABSTRACT

Thirty-four specific pathogen-free (SPF) Swiss mice were intranasally inoculated with a suspension containing about 3 x 10^7 colony-forming units (CFU)/ml of a **B. parapertussis**-like organism isolated from pneumonic ovine lung. Eleven per cent of the animals died between 2 and 3 days of inoculation and over 90% of infected mice developed a subacute bronchopneumonia morphologically similar to early lesions of naturally-occurring ovine chronic non-progressive pneumonia (CNP). The sequential pulmonary changes were examined by light microscopy and transmission electronmicroscopy from 12 hr to 29 days after inoculation. The early stages were characterized by alveolar septal congestion and oedema, focal intra-alveolar haemorrhage, and intra-alveolar and septal infiltration by neutrophils and macrophages. Later, hyperplasia of perivascular and peribronchiolar lymphoid tissue and the deposition of collagen in the interalveolar septa were prominent. The bronchial and bronchiolar epithelium remained intact throughout the experiment, but bronchiolar lumina became occluded by inflammatory exudate at an early stage. Ultrastructural changes consisted of the degeneration of the alveolar type I and type II epithelial cells and marked degeneration of alveolar macrophages. Pure cultures of the **B. parapertussis**-like organism were consistently recovered from mouse lungs 12 hr to 6 days after inoculation. Both intact and degenerating organisms were found free in alveolar spaces and within phagocytic vacuoles of alveolar macrophages. However, replication of organisms was not observed at any stage of infection and no special association was observed between organisms and the ciliated or non-ciliated respiratory epithelium.

Injury to ovine respiratory tract was demonstrated when a similar bacterial suspension to that given to the mice was given by intratracheally to colostrum-deprived lambs. The lesions produced in the pulmonary parenchyma of the lambs were similar to those seen in both early naturally-occurring ovine CNP and the experimental infection with this organism in mice. They consisted of an acute

mild tracheobronchitis, severe alveolar collapse and acute bronchopneumonia which developed within 24 hr and was most severe at 1 to 3 days after inoculation. Ultrastructurally, the alveolar epithelium exhibited extensive degenerative changes and necrosis of individual epithelial cells. Topographical studies revealed extensive coverage of the infected tracheobronchial epithelium with a dense layer of inflammatory cells mixed with mucus, and focal extrusion of ciliated cells. Occasionally, moderate numbers of the **B. parapertussis**-like coccobacilli were seen closely associated with cilia. Inoculated lambs showed a marked elevation in the numbers of cells in bronchoalveolar lavage 24 hr after infection. Up to 93% of the cells in the lavage at 24 hr were neutrophils. However, no close interation between phagocytic cells and the organisms was detected. Many of the macrophages in the lavage exhibited cytoplasmic vacuolation from five days after inoculation onwards. Blood leucocyte and neutrophil counts in infected lambs gradually rose to reach peaks at five and three days after inoculation, respectively. The **B. parapertussis**-like organism was recovered in pure culture from the nasal cavity of lambs killed on days one, three, five and nine. The viable bacterial count in bronchoalveolar lavage fluid decreased from 24 hr to 5 days with almost complete elimination of organisms nine days after inoculation.

The retention of the **B. parapertussis**-like organism in the mouse trachea was compared to that in the mouse lung from 0 to 48 hr after intranasal inoculation. Although there was greater bacterial deposition in the trachea than the lung there was a faster clearance from the trachea. At 48 hr after instillation, almost all organisms were eliminated from the trachea but about 45% of organisms were retained in the lung.

The current investigation has shown that the **B. parapertussis**-like organism can infect SPF mice and colostrum-deprived lambs and induce a subacute bronchopneumonia. The morphological changes seen suggest that this organism has the potential to predispose the ovine respiratory tract to further infection by other microorganisms and that the organism itself may also be able to cause severe pulmonary damage. The relevance of these observations to the problem of CNP in sheep in the field has yet to be determined.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Pneumonia is one of the most important infectious diseases of sheep, especially feedlot lambs, in sheep-raising countries throughout the world. As New Zealand is one of the largest exporters of lamb in the world, ovine pneumonia is of special economic importance in this country. The disease can infect animals whether they are fattened intensively indoors, are grazed extensively on pasture for all or part of the year, or are reared under nomadic conditions (Davies, 1985).

Ovine pneumonia in New Zealand is usually divided into two forms (Alley, 1975a). One is an acute fibrinous pneumonia (AFP) as has been described by Salisbury in 1957, and the other more common form is chronic non-progressive pneumonia (CNP) which was described initially by Alley (1975a) and comprehensively studied **in vivo** and **in vitro** by Alley (1975a & b) and Al-Kaissi (1986).

Fortunately, outbreaks involving mortalities from acute pneumonia in sheep are sporadic in New Zealand, so overall losses are low (Manktelow, 1984) although in some individual flocks mortality may reach up to 47% (Sorenson, 1976). The disease was second only to pregnancy toxaemia as a cause of death in a survey conducted in 1974 (Davies, 1974) and caused 1-8% mortalities of sheep 3 years and older according to surveys by Salisbury (1957) and Downey (1957). The surviving animals may lose an average of 1.4 kg body weight per animal and 0.28 kg wool per animal (Nikitin et al., 1981).

The most commmon form of ovine pneumonia in this country is CNP and up to 70 to 80% of the lambs in some flocks may be affected (Alley, 1975a). Since CNP is usually subclinical, it is difficult to estimate its real economic importance. The initial studies in this country showed that when lambs were affected at an early age, survivors were likely to take longer to reach a predetermined weaning weight (Kirton et al., 1976). More recent weight gain trials

have shown that under pastoral conditions, lambs affected by CNP had a mean liveweight gain of 1.74 kg less than controls after 30 days, and 2.19 kg after 60 days (Alley, 1987). In poor growing conditions, some affected animals even lost weight. A significant linear relationship was established between liveweight gain and the extent of the pneumonic lesions.

Another important indirect economic loss is the development of pleural adhesions, a common sequela in both AFP (Alley, 1975a) and CNP (Jones & Gilmour, 1983). Carcasses with pleural adhesions, are unacceptable in several major overseas markets such as the U.S.A., Canada, and European Economic Community (EEC), and are therefore downgraded (Brain, 1980). The pleural adhesions may sometimes account for 31.4% of the carcass defects, second only to sarcocystis (Central Districts Farmer, 1985). The annual loss attributed to pleurisy alone in the industry in New Zealand, excluding the cost of treatment or preventive measures, has been estimated at 1.8 million New Zealand dollars in 1974/1975 season (Dysart, 1976), and 26 million in 1983 (Alley, 1983).

The aetiology and details of the pathogenesis of CNP have however, not been unequivocally determined. It is currently believed that the disease is multifactorial, and that the presence of bacteria is essential for the development of the lesions (Alley & Clarke, 1979; Jones & Gilmour, 1983; Davies, 1985). A wide range of bacteria have been isolated from pneumonic ovine lungs (Steveson, 1969; Alley, 1975b; Robinson, 1983; Davies, 1985), but the aetiologic importance of many of them is doubtful. Recently, a **Bordetella parapertussis**-like organism was isolated from both pneumonic and healthy ovine lungs as well as the nasal cavities of healthy ewes (Manktelow, 1984; Alley, 1986; Cullinane et al., 1987). The organism was demonstrated to be able to attach and damage the ovine ciliary epithelium in tracheal organ culture (Al-Kaissi, 1986) and it was proposed that this organism may have a role in initiating or prolonging CNP in sheep in New Zealand (Al-Kaissi et al., 1986).

The objective of the current research was to study the possible role and pathogenesis of the **B. parapertussis**-like organism in CNP and to accumulate preliminary information on its pathogenicity. A

three-step project was designed, aimed at firstly establishing a laboratory animal model to study the pathogenesis of any disease caused by the organism. Secondly, to determine the pathogenicity of the organism for colostrum-deprived lambs and finally to investigate the persistence of the organism in the trachea and lungs of SPF mouse.