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THE ROLE OF *MYCOPLASMA* SPECIES IN BOVINE RESPIRATORY DISEASE COMPLEX IN FEEDLOT CATTLE IN SOUTH AFRICA

By

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DEDICATION

This thesis is dedicated to:

my wife Dawn for the love and support through all the years;

my kids Beverly, Hilary and Shaun who often had to give up some of the pleasures of life as I followed a dream;

and my parents who always encouraged me to follow that dream.

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DECLARATION

I hereby declare that the work done in this dissertation is my own work except where acknowledgements indicate otherwise and for the advice from my promoter. Neither the full dissertation nor any part of it is to be submitted for another degree at this or any other University

CAP Carrington

Résumé

Bovine respiratory disease complex (BRD) consists of a largely single clinical entity of bronchopneumonia that is usually associated with the assembly of large numbers of especially weaner cattle into a feedlot environment. It has a multifactorial aetiology and develops as a result of complex interactions between environmental factors, host or animal factors and pathogens. It is often difficult to determine the exact role played by the various pathogens involved in an individual outbreak of disease. None of the many organisms isolated will on their own, reliably reproduce the natural disease in experimental animals. Observations from research studies and clinical experience have indicated that the presence of mycoplasmas increases the severity of respiratory disease. However, the role of *Mycoplasma* spp. in BRD complex as a primary or secondary pathogen remains controversial.

The various stresses associated with the feedlot causes a breakdown of the defense mechanisms that normally hold the nasal infections in check, resulting in a rapid proliferation of virulent *Mannheimia haemolytica* serotype A1 in particular and the spread to the lower respiratory tract. The various viruses and mycoplasmas have however been shown to have the same effect as stress on the *Pasteurella* populations of the nasal mucosa.

More than 10 species of *Mycoplasma* have been isolated from the bovine respiratory tract, but not all are pathogenic. They are able to act as a stress-causing agent, leading to a decreased host defense mechanism by altering the immune responsiveness or by causing tissue damage and thereby allowing bacteria to invade and colonise the lung and so causing a severe pneumonia. *M. bovis* and *M. dispar* are the more important lung isolates, with *M. bovis* being the most invasive and destructive and has been shown to increase the severity of calf pneumonias. *M. bovis* has been isolated from bovine pneumonias, arthritis, mastitis, tendosynovitis, genitalia, keratoconjunctivitis and is considered to be the primary pathogen in endemic pneumonia in younger calves.

According to the literature, mycoplasmas are isolated from 25% to 80% of pneumonic lungs in feedlot cattle and the aim of the study was to identify the isolation rates in South African feedlots over a period of 2000 to 2004. To achieve this, 446 transtracheal aspirates (TTA's) were collected from more than 25 feedlots around South Africa, except for the western Cape. Collection criteria included: pulled for respiratory disease; febrile (≥40ºC); depressed; anorexia and/or lack of rumen fill; nasal discharge or failure to clean muzzle; cough; increased respiratory rate >40 and most importantly, no prior treatment. Samples were also collected from 31 'healthy' animals as controls. Samples collected were used for *Mycoplasma* isolations, as well as the aerobic bacteria to establish an antibiogram profile of bacteria commonly isolated in cattle feedlots.

Mycoplasma spp. were isolated from 52.8% of samples taken from sick animals, with 67 out of 201 isolates (33.3%) being identified as *M. bovis.* According to the literature, *M. bovis, M. haemolytica* or *P. multocida* are isolated from bronchial lavage fluids from healthy calves in only a few cases, with estimates being put at 5 – 10% levels for *Mycoplasma*. Isolation rates of *Mycoplasma* spp. from healthy animals in this study was 22.7%, which was considerably higher than anticipated and could possibly be due to problems with the definition of a healthy animal. Although the number of samples from healthy animals was relatively small in this study, it was possible to show that there was a statistically significant association between *Mycoplasma* isolation and respiratory disease, p = 0,001 and with an odds ratio (OR) of 3,75 in cattle from those feedlots included in the study and thereby proving the hypothesis put forward.

Samevatting

Beesasemhalingsiekte kompleks is hoofsaaklik 'n enkele kliniese entiteit van brongopneumonie wat gewoonlik met die samekoms van groot getalle van veral speenkalwers in 'n voerkraal omgewing geassosieer word. Dit het 'n multifaktoriale etologie en ontwikkel as gevolg van 'n komplekse interaksie tussen omgewingsfaktore, gasheer of dierlike faktore en patogene. Dit is dikwels moeilik om die presiese rol van die verskeie patogene in 'n siekte uitbraak te bepaal. Geen een van die baie organismes wat geisoleer kan word, kan op hulle eie die natuurlike siekte op 'n gereelde basis in eksperimentele diere veroorsaak nie. Alhoewel navorsingsresultate en kliniese ervaring aandui dat die teenwoordigheid van mikoplasmas die graad van asemhalingsiekte kan vererger, bly die rol van *Mikoplasma* spesies in die asemhalingsiektekompleks as 'n primêre of sekondêre patogeen kontroversieël.

Die verskeie stresfaktore wat met voerkrale geassosieer is, lei tot die afbreek van die natuurlike verdedigingsmeganismes in die boonste asemhalingsweë, met 'n vinnige vermenigvuldiging van veral *Mannheimia haemolytica* serotipe A1 en 'n verspreiding tot in die onderste lugweë. Dit is aangetoon dat die verskillende virusse en mikoplasmas dieselfde effek uitoefen as stres op die *Mannheimia/ Pasteurella* populasies van die neusslymvlies.

Meer as tien *Mikoplasma* spesies is in die beesasemhalingstelsel geisoleer, maar hulle is nie almal patogenies nie. Hulle is in staat om as 'n stres-veroorsakende agent op te tree, wat aanleiding kan gee tot 'n verlaagde verdedigingsmeganisme, hetsy deur die

immuunrespons te onderdruk, óf deur weefselbeskadiging en daardeur toelaat dat bakterieë die longe kan binnedring en pneumonie veroorsaak. *M. bovis* en *M. dispar* is die belangrikste van die longisolate by beeste. *M. bovis* is die mees patogeniese en het bewys dat dit die graad van kalfpneumonie verhoog. *M. bovis* is geisoleer by beeste met longontsteking, artritis, mastitis, tenosinovitis, geslagsorgane, keratokonjunktivitis en word beskou as die primêre patogeen by endemiese pneumonie by jong kalwers.

Volgens die literatuur, word mikoplasmas vanaf 25% tot 80% van pneumonielonge by voerkraalbeeste geisoleer en die doel van hierdie studie was om die isolasie tempo by Suid Afrikaanse voerkrale te bepaal vir die periode 2000 tot 2004. Om dit te verrig, is 446 transtrageale-aspirate geneem by meer as 25 voerkrale in Suid Afrika, uitsluitend die Weskaap. Kriteria wat gebruik is vir bemonstering sluit in: duidelike tekens van asemhalingsiekte; koors (≥40ºC); depressie; anoreksie en/of leë rumen; neusuitloopsel of versuim om die snoet skoon te maak; hoes; verhoogde respirasiekoers (>40) en mees belangrik, geen vorige behandeling. Monsters is ook by 31 'gesonde' diere geneem as kontroles. Monsters is geneem vir Mikoplasma isolasies, sowel as vir aërobiese bakterieë sodat 'n antibiogram profiel vir beesvoerkrale opgestel kon word.

Die resultate toon dat 52.8% van monsters vanaf siek diere mikoplasmas bevat het, met 67 uit 201 isolate (33.3%) geidentifiseer as *M. bovis*. Volgens die literatuur, word *M. bovis, M. haemolytica* of *P. multocida* slegs by enkelle gevalle in brongiale vloeistof by gesonde diere geisoleer, met 'n raming van 5–10% vir Mikoplasma. Isolasietempo vir Mikoplasma spp. by gesonde diere in hierdie studie was 22.7%, wat aansienlik hoër as verwag was en moontlik 'n aanduiding mag wees van probleme met die definisie van gesonde diere. Alhoewel die monsters vanaf gesonde diere

relatief min was, was dit moontlik om ‘n statisties betekenisvolle assosiasie tussen Mikoplasma isolasie en longontsteking te bewys (p=0,001) en ‘n waarskynlikheids verhouding van 3,75 in beeste vanaf die voerkrale ingesluit in die studie en daarby die hipotese te bewys.

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Abbreviation

ADG	=	average daily gain
ARDS	=	acute respiratory distress syndrome
AUBRD	=	acute undifferentiated bovine respiratory disease
AUC	=	Area under the curve
BAL	=	bronchoalveolar lavage
BALT	=	bronchus-associated lymphoid tissue
BCV	=	bovine coronavirus
BHV-1	=	bovine herpesvirus type 1
BRCV	=	bovine respiratory coronavirus
BRD(C)	=	bovine respiratory disease (complex)
BRSV	=	bovine respiratory syncytial virus
BVDV	=	bovine viral diarrhoea virus
CI	=	confidence interval
CBPP	=	contagious bovine pleuropneumonia
CO_2	=	carbon dioxide
COX	=	cyclo-oxygenase
C_{max}	=	maximum concentration
CVBP	=	cranial ventral bronchopneumonia
DCP	=	dairy calf pneumonia
DNA	=	deoxyribonucleic acid
ELISA	=	enzyme-linked immunosorbent assay
EP	=	endemic pneumonia
EU	=	European Union
FA	=	direct fluorescent antibody test

FCA	=	Freund's complete adjuvant
FDO	=	median fatal disease onset
HSS	=	hypertonic saline
IBR	=	infectious bovine rhinotracheitis
IF	=	immunofluorescence
IFA	=	indirect fluorescent antibody test
Ig	=	immunoglobulins
im	=	intramuscularly
it	=	intratracheally
Km	=	kilometers
LOS	=	lipo-oligosaccharide
LPS	=	lipopolysaccharide
mg/kg	=	milligrams per kilogram bodymass
MIC	=	minimum inhibitory concentration
ml	=	millilitres
mm	=	millimetres
MMP	=	major mastitis pathogens
NaCl	=	sodium chloride
NCCLS	=	National Committee for Clinical Laboratory Standards
NO	=	nitrogen monoxide
NPS	=	nasopharyngeal swabs
NSAID	=	non-steroidal anti-inflammatory drugs
OIE	=	Office des Epizooties
OR	=	odds ratio
PaO_2	=	partial pressure of oxygen
PBS	=	physiologically buffered saline

PCA	=	procoagulant activity
PCR	=	polymerase chain reaction
PG	=	propylene glycol
PI	=	persistently infected
PI-3	=	parainfluenza virus type 3
PMN	=	polymorphonuclear cells
PVP	=	polyvinylpyrrolidine
RNA	=	ribonucleic acid
RSV	=	respiratory syncytial virus
RR	=	risk ratio
RV	=	rhinovirus
sc	=	subcutaneously
SCC	=	somatic cell count
T(E)ME	=	thromboembolic meningoenchephalitis)
TNF-α	=	tumour necrosis factor-α
TTA/TTW	=	transtracheal aspirate / washes
UF	=	undifferentiated fever
UK	=	United Kingdom
USA	=	United States of America
µg/ml	=	microgram per millilitre of fluid
µm	=	micrometres
V_d	=	Volume of distribution
vsp	=	variable surface lipoproteins
Vsps	=	variable membrane surface lipoproteins

CHAPTER 1.

LITERATURE REVIEW

BOVINE RESPIRATORY DISEASE COMPLEX

1.1. INTRODUCTION

Bovine respiratory disease complex (BRD) consists of a largely single clinical entity of bronchopneumonia that is usually associated with the assembly of large numbers of especially weaner cattle into a feedlot environment. It has a multifactorial aetiology and develops as a result of complex interactions between environmental factors, host or animal factors and pathogens[13,20,52,101,143,176,186,203,285,292]. It is often difficult to determine the exact role played by the various pathogens involved in an individual outbreak of disease [95,203,251], and most of these agents, including *Mannheimia haemolytica* (formerly *Pasteurella haemolytica* biotype A), appear to be insufficient, singly, to produce BRD except under contrived laboratory conditions [157]. The numerous infectious agents associated with BRD are ubiquitous in the cattle population and the bacteria most often associated with pneumonic lesions are part of the normal resident flora of the nasopharynx of cattle[13,20,101]. Individually, these pathogens do not appear capable of causing severe disease in healthy cattle [251]. Observations from research studies and clinical experience have indicated that the presence of mycoplasmas increases the severity of respiratory disease. The role of *Mycoplasma* spp. in BRD complex as a primary or secondary pathogen however remains controversial [234].

BRD needs to be distinguished from dairy calf pneumonia (DCP) or endemic calf pneumonia (ECP) which has traditionally been described as affecting dairy calves in particular from 2 to 6 months of age and more common in housed calves than those raised outside [12,13,20,203].

Although *M. haemolytica* is the most frequently isolated organism in BRD, there are other organisms such as *Mycoplasma* spp. which are increasingly being isolated from clinically ill animals and cattle that have died from BRD. Whether they are primary aetiologic agents or secondary pathogens, their isolation, identification and *in vitro* susceptibility to antimicrobials are essential for selection of an appropriate therapeutic strategy. It has been stated that the assumption that *M. haemolytica* is the only important causative bacterium causing BRD may lead to inappropriate antibiotic selection and high treatment failure rates [51].

The current increasing importance and prevalence of quality assurance schemes, encompassing better animal health monitoring and promoting preventive medicine programmes, emphasises the need to develop and monitor alternative strategies of disease control [26,27,69]. This could include the need to correctly identify the organisms playing a role in BRD and the development of vaccines. Vaccines are currently available for the major viruses implicated, as well as *M. haemolytica, Pasteurella multocida* and *Histophilus somni* (formerly *Haemophilus somnus*), but not for the mycoplasmas.

Mannheimia haemolytica, P. multocida and *H. somni* are considered to be normal inhabitants of the nasal pharyngeal mucosa [13,292], but not of the lung and are considered to be “opportunistic pathogens”. *M. haemolytica* serotype 1 is considered to be the major cause of BRD but is rarely isolated on nasal swabs in healthy animals. The various stresses associated with the feedlot causes a breakdown of the defense mechanisms that hold the nasal infections in check, resulting in a rapid proliferation of virulent *M. haemolytica* serotype 1 and the spread to the lower respiratory tract. This is the most common and virulent serotype, but other serotypes have also been implicated. It is also the one on which most of the experimental work has been done. The various viruses and

mycoplasmas have been shown to have the same effect as stress on the pasteurella populations of the nasal mucosa [13,24,292]. Once the bacterial pathogens become established in the lungs, there is an interaction between the bacteria and the host defense.

Mannheimia haemolytica serotype1 is considered to be the primary bacterial pathogen in BRD in the United States. In a survey of lung samples, *M. haemolytica* was isolated in 49.7% of the cases, *Mycoplasma* spp. in 33.3%, *P. multocida* in 14.7%, *A. pyogenes* in 5.7%, *H. somni* in 5.5% and *Salmonella* spp. in 3.2%. Mycoplasmas in this survey were all isolated in conjunction with one of the other pathogens [186,282]. The respiratory viruses usually act in combination with other infectious agents, in particular bacteria, in the production of respiratory disease. *Mycoplasma haemolytica* 1 was capable of causing disease as a primary pathogen, firstly in calves that are susceptible or non-immune and secondly in the logarithmic growth phase when administered to the trachea or lungs of calves [222].

The clinical signs of BRD are characterized by fever, coughing, depression, anorexia, weight loss and death. All ages and types of cattle can be affected, but the highest morbidity and mortality rates occur in feedlots [95].

It is now recognised that there are considerable similarities between the pulmonary pathophysiology of human adult respiratory distress syndrome (ARDS) and BRD. In both diseases, neutrophil infiltration, fibrin deposition and thrombi associated with local areas of necrosis are consistent observations [55,60].

Bovine respiratory disease is one of the most economically important diseases of beef cattle and is known under various guises as Bovine Respiratory Disease Complex (BRD), Acute

Undifferentiated BRD (AUBRD)[5,157], Undifferentiated fever (UF –animals that are febrile with a lack of abnormal clinical signs referable to organ systems other than the respiratory system)[32,33], 'shipping fever', 'pneumonic pasteurellosis'[171,176], 'transit fever' [16] and 'stockyards pneumonia' [24,171]. The very fact that no uniform terminology is accepted for the disease hints at the problem in identifying the precise agents involved [47,292], thereby correctly reflecting the complexity of the condition. It is the single most important cause of morbidity and mortality among feedlot cattle worldwide and reported to account for up to 65% of diseases in feedlots, with 15 to 45% of animals entering a feedlot requiring treatment for BRD and 0 to 5% dying, with the peak incidence of disease within the first three weeks after arrival in the feedlot [4,13,14,16,20,52,80,82,95,96,99,103,131,157,176,186,203,207,209,292]. In one survey, 58% of the lower respiratory tract cases developed in the first 40 days on feed, with the peak incidence prior to 20 days on feed [4]. In a retrospective study in Canada, it was found that 75% of calves that died from fibrinous pneumonia were already sick within two weeks of arrival in a feedlot. The median was 19 to 22 days with peak mortality at day 15, indicating that preventative measures should ideally be instituted before arrival [207]. The peak incidence of BRD occurs so reliably that feedlot operators concentrate their attention on the new arrival calves up to three weeks to allow for earliest detection and prompt treatment. Morbidity and mortality rates are highest in autumn, less in winter and least in spring and summer [131,203].

1.2. ECONOMICS

BRD is reported to be the most costly disease of beef cattle and the annual loss to respiratory disease is higher than any other cause of animal death [96,126,143,186,227,285,288,292]. It has been estimated that 1,3 million cattle are lost to the U.S. market as a result of respiratory disease [143]. More recent estimates on the costs of infectious respiratory disease to the US beef industry is $ 1 billion per annum[96,285], while the cost of preventative

measures and treatment are estimated at $ 3 billion[96,227,288]. The cost to the Dutch cattle industry is US$ 75 million and £ 80 million per annum to the UK cattle industry[26]. Estimates are that non-fatal production losses alone may cost the European cattle industry up to €576 million a year[25,26]. In spite of major advances in respiratory vaccines, pharmaceuticals and management systems, the problem still remains [227].

In a large survey conducted in Colorado (58 000 head of cattle over a 12-month period), the total cost of lower respiratory tract diseases contributed approximately 46% of the total disease costs in terms of deaths, culling losses and treatment costs, but did not include loss of production costs [80]. The majority of estimates of the cost of respiratory disease are based on morbidity and mortality, usually based on the clinical appearance of the animals, the assumption being that clinically normal animals are not affected by the disease, and do not contribute to the economic impact of the disease outbreak. It has however been shown that many animals exposed to respiratory pathogens will seroconvert and might be sub-clinically affected, which has negative effects on performance and these costs are likely to be underestimated [26,27]. Griffin has shown that 50% of cattle that had never been diagnosed as having BRD, had lung lesions at slaughter[96]. This study also showed a significant difference in average daily gain (ADG) between those with and without lung lesions and might account for why there is often so little difference in growth rates between pneumonic and clinically healthy control cattle. Affected and control animals should be grouped on the basis of their lung lesions rather than clinical signs. These results also underline the economic and welfare importance of disease prevention over and above treatment [25].

Numerous workers have looked at a lung scoring system at slaughter, and tried to correlate this with growth performance of the calves. It has been shown that lesions resulting from

cranial ventral bronchopneumonia (CVBP) are significantly associated with growth performance [40]. Cattle with lung lesions had a 0,06 to 0,3 kg lower ADG [40,185], while Wittum *et al* reported a 0,08 kg/day lower ADG [288] than animals without lesions. Given the large proportion of animals observed to have lesions, this represents a substantial production loss from feedlots. Conversely, 22% of calves treated for pneumonia had no lesions at slaughter [185]. In another study, it was found that there was a 0,14 to 0,23 kg decrease in ADG in calves that required two or more courses of therapy for BRD [227], with reports of up to 0,33 kg loss daily. This translates into 40 kg over a 120-day feeding period or nearly SA Rand 500 loss per head that are repulled. Using a very conservative estimate of a 25% morbidity and a loss of 10 kg per treated head, this would represent a loss of Rand 45 million to the South African feedlot industry, which is a figure put forward previously by the industry, but is probably closer to R 70 million [153,168]. These losses do not include factors such as longer days on feed, and lower carcass gradings [39,82,96,97], as data suggests that catabolic events, such as BRD, can have long term effects on carcass quality and decreased marbling scores. Treated animals had lower ($p<0,05$) final live mass, ADG, hot carcass mass, less external and internal fat and lower marbling scores [82,227,238]. In another survey however, there was no significant difference between 'sick' and 'well' groups for growth performances nor carcass qualities, possibly because the sample size was not big enough [126]. In the Texas Ranch to Rail study, 28% of cattle received treatment for BRD and there were 2,88% mortalities. When compared to cattle not diagnosed with BRD, sick cattle gained 7% and weighed 3% less at shipping, cost 18,5% more to feed and 25% fewer graded "choice" [96]. In a similar study, heifers treated for BRD had lower marbling scores resulting in a 37,9% reduction in the percentage of choice grade carcasses [238].

In a slaughter-house study, so-called mycoplasma-like lesions were found in 37% of the animals and the authors question the current dogma which suggests that although *Mycoplasma* spp. are frequently isolated from pneumonic bovine lungs, they do not cause clinical respiratory disease [40]. Yates *et al* reported that 63% of feedlot cattle at slaughter had histological changes indicative of pneumonia [293], while Wittum *et al* reported 72% [288]. Thirty five percent of the population was treated for clinical respiratory disease from birth to slaughter and 78% of this subpopulation had pulmonary lesions. However, 68% of the animals never showing clinical respiratory disease had similar lesions ($p<0,05$) [288]. This is confirmed by Griffin *et al* [97], who found a lack of association with performance and clinical BRD and concluded that asymptomatic or subclinical respiratory disease was common in feedlot cattle. Fifty percent of the cattle that had never been clinically diagnosed with BRD had gross lung lesions [96]. They stated that a greater emphasis on prophylactics was needed to reduce clinical and subclinical respiratory disease. Research data supports the use of metaphylaxis in high-risk cattle as a disease management tool, usually on arrival at a feedlot or when the pen incidence goes over a predetermined level [99]. In a summary of fifteen trials using tilmicosin metaphylactically, the respiratory disease morbidity was 47,7% for non-medicated animals and 22,6% for medicated; mortality was 2,3% vs 0,5% and daily gain was 1,027 kg vs 1,11 kg which is a 8,4% improvement. The probability for the three were all $p<0,01$ [99]. One possible reason put forward for the efficacy of this metaphylaxis is that tilmicosin and related antibiotics are more effective against mycoplasmas than against many of the other pathogens involved [108].

The estimated $ 3 billion per annum for preventatives and treatment represents only about 2 to 6% of the total production costs in the USA. These measures include practices such as preconditioning, processing and vaccinations, mass medications, especially when a large percentage of animals in a group start exhibiting signs of BRD. Many strategies have been

developed in an attempt to reduce the risk of BRD, but the complexity of factors associated with the development of BRD are so varied, even in animals that have been preconditioned or mass medicated, that the problem remains [96,229].

1.3 EPIDEMIOLOGY / PATHOPHYSIOLOGY

The high incidence of BRD in feedlot cattle is generally attributed to the fact that these animals are exposed to a wide range of pathogenic agents including bacteria, viruses, mycoplasmas, rickettsias, chlamydias, fungi and toxins at a time when their resistance is impaired by environmental factors such as the stresses of recent weaning, transportation to the feedlots, crowding, commingling, dietary upsets and water deprivation. These stressors lead to imbalances in homeostasis and adversely affect the immune and non-immune defense mechanisms of the animal, which reduces the body defense mechanisms, thereby making the animals more susceptible to pathogens, which further reduces the integrity of the lung cells, establishment of bacterial colonies and fibrinopurulent pneumonia. [13,16,42,52,59,95,131,170,176,203,204,207].

1.3.1 HOST FACTORS

1.3.1.1. INTRODUCTION

The bovine respiratory system is continuously exposed to potentially pathogenic organisms, but most cattle remain healthy because of the efficient clearance of these organisms by pulmonary defenses. When pulmonary function is impaired or pulmonary tissues are damaged, then the organisms can establish a foothold to initiate disease [68,170,185,214]. The outcome of an infectious challenge on the respiratory tree depends on the ability of infectious agents acting alone or in combination, to overwhelm the inherent defense system of the lungs. The proper functioning of the defense mechanisms depends

on factors such as the level of nutrition, hydration, stress and immunity of the host [68,143,185,186,214]. The risk factors relating to pathogens in respiratory disease are many and complex. Some pathogens like the viruses and mycoplasmas primarily play a role in upsetting the defense mechanisms of the animal, while others such as the bacteria and their toxins play a crucial role in the development of pulmonary lesions [143].

The lung is normally sterile distal to the first bronchial division due to the effects of the numerous defense mechanisms of the upper and lower respiratory tracts [146]. The airway defense mechanism can be divided broadly into non-immunologic and immunologic components. The non-immunologic components include cough reflex, filtration systems and airflow patterns, the mucociliary clearance mechanisms and the pulmonary macrophages with their naturally occurring antibacterial proteins (lysosomes, lactoferrins, interferon). The immunologic components include antibody- and cell-mediated immune effector systems that are present both locally as immunoglobulin A, G and M antibodies on the respiratory epithelial surfaces, the alveolar macrophages and lymphocytes on the respiratory epithelium and alveoli or the immunity mediated primarily by the T-lymphocytes [24,68,176,203,214,272].

1.3.1.2. NON-SPECIFIC DEFENSE MECHANISMS

- **SUSCEPTIBILITY OF THE LUNG**

The bovine respiratory system is particularly predisposed to disease. Cattle have small lungs relative to their body size and their metabolic demands, with a smaller physiological gaseous exchange capacity relative to other mammals. Bovine lungs have about 25% of the lung volume per unit of body mass as compared with the mammalian mean. The

bovine lung also has a lower number of pulmonary capillaries per alveolar section with a decreased gaseous exchange capacity per unit of alveolar surface. There are also many septa dividing the lungs into secondary lobules, and the presence of fewer interalveolar pores decreases the ability of the lung to adapt to reduced ventilation when lung consolidation begins to occur due to pneumonia.

Bovines use 2,1 times more of its total lung volume for basal breathing than the mammalian mean, but has a similar proportion of tracheal volume to total lung as does the horse. This represents a greater tidal volume excursion into the lower airways and greater respiratory epithelial exposure, especially if there is a significant environmental air contamination as is frequently experienced in feedlots [272]. The ventilatory reserve appears to be inadequate in cattle used in meat production [143].

Mammalian regional blood perfusion tends to parallel the regional airway oxygen levels and the ventral bovine lung is reported to have less blood perfusion than the dorsal lung, which might account for the decreased alveolar macrophage activity in the most commonly affected areas of the bovine lung [272]. Decreased phagocytic activity slows pulmonary clearance of infectious agents, allowing more time for other pathogens to colonise and grow.

The functional maturity of the bovine respiratory tract is also not achieved before one year of age and therefore, regardless of immunological and managemental considerations, respiratory disease will be more severe in younger cattle than in mature cattle[143,186].

- **FILTRATION AND MUCOCILIARY ESCULATOR**

The filtration system is capable of removing particles as small as 5 µm before they reach the alveoli [69] and the mucociliary apparatus produces a mucous flow equal to 15 mm per minute[272]. Destruction of the ciliated cells by infectious agents, noxious gasses or extremes in temperature or humidity will hinder this flow rate and therefore the clearance of infective agents [68]. Smaller particles that reach the distal non-cilated regions are removed by one of three mechanisms: some remain on the thin layer of fluid and are moved proximally until they gain access to the mucociliary mechanism. Others may be phagocytosed by the alveolar phagocytes in the airway lumen and transported back to the mucociliary apparatus, while others penetrate the mucosal barrier to enter the interstitial compartment where they are phagocytosed by interstitial phagocytes [68].

The mucociliary apparatus prevents the contact of harmful substances or infectious agents with the epithelial cells of the airways by removing them from the respiratory tract, by means of the trapping capacity of the mucous layer and the propelling effect of the ciliary blanket. Pathogenic bacteria have been shown to be able to reduce the viscoelastic properties of the mucous layer and impairing ciliary activity [9] such as *P. multocida* and *A. pyogenes* that produce neuramidases or extracellular proteases.

- **ALVEOLAR MACROPHAGE ROLE**

The alveolar macrophage is the most important phagocyte in normal mammalian pulmonary clearance and comprises over 85% of the leukocytes in the normal alveolus. Cattle, however, have fewer pulmonary alveolar macrophages compared to other animals[68,203,259,272,281].

Macrophages function to maintain normal alveolar sterility, despite constant environmental challenges to the lung and have two major functions, namely phagocytosis of trapped particles and elaboration of numerous inflammation mediators. Lymphocytes comprise about 12% of alveolar leukocytes and become important in the formation of helper- and suppressor-T cells, cytotoxic cells and antibody-producing B cells. Neutrophils comprise about 2% of the alveolar leukocytes and eosinophils less than 1% [24,68,146,272].

The potential adverse effects of polymorphonuclear cells (PMN's) in the pathogenesis of pneumonic pasteurellosis in bovines has been studied and shown that a depletion of peripheral blood neutrophils reduced the severity of lung damage in cattle with experimental pasteurellosis due to the lowered release of the inflammation mediators [36,226,275]. There is also evidence from experimental animal models that neutrophil-platelet interactions may play a significant role in the inflammatory response and that platelets may contribute directly to the loss of integrity of the alveolar-capillary wall [60]. The activated neutrophil is equally toxic to pathogens and to host tissues [275].

- **NON-SPECIFIC CHEMICALS**

Interferon induces a virostatic cellular resistance and is produced by the respiratory epithelium and alveolar macrophages and released into the respiratory secretions in response to penetration of the cytoplasm with poly-RNA materials. It then enters unaffected cells, providing protection by blocking protein required for viral replication in the cells[272]. Interferon also participates in the regulation of both humoral and cellular immunity by enhancing macrophage phagocytic function and regulating B- and T-lymphocyte responses [146].

Lysozymes (muramidases) are a group of enzymes that are especially effective against certain Gram-positive bacterial cell walls. An apparent low level presence and atypical bioactivity in cattle may be significant in causing susceptibility to infectious bovine pulmonary disease [272]

1.3.1.3. SPECIFIC DEFENSE MECHANISMS

The bovine respiratory tract can produce immunoglobulins (Ig) locally, primarily IgA, IgG and small amounts of IgM. Immunoglobulins are produced by secretory cells in the submucosa, lamina propria and submucosal glands in lymphocytic aggregations that form the 'bronchus-associated lymphoid tissue' (BALT).

IgA is the predominant antibody found in the nasal, pharyngeal and tracheal airways, with the levels of IgA decreasing in the more distal airways and are virtually nonexistent in the alveoli. IgA is important for mucosal immunity[214] and it is thought that the major mode of action of IgA is to prevent the adherence of antigens to body surfaces [255].

IgG levels on the other hand increases in the more distal regions. IgG aids the lung defenses by agglutinating viruses and bacteria, thereby rendering them less able to attach and infect epithelial surfaces. IgG also coats foreign materials, transforming them from poorly recognised substances to substances that can be more readily phagocytosed by the alveolar macrophages and neutrophils (opsonization). IgG also activates complement proteins which facilitate the lysis of bacteria [68,146,272].

1.3.2. ENVIRONMENT

Abrupt changes in climate or cold stress has been shown to alter pulmonary function and to influence the incidence of BRD [16,57,188,208,20959,203,224,225]. Calves subjected to an abrupt change in climate after aerosol challenge to *M. haemolytica*, had higher respiratory rates eight to 14 hours later and rapid bacterial proliferation and colonisation in the nasopharynx than calves kept at a constant climate [127]. Cold air has been shown to reduce tracheal mucociliary clearance, thereby increasing pulmonary deposition of pathogens. Aerosol particles are also smaller in cold weather. Diesel *et al* [16,63,65] showed a 24% lower nasal mucous velocity during cold exposure and a 66% increase ($p<0.05$) in bacterial deposition. Other changes to cold is a slight hypoxaemia and the resultant pulmonary oedema associated with the hypoxic pulmonary vasoconstriction would be an excellent growth medium for pathogens deposited in the lungs [16,63,65]. A fairly consistent observation in South African feedlots is a day-night temperature differential of 17 or 18ºC a few days prior to an outbreak (personal observation) [168].

Mucociliary flow is also slowed by irritating gases such as ammonia. Ammonia can increase ciliated epithelial degeneration, or changes in the physical properties and pH of the mucous [68,272].

Dust-particle concentrations in crowded lots may also be high. High concentrations of aerosolised particulate matter of 2.0 – 3,3µm will irritate the respiratory tree and could also serve as a vehicle for virulent organism transportation [16,20,151].

1.3.3. STRESS

Stress, a vaguely defined neuroendocrinologic reaction, arises when animals encounter a variety of social or environmental conditions to which they are not accustomed. Adaptations imposed on new cattle arriving in a feedlot, such as crowded conditions, commingling and social acclimatisation, physical abuse, dietary changes, branding, castrations and dehorning, vaccinations and deworming procedures ensure that the pulmonary clearance of micro-organisms are stretched to the limit. Animals subjected to stressful exercise were more susceptible to experimental pneumonic pasteurellosis [15,20].

Stress produces an elevation in the endogenous steroid levels of cattle. These levels may remain elevated for prolonged periods and result in significant reductions in neutrophil and antibody levels in the blood. Corticosteroids block the release of chemotactic factors from alveolar macrophages, inhibiting the binding of chemotactic factors to the granulocyte, and producing a chemical paralysis of the macrophages' ability to migrate after contacting chemotactic factors [68,186,214]. Transportation of cattle also results in a rise in plasma fibrinogen levels, which is also an indication of stress [16,191].

It would appear that bovines might be particularly susceptible to stress, when compared to other species. A dexamethasone dose of 0,04 mg/kg (2mg per 45kg) given once daily is used as an immunosuppressive model in cattle [18,213]. Pigs on the other hand require a dose of 2 mg/kg to achieve the same immunosuppressive effects [18].

1.3.4. TRANSPORTATION

The transportation of animals has also been shown to be an important contributing factor in BRD, other than the commingling of the animals [4,208]. Transportation has been shown to depress the immune response capability of the body, particularly the lymphocytes

(blastogenic response), which correlated with elevated serum cortisol levels for at least three days after transportation of calves to a feedlot. In this study, the stress of transportation and handling at processing was sufficient to make the calves susceptible to an aerosol BHV-1 challenge, but not to *M. haemolytica* [73,74]. In another study, calves were sampled for *M. haemolytica* at the farm of origin, at an auction barn and the feedlot after they were transported 1 600 km. The frequencies of *M. haemolytica* were lowest at the farm of origin, greater at the auction barn, but markedly increased at the feedlot. Serotype 2 was the predominant isolate at the farm of origin, while serotype 1 was isolated at the feedyard and from pneumonic lungs [79].

A similar situation is noted with *M. bovis*. In a study, *M. bovis* was not isolated from nasal swabs of calves on the farm of origin, but was isolated from the nasal swabs of 20% and from lung lavage of 53% of these calves after transport [136]. It has been reported that *M. bovis* is seldomly isolated from clinically normal calves [29,136,230] and that nasal swabs are not a reliable method of determining the mycoplasmal status of calves [136].

Although the stress of transport is cited as being a major influence in the development of BRD, not all studies have shown that the distances or length of travel affected the risk. The risk of pneumonia for calves from nearby markets was just as high as that for calves transported much greater distances (distances varied from 90 to 1300 km with an average of 150 km), however, the mean shrinkage of animals increased with increasing distances travelled [208].

1.3.5 AETIOLOGY

Many viruses, bacteria and mycoplasmas have been associated or isolated with BRD and include the pathogens mentioned in Table 1 below[12,16,20,143,176,186,187,203,234,251]: It must be

noted that many of the bacteria cultured are apathogenic and happen to enter the respiratory tract due to aspiration and a damaged mucociliary esculator. Bacteria isolated at necropsy may also be contaminants.

Table 1. Micro-organisms isolated from the bovine lung

Viruses	Mycoplasmas	Bacteria
Infectious bovine rhinotracheitis	*Mycoplasma bovis*[a]	*Mannheimia (Pasteurella) haemolytica*[a]
(IBR)[a] /Bovine Herpes virus type1	*M. dispar*[a]	*P. multocida*[a]
and possibly type 4	*M. bovirhinis*[a]	*Histophilus somni (Haemophilus*
Parainfluenza virus III (PI3)[a]	*Ureaplasma* spp.[a]	*somnus)*[a]
Bovine viral diarrhoea virus	*M. alkalescens*	*Arcanobacterium (Actinomyces,*
(BVDV)[a]	*M. arginini*	*Corynebacterium) pyogenes*[a]
Respiratory syncytial virus	*M. canis*	*Fusobacterium necrophorum*[a]
(BRSV)[a]	*M. bovigenitalium*	*Chlamydophilia* spp.
Respiratory bovine coronavirus	*Acholeplasma laidlawii*	*Salmonella* spp.
Parainfluenza virus II	*A. modicum*	*Moraxella* spp.
Reovirus types 1, 2, 3 (Reo)	*A. axanthum*	*Streptococcus pneumoniae*
Adenovirus types 1, 2, 3, 4	*M. mycoides* subsp. *mycoides*	*Staphylococcus aureus*
Enterovirus	*Ureaplasma diversum*	*Strep. bovis*
Rhinovirus type 1 (RV)	Leach's group 7 mycoplasmas	*Staph. epidermidis*
Calicivirus		*Strep. mitis*
		Enterococcus faecalis
		Aerococcus viridans
		Acinetobacter spp.
		Micrococcus luteus
		Staphylococcus spp.
		Neisseria spp.
		Actinobacillus lignieresii
		Klebsiella spp.
		Corynebacterium bovis
		C. xerosis
		Streptococcus spp.
		Aerococcus spp.
		Haemophilus spp.
		Aeromonas spp.
		Bacillus spp.
		Alcaligenes faecalis
		Micrococcus roseus
		Micrococcus spp.
		Escherichia coli

[a] Thought to be the most important causes in BRD
Adapted Andrews[16]

1.3.5.1. VIRUSES

The primary role of the viruses in BRD lies in their capacity to predispose to secondary bacterial infections. Viruses involved have a direct killing effect on epithelial cells, leading to an inflammatory exudate, which is an ideal nutrient broth for bacteria, with a

resultant increase in bacterial growth. Viruses also have an effect on the host tissue by degrading the fibronectin layers and thereby exposing the adhesin receptors and depressing the mucociliary apparatus [50,176]. Viruses also lead to dysfunction of the neutrophils, lymphocytes and alveolar macrophages, all of which facilitate enhanced susceptibility of bacterial attachment and colonisation. Loss of adequate function of the alveolar macrophage would appear to be the most important determinant [176].

There is also considerable synergy between overlapping viral infections. Calves infected with both BRSV and BVD virus displayed more severe clinical signs than calves infected with either virus alone [185].

1.3.5.1.1 Bovine Herpes Virus

Bovine herpes virus-1 (BHV-1) is considered by many researchers to be the most important of the respiratory viruses in feedlot cattle and there is much seroepidemiologic evidence of its role in BRD [32,69], and was the first virus shown to definitely cause respiratory infection in cattle [292]. The disease is known as infectious bovine rhinotracheitis (IBR) [16,95] and the virus has a particular affinity for mucous membranes [186]. Infectious bovine rhinotracheitis usually affects cattle over the age of six months and several strains of differing pathogenicity not only cause lesions in the respiratory tract, but also in the reproductive tract and rarely the central nervous system[16,20,27]. The viral infection alone is not life-threatening and rarely results in pneumonia in non-stressed calves [20,133], but predisposes to secondary bacterial pneumonia. The virus is rapidly spread by aerosol to susceptible animals because of the sustained close contact between feedlot cattle. Latent infections are also relatively common and infection can be stimulated by corticosteroid

administration or stress[16,23]. High cortisol levels due to transport stress, have been shown to render cattle more susceptible to BHV-1, but not necessarily to *M. haemolytica* 1 [74,176].

Signs of infection vary considerably from mild disease with a conjunctivitis and a watery ocular discharge; subacute to acute with a febrile reaction, conjunctivitis and reddening of the nasal mucosa ("red nose"), expressive cough, rapid and shallow breathing. The discharge from the eyes and nose tends to be purulent. A peracute form with pyrexia, eye and nasal discharge, respiratory distress, cough and death have also been described [16,20]. In uncomplicated IBR infections, most lesions are restricted to the upper respiratory tract and trachea [20].

Intranasal inoculation with BHV-1 prior to *M. haemolytica* 1 inoculation caused a more severe clinical illness and resulted in a greater degree of bacterial colonisation than developed after inoculation with PI-3 [78,186]. Impairment of bacterial pulmonary clearance is maximal about 4 days after BHV-1 infection [154,176]. At least 10^6 plaque-forming units of BHV-1 are required to induce sufficient impairment of pulmonary clearance for significant bacterial impact [176].

Virus results in the alveolar macrophages being unable to produce chemotactic factors. These cells also show reduced complement receptor activity and phagocytosis, thereby predisposing to secondary bacterial infection [23]. The virus also increases activity of the enzyme, elastase in nasal mucous, facilitating the colonisation with *M. haemolytica* [185,186]. One possible mechanism that might explain BHV-1/ *M. haemolytica* synergism in BRD is that BHV-1 infection stimulates the release of inflammatory cytokines that activate lymphocyte function-associated antigen-1 (LFA-1) on bovine leukocytes and thereby increases their ability to bind and be affected by the leukotoxin [142].

1.3.5.1.2. Parainfluenza-3

Parainfluenza virus type 3 (PI-3) is also a consistent viral isolate in BRD. Uncomplicated PI-3 infection is not normally an important cause of death in calves, causing only mild to inapparent clinical signs on its own [20,77,130,203,271]. Clinical signs include pyrexia, cough, serous nasal and lachrymal discharge, increased respiratory rate and increased lung sounds [20]. Strain differences are recognised and infection with virulent strains may cause severe bronchiolar and alveolar damage, though mortality in uncomplicated PI-3 virus infection are rare [20,27]. The mechanism by which PI-3 predisposes to secondary bacterial pneumonia is poorly understood, but research suggests that viral-mediated alterations in bacteriocidal ability of macrophages, decreased bacterial clearance and immunosuppression are important [69,130] , although there appears to be some doubt about this [271].

PI-3 infects ciliated respiratory epithelium of the upper and lower airway, facilitating pulmonary bacterial colonisation. It also infects alveolar epithelium and macrophages. Macrophages support PI-3 replication, resulting in cell death or altered function [130,133,271]. These infected macrophages have decreased phagocytosis and killing of bacteria, as well as depressing lymphocyte proliferation. Infected macrophages also have altered fatty acid metabolism and secretion, resulting in the release of immunosuppressive prostaglandins [130].

Studies have shown that prior PI-3 infection impairs the clearance of *M. haemolytica*. The suppression was greatest during the acute phase of viral replication and most pronounced 7 days post-viral exposure [68].

1.3.5.1.3. Bovine Respiratory Syncytial Virus

Bovine respiratory syncytial virus (BRSV) infection is common in cattle, as evidenced by reports of 65 to 81% of cattle being seropositive in the USA, Canada and Europe before the introduction of vaccines. There is also a high correlation between infection by BRSV and the occurrence of respiratory tract disease [20,21,176,203]. BRSV has shown to act synergistically with bacteria and other viruses to cause pneumonia [27,37,147,154,186].

Bovine respiratory syncytial virus infects several ciliated and non-ciliated epithelial cell types in the pulmonary airways. Cytopathic changes and necrosis of the infected cells result in necrotising bronchiolitis that is characteristic of BRSV infection [21,84,132,270]. The mechanisms involved in the mixed inflammatory cell infiltration with a predominance of neutrophils in infected bronchioles is not well understood, but it is likely to involve cytokines and chemokines that are produced in response to BRSV infection and serve as chemoattractants for inflammatory cells [21].

Infection with BRSV is considered to be inapparent in the majority of animals, but may cause mild to more severe respiratory tract disease characterised by fever, coughing, serous nasal and ocular discharges and dyspnoea [270]. Severity of disease in calves decreases with age, the most severe BRSV-associated disease is observed in calves less than 6 months of age [265].

The finding that pneumonia associated with BRSV is often complicated by secondary bacterial infections, implies that BRSV may suppress the immune system similar to other viruses involved in BRD [21,27]. The role played by the host’s immune system in the pathogenesis of human RSV was recognised in children in 1970 and it is now accepted

that the calf's immune response to BRSV may also be similarly affected [27,132,133]. Complement activation following BRSV infection may have both adverse and beneficial effects (see Table 2). Activated complement components may have severe spasmogenic effects on airway and blood vessel musculature and may in addition cause mast cell degranulation [27,132]. Ultimately the action of these inflammatory mediators leads to the development of severe oedema and emphysema throughout the whole lung. Alternatively, the complement activation and the antibodies may lead to lysis of infected cells and clearance of infection [42,132]. BRSV is now considered to be the most important of the viral respiratory pathogens in both beef and dairy calves under UK management systems [41].

Table 2. Bovine Respiratory Syncytial Virus activation

Signs indicative of Complement Activation and Mast Cell Degranulation in BRSV-induced Respiratory Disease

- The simultaneous presence of BRSV antigen and BRSV antibodies in the lungs of infected animals
- The deposition of complement factor C3 in the virus-infected cranioventral parts of the lung
- Neutrophil influx into the virus-infected regions of the lungs
- A lowered number of mast cells and mast cell granules in the lungs
- A lowered level of histamine, which is indicative of mast cell degranulation
- The presence of oedema throughout the lungs
- The presence of severe emphysema throughout the lungs, resulting in marked dyspnoea

Adapted Kimman[132]

1.3.5.1.4. Bovine Viral Diarrhoea Virus

Whether BVD virus is a pneumotropic virus and therefore a true respiratory pathogen is somewhat debatable [195]. Bovine viral diarrhoea virus is however commonly isolated from cattle with BRD and a synergistic interaction has been demonstrated between the virus and

M. haemolytica and other respiratory viruses [20,22,30,37,69,102,147,186,195,196]. Of the pathogenic vruses, BVDV had the most consistent association with an elevated risk of BRD and lower mass gains [159,182]. It has also been reported to be the virus most frequently associated with multiple virus infections of the respiratory tract of calves [27] and has caused a mild interstitial pneumonia when administered endobronchially[133,197]. Severe fibrinopurulent bronchopneumonia and pleuritis developed in calves inoculated sequentially with BVDV and *M. haemolytica* five days later [197]. The role of BVDV in BRD appears to be that the virus is capable of inducing immunosuppression, by causing a leukopaenia and lymphoid depletion, thereby allowing for the development of secondary viral or bacterial pneumonia [20,27,69,102,195,196]. An important consideration in the control of BRD, is that modified-live BVDV vaccines have also been associated with immunosuppression, particularly in stressed calves[22]. Notwithstanding this, vaccination with BVDV vaccines has demonstrated major disease-sparing effects against BRD [69].

An association between BVDV and *M. bovis* in respiratory and arthritic infections in cattle have also been shown [100].

Animals entering a feedlot will usually seroconvert to BVDV without clinical evidence of BVD [32,32,155]. While both innate and acquired features of immunity are observed with a primary infection of the bovine lung with BVDV, it is the cell-mediated T-lymphocyte response that is necessary for clearing the virus within 2-3 weeks. Populations of immune-activated and memory T-lymphocytes, combined with BVDV specific antibody production, contribute to rapid BVDV clearance with secondary exposure to the virus [223].

About 1-2% of calves entering a feedlot are persistently infected (PI). They are prolific shedders of BVDV, resulting in many negative calves seroconverting. Persistently

infected calves are also at greater risk of suffering fatal disease after leaving the farm of origin [227].

1.3.5.1.5. Bovine Coronavirus

Bovine respiratory coronavirus (BRCV) appears to be an emerging pathogen causing upper and lower respiratory tract disease in feedlot cattle, with clinical disease occurring particularly when the animals are stressed [20,63,107,115,119,130,160,182,237,251]. Whether this pathogen has emerged in the wake of improved control of the other respiratory viruses or is simply better recognised or diagnosed remains to be determined [69]. There is some evidence that maternal antibodies have a sparing effect with BCV-associated respiratory disease in calves [69], as is found with BCV-associated enteric disease [130].

During investigations into two natural outbreaks of shipping fever, BCV was isolated from 88% of the animals while BHV-1 and PI-3 were isolated from only a few of the animals involved [236].

1.3.5.1.6. Other Bovine Respiratory Viruses

A number of other viruses have been identified as being involved in BRD

- Bovine herpesvirus 4
- Bovine adenovirus type 3
- Bovine rhinovirus
- Bovine reovirus
- Bovine enterovirus

These viruses appear to have a similar role to the other viruses described and in combination with other stressors, can serve as initiators of bacterial pneumonia [20,107,203].

1.3.5.2 BACTERIA

1.3.5.2.1 INTRODUCTION

The aetiology of BRD is very complex, but the consistent finding by the majority of researchers is that *Mannheimia* (formerly *Pasteurella haemolytica* biotype A) *haemolytica* serotype 1 is considered to be the primary pathogen. The organism has been reported to occur in more than 75% (with reports up to 90%) of cases from natural outbreaks of the disease [176]. It has been shown experimentally that *M. haemolytica* 1 can produce disease as a primary agent in non-immune calves [16,87,222]. Isolations of *Pasteurella multocida* and *Histophilus somni* and others are often considered as being secondary [94,176]. These three bacterial species belong to the family Pasteurellaceae and are characterised as:

- Gram-negative
- non-motile
- pleomorphic cocco-bacilli, 0,2-0,4 X 0,4-2,0µm
- aerobic / facultatively anaerobic
- grow optimally at 37ºC
- mostly pathogenic
- often found on the mucosae in clinically healthy animals [66]

DNA hybridisation studies have shown the heterogenecity of the species within the group, which led to frequent taxonomic changes [105,175] and confusion amongst non-bacteriologists at times.

1.3.5.2.2 PATHOPHYSIOLOGY

Endotoxins are released on the death of the bacteria and the most important is lipopolysaccharide (LPS) in the outer membrane of Gram-negative cell walls, including the genera *Mannheimia, Pasteurella* and *Histophilus*. It is an important cause of pulmonary injury due to the initiation of the complement and coagulation cascades. Toxicity is due to the lipid A portion of the LPS molecule, whereas the polysaccharide side chains are responsible for major antigenicity, antiphagocytic or cell attachment functions. Endotoxin has a wide array of toxic effects *in vivo,* including initiation of complement and coagulation cascades. This results in increased vascular permeability, coagulation, accumulations of inflammatory cells, oedema and both intravascular and extravascular fibrin in the lung. Pulmonary instillation of LPS produces fibrinopurulent inflammation, oedema, haemorrhage, platelet and neutrophil aggregation within the pulmonary capillaries. Additionally, the vascular endothelium undergoes toxic and degenerative changes in response to *M. haemolytica* LPS [61,170,285].

In summary, the pathogenic effects of LPS at the alveolar level [58,175,284,285] include:

- direct damage to endothelial cells, with induction of increased vascular permeability and oedema
- activation of the clotting mechanism, resulting in fibrinogenesis
- activation in complement, resulting in activation of alveolar macrophages as well as chemotaxis and activation of neutrophils, and
- complex formation with surfactants leading to increased surface tension and predisposing to atelectasis, oedema and haemorrhage.

1.3.5.2.3. *Mannheimia haemolytica*

Mannheimia haemolytica is classified into 13 serotypes, namely 1, 2, 5-9, 11-14, 16 and 17 as well as several untypable strains. The formerly named *Pasteurella haemolytica* biotype T is now known as *Pasteurella trehalosi* and has four known serotypes: 3, 4, 10 and 15 [66,175].

In a South African study, *M. haemolytica* yielded a relatively low 45% isolation rate from clinical cases [261], while in another survey *M. haemolytica* 1 accounted for 80% of the isolations, 12% for serotype 6 and 2% each for serotypes 4, 9, 12, and 13. Serotype A was the predominant *P. multocida* isolated [176,262]. In a similar survey in the USA, *M. haemolytica* serotype 1 was isolated from 60% of samples, serotype 6 from 26%, serotype 2 from 7% and the remaining 7% from serotype 9, 11 or untypable [3]. During 1998 a new and particularly virulent serotype 17 was isolated in South Africa [184].

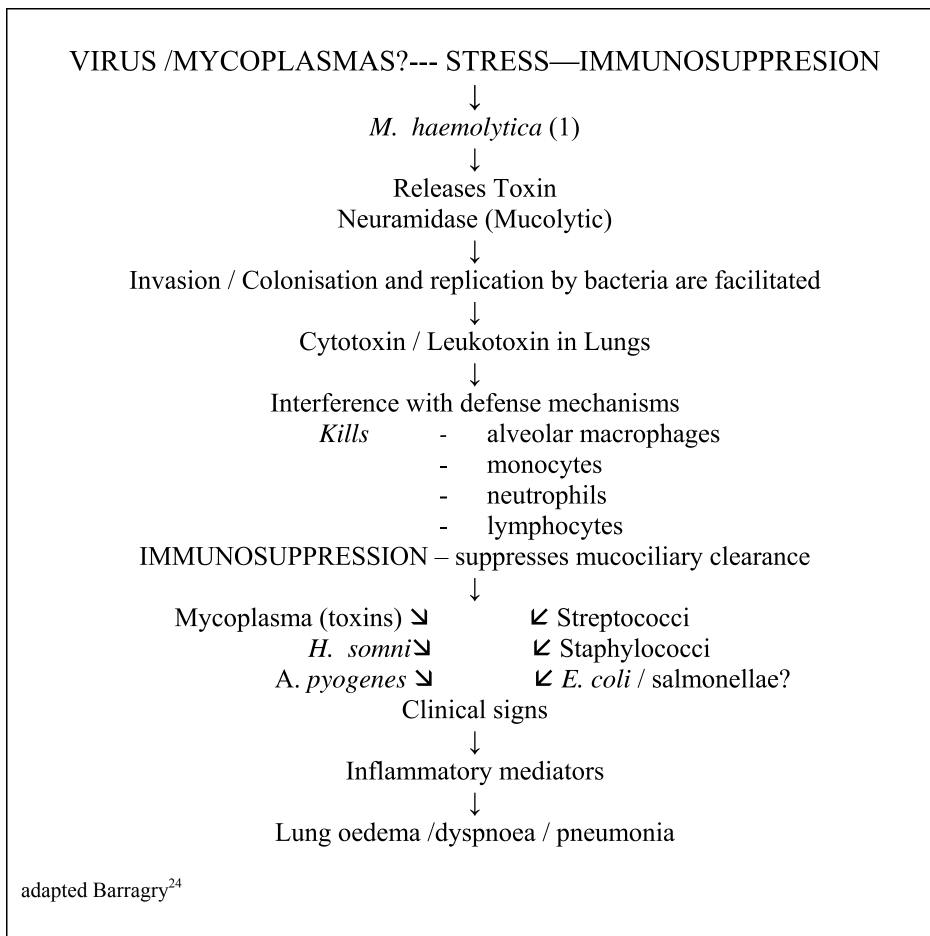
M. haemolytica 2 is frequently isolated from the nasopharynx of normal animals but does not usually colonise the lung unless the animal is subjected to stress and/or viral/mycoplasmal infections [16,52,59,61,170,186,203]. It has been shown experimentally to be less adherent to bovine cells than 1 strains [54]. Serotype 1 is the more common isolate with respiratory disease [50,51,184,186], which again emphasises why tracheal samples are more important than nasopharyngeal swabs. The organism is more susceptible to colonisation and adherence in the upper respiratory tract and is then able to be transferred to the lung by inhalation of infective droplets or retrograde flow of bacteria-laden mucus. Actively growing *M. haemolytica* (ie. in the log phase) produces several adherence factors or toxins and enzymes, plus other defense mechanisms, which contributes to the severity of the disease [21,50,203].

Although the virulence determinants of *M. haemolytica* are not fully understood, the pathogenicity of *M. haemolytica* 1 rests with certain cellular components or virulence factors that include [58,170,175,203,284,285]:

- fimbriae (cell-surface pili)
- proteolytic enzymes, neuramidase in particular
- capsular polysaccharides
- leukotoxin (cytotoxin), and
- endotoxin or lipopolysaccharide (LPS) of the outer cell wall

The role of *Mannheimia haemolytica* in the pathophysiology of BRD has been summarized in the following flow diagram (Figure 1):

Figure 1. Significance of *Mannheimia haemolytica* in bovine respiratory disease



The fimbriae or pili act as specific adhesins facilitating bacterial attachment and colonisation to the ciliated epithelium of the respiratory tract.

Neuramidase or extracellular protease is mucolytic, thus making the mucus less viscous and adhesive. This interferes with the mucociliary clearance function and thereby increasing the spread of bacteria and decreasing the trapping of foreign matter. The enzymes are also responsible for the removal of the fibronectin from the surface of the epithelial cells lining the airways, facilitating bacterial attachment and colonisation [24,58,61,175,203,285]. Further enzymes produced by *M. haemolytica* include gelatinases, metalloproteinases and *O*-sialoglycoprotease, all of which play a role in the destruction of the basement membrane and thereby damage of alveolar epithelium and vascular endothelial cells seenduring inflammation [233].

The production of capsular polysaccharides is maximal during the log growth phase and are apparently more invasive than the nonencapsulated forms [24,285]. Their pathogenic functions include:

- n of complement-mediated microbicidal mechanisms

These functions are largely due to the net negative surface charge and hydrophilic properties of the capsular polysaccharides, which are weak antigens in comparison with proteins [61,175].

During the logarithmic growth stage, *M. haemolytica* also produces a heat-labile exotoxin or soluble cytotoxin called leukotoxin, that is a potent leukocyte-modulating agent and causes cytolysis of platelets, macrophages and neutrophils [13,24,203,285]. The destruction of neutrophils are important in the pathogenesis of acute lung injury induced by *M. haemolytica* [226,285]. This leukotoxin is also capable of activating macrophages to produce inflammatory cytokines (glycoprotein molecules that activate other cells of the immune

system to be more aggressive, including macrophages, neutrophils and lymphocytes) [214,285], resulting in a massive influx of neutrophils, which is a key factor in lung tissue destruction, as the destruction of neutrophils results in the release of lysosomal products such as elastase, collagenase and reactive oxygen intermediaries [13,24,52,59,203,285].

When the initial bacterial invaders begin to multiply in the lung and release small amounts of leukotoxin, there is stimulation of bovine neutrophils and mononuclear phagocytes to produce reactive oxygen intermediates ('oxidative burst'). These degranulate and release eicosanoids and cytokines that are potent inflammatory mediators [52,55,62,104,152,285] as well as proteolytic enzymes which leads to further tissue destruction and reduced lung defense, while at the same time causing death of cells by apoptosis (programmed cell death) or cell necrosis [141,142,235,239,285,294,295]. With continued bacterial multiplication, higher concentrations or with exposure for longer periods, the toxin causes rapid swelling and loss of cell viability, possibly from the ability of leukotoxins to form pores in cell membranes, resulting in increased membrane permeability, cells swelling and allowing calcium uptake and ultimately leads to loss of cytoplasmic constituents and cell death [62,233,235]. It would appear that there are specific leukotoxin binding sites on the bovine but not on canine, equine, porcine or human leukocytes and these might be involved in the activation and lytic activities of the leukotoxin [38,58,175,239,285].

There have been reports of some *M. haemolytica* and *H. somni* isolates from pneumonias that produced lysis of bovine erythrocytes and therefore haemolysins may also be involved in pulmonary injury [61].

1.3.5.2.4. *Pasteurella multocida*

Pasteurella multocida is generally considered to be less virulent for the respiratory tract than *M. haemolytica* and necessitates more organisms to initiate primary infection [170,186]. This may be attributable to the number and efficacy of its virulence factors and the fact that a potent leukotoxin has not been identified in *P. multocida*.

P. *multocida* serogroup A is the most significant bacterial component of enzootic pneumonia in young dairy calves and it is believed to cause less fulminating respiratory disease in older feedlot cattle and is most often associated with subacute to chronic suppurative bronchopneumonia rather than acute fibrinous pneumonias [69,95,170,186]. *Pasteurella multocida* is, however, thought to be capable of significant contributions to lung disease when present [186].

1.3.5.2.5. *Histophilus somni* (formerly *Haemophilus somnus*)

Histophilus somni has been reported as a major cause of respiratory disease in cattle and accounted for more than 40% of mortalities in Canada [186,267], but is less important in the United States and elsewhere.

Histophilus somni is the aetiologic agent of thromboembolic meningoenchephalitis (TEME), a usually fatal disease in ruminants, but can also cause pneumonia as a primary pathogen in feedlot cattle, endometritis, abortions, mastitis and arthritis [107,109,158,170,186]. A septicaemic manifestation of *H. somni* has also been described [123,266]. It is an important cause of meningitis in humans, especially in young children, where it is a very serious condition [24].

In Canada, the median fatal disease onset (FDO) for pneumonia caused by *H. somni* was day 12; for septicaemia, day 17; for polyarthritis, day 18; for myocarditis and pleuritis, day 22; and for TME, day 29, despite routine immunization against *H. somni* with a commercial bacterin vaccine [266]. This lead to the use of post-arrival prophylactic mass medication in order to control the high incidence experienced. This reduced the need for BRD treatment by 14% and the risk of BRD mortality by 71% [267].

Virulence factors for *H. somni* include a lipo-oligosaccharide (LOS)[120,231]and immunoglobulin binding proteins[231,241]. It is also thought to be able to function as a facultative intracellular parasite, with the ability to inhibit the oxidative burst in bovine neutrophils[120,186]. *Histophilus somni* has also been shown to produce histamines, which may partially explain some of the post-vaccination reactions occasionally observed with *H. somni* bacterins. Its role in the pathogenesis of BRD is however uncertain [215]. Lipo-oligosaccharide is the major outer membrane component of many Gram-negative bacteria inhabiting the mucosal membranes and in *H. somni* been shown to undergo phase variation and thereby evading or delaying recognition by the host immune response[120,231]. Pathogenic strains of *H. somni* have also been shown to induce apoptosis of bovine endothelial cells in a time- and dose-dependent manner[241]. *Histophilus somni* has also been shown to resist killing by bovine neutrophils, by causing the latter to undergo morphological changes consistent with apoptosis [291].

Histophilus somni is found commonly in the respiratory and urogenital tracts of clinically normal animals [98,170] and it is most commonly isolated in association with *Mannheimia / Pasteurella* spp. in cases of pneumonia.

The lesions associated with *H. somni* are generally more subacute or chronic [170], while fatal cases are considered more common in the colder climates of North America [96]. There is also increasing evidence linking *H. somni* infections with an increase in myocarditis in feedlot cattle [14,123,170,207], especially towards the end of the feeding period.

Although *H. somni* has been implicated in joint conditions in feedlot cattle [14], it appears not to be significant [1,100,204,266,267]. In a study in Canada of samples from cases unresponsive to antibacterial treatment, *H. somni* was found in only one of four heart samples and *M. bovis* in three of the four. In the same study, *M. bovis* was isolated from 80% of the lung and/or joint samples and *H. somni* only from 14% of the lung samples and none from joint samples [100]. In the studies done by Adegboye [1], only mycoplasmas were isolated from the joints.

Tegtmeier *et al* [242], attempted to induce pneumonia in calves by aerosol administration of *M. dispar* followed by *H. somni* 11-14 days later. Although mild responses were noted, only one calf that received *H. somni* developed pneumonia.

1.3.5.3. MYCOPLASMAS

Mycoplasmas are classified under the Class Mollicutes and are the smallest self-replicating organisms and there is now solid genetic support for the hypothesis that mycoplasmas have evolved as a branch of *Clostridia*. They lack a cell wall and are therefore highly pleomorphic. Mycoplasmas require close interaction with their host because of their fastidious nature and requirement for a source of lipids and nucleic acid precursors for growth. They also have the ability to adhere to the surface of the host's mucous membranes, as well as being able to incorporate with the host antigens within the cytoplasmic membrane [277].

Mycoplasmal diseases remain among the most intractable infectious diseases of cattle. Difficulties in diagnosis, poor response to treatment, few preventive measures and increasing incidence make them an important consideration in disease management [211,246].

Mycoplasmal infections are rarely of the fulminant type, but rather follow a more chronic course, indicating a frequent failure of the host defense to eradicate the parasites [149].

Studies have also shown that it is more common to find mycoplasma in conjunction with other bacteria than alone [273]. *Mycoplasma bovis* is receiving more attention in the aetiology of BRD and many practitioners are giving serious consideration to this organism in their treatment regimens [18,144]. Mycoplasmas have been reported to be isolated, usually in combination with other pathogens from 50% to 90% of beef and dairy cattle with pneumonias [13,108,172,173,177,192,242]. Knudson [136] reports that the upper respiratory tract of both normal and pneumonic calves are frequently colonised with mycoplasmas, however *M. bovis* is seldomly isolated from clinically normal calves [29,136,230]. The contention is that because *M. bovis* is not commonly found in the respiratory tract of normal calves, it should be considered of primary importance when isolated from calves with pneumonia [136]. Serological results indicate a high prevalence of *M. bovis* or *M. dispar* in feedlot calves and calves with increasing titres to these organisms are at increased risk of being treated for respiratory disease [212].

More than 10 species of Mycoplasmatacaea have been reported to have been isolated from the bovine respiratory tract, including: *M. bovis* (formerly *Mycoplasma agalactiae* subsp. *bovis*), *M. dispar, M. bovirhinis, M. bovigenitalium, M. arginini, M. alkalescens, M. canadense, M. canis, Acholeplasma laidlawaii, A. axanthum, A. modicum* and *Ureaplasma* spp. [16,86,101,124,127,131,165,205,217,25592,108,133,136,140,177,221,234,277].

Mycoplasmas cause some of the most serious and economically most costly diseases of cattle. *Mycoplasma mycoides* subsp. *mycoides* small colony, the causative organism of contagious bovine pleuropneumonia (CBPP), is probably the most pathogenic bovine mycoplasma, but has been specifically excluded in this study, as it has been eradicated from South Africa and is not associated with BRD in feedlots. Contagious bovine pleuropneumonia is the only bacterial disease classified by the Office des Epizooties as a List A disease[108,119,179] and is a controlled disease in South Africa.

The role of mycoplasmas in calf pneumonia is considered by some researchers to be under-rated in comparison to that of viruses and bacteria as it is not uncommon to isolate two or more of the *Mycoplasma* spp. from a single incident of pneumonia [42,69,136,172,177,179,243]. Since Mycoplasmas require complex media and elevated CO_2 levels for isolation, their incidence in BRD may have been underestimated in the past [170,186,190,234,277,282].

Nine different species of *Mycoplasma* and *Acholeplasma* were inoculated intratracheally into gnotobiotic calves. Strains of *M. bovirhinis, M. canadense, M. verecundum, A. axanthum* and *A. modicum* did not produce visible pneumonic lesions and were not re-isolated from the lungs. Strains of *M. alkalescens* and *M. arginini* colonised the lower respiratory tract but failed to produce visible pneumonia. *M. bovigenitalium* and *M. dispar* both colonised the respiratory tract and induced pneumonic lesions estimated to involve up to 8% (*M. bovigenitalium*) and 17% (*M. dispar*) of the lung. Histologically *M. bovigenitalium* produced a cuffing pneumonia. Data would suggest that *M. bovirhinis* possesses limited tropism for the bovine lung [91,136,140]. The pathogenicity of *Ureaplasma diversum* could be demonstrated after endobronchial but not after intranasal inoculation [245]. Not all mycoplasmas appear to be pathogenic, but seem to decrease the host's

defenses by altering the immune responsiveness or by causing tissue damage and thereby allowing bacteria to colonise and invade the lung and cause a severe pneumonia [161].

Workers have more recently isolated *M. canis* from pneumonic calf lungs in Europe and Canada[42,164,177,178,244]. In the Netherlands *M. canis* was recovered from 5,5% of lung samples from 143 herds, but the role in bovine pneumonia is not clear, nor could it be clarified with experimental endobronchial inoculation [244,245]. It was originally assumed that the infections were as a result of close contact between calves and dogs, but was subsequently found not to be the case and can occur independently of each other and been shown to be capable of causing pneumonia in the absence of other bacterial pathogens [177]. *Mycoplasma canis* is usually isolated from the upper respiratory and urogenital tracts of dogs [42,164,178].

After *M. bovis*, *M. bovirhinis* is the next most frequently isolated mycoplasma in Europe. It has however been shown to occur equally in healthy and pneumonic calves and its role in pneumonia is questioned, as are *M. arginini* and *A. laidlawlii* [177]. Booker *et al* [32] found that an increase in *M. alkalescens* antibody titre after arrival at the feedlot was associated with an increase risk of BRD (OR = 1,10). This confirms findings from similar studies with other *Mycoplasma* spp. [212].

Pathogenic mycoplasma species have also been shown to induce proliferation of goblet cells and decreased viscosity of the mucous layers which reduces the efficiency of trapping and removal of inhaled substances. Mycoplasmas are also capable of suppressing the alveolar macrophages, thereby inhibiting the activation and phagocytic activity of these cells [9]

1.3.5.3.1 *Mycoplasma bovis*

Among the bovine mycoplasmas isolated from feedlot cattle, *M. bovis* stands out as being the most invasive and destructive[2,42,69,98,177,234,246] . This mycoplasma has been isolated from cases of pneumonia, as well as arthritis, tenosynovitis[1,9,12,43,106,111,121,139,204,234], mastitis [29,43,121,137,190,221] and the bovine genital tract[2,92,111,121,140,179,190,277], keratoconjunctivitis [134], otitis media [211,277,278], decubital abscesses [98,170,277] and has been shown to increase the severity of calf pneumonias and the lung lesions [42,90,93,111,177,206,210,246,277].

Opinion varies from merely being present, to being a primary or major pathogen capable of causing mortalities[1,32,69,122,136,156,161,177,179]. Work by Gourlay *et al* and other workers showed that *M. bovis* was capable of increasing the morbidity and mortality on farms with low grade respiratory disease [92-94,111,112,115,118,119,177,179].

Research has shown that *M. bovis* can act as a primary pathogen. A pure culture of *M. bovis* isolated from an outbreak of calf pneumonia in England [252] in 1975, was inoculated endobronchially or intratracheally into gnotobiotic calves. The pure culture was able to cause a subclinical (and occasionally clinical) pneumonia. Some of the calves used in the study also developed clinical lameness. No other bacteria considered to be pathogenic were isolated from the lungs, nor the joints [91].

Synergism has been demonstrated between *M. haemolytica* and *M. bovis* [94]. Mild clinical disease was produced in calves inoculated intranasally with *M. haemolytica* and *M. bovis* simultaneously, and resulted in moderate pneumonic consolidation at necropsy seven days later. The most severe lesions were produced by inoculating *M. haemolytica* one day after *M. bovis* inoculation [90]. Among lungs infected with *Mannheimia* and/or *Pasteurella*

species, more than 50% were mixed infections with *M. bovis* [248]. This emphasises the role of mycoplasmas in BRD without the need for a preceding viral infection [179,199,203]. In a study conducted by Poumarat *et al*, calves were experimentally inoculated intratracheally with *M. bovis*. Follow-up procedures included regular bronchoalveolar lavages (BALs) and clinical examinations. Counts were made of the mycoplasmas and other bacteria isolated from the BAL liquids. All animals developed a persistent *M. bovis* infection with a maximum BAL count on day 6 (from the start of treatment) and a secondary co-occurring *P. multocida* infection was found in most animals with maximum numbers reached on the 14th day [199].

The isolation rates of *M. bovis* from pneumonic lungs varies considerably, but the incidence would appear to be increasing worldwide. *Mycoplasma bovis* was not reported in Ireland until 1994, which coincided with the lifting of import restrictions from other EU countries in 1992. In an investigation to establish the extent of *M. bovis* involvement in clinical disease, the organism was isolated from 18% of 736 bovine lung samples collected from fatal pneumonia cases between 1995 and 1998 from 95 herds. In 34% of these positive samples, *M. bovis* was the only microorganism, including viruses, of known pathogenicity isolated[43,206]. In Denmark, 86% of pneumonic bovine lungs examined were infected with mycoplasmas, and there appears to be an increasing prevalence of *M. bovis* (24% of isolates), which was first isolated in Denmark in 1981. This is considerably up from previously reported 0,6 – 2% [137,242]. Other species isolated were *M. dispar, M. bovirhinis, M. bovigenitalum* and *Ureaplasma* spp. In a survey by the OIE of over 48 countries, *M. bovis* was seen as a major impediment to the cattle industry. In a USA survey, the organism was isolated from a third of over 400 pneumonic lungs. In France, *Mycoplasma bovis* was isolated in 25 –30% of pneumonic lungs in fattening calves and in Northern Ireland the incidence in pneumonic lungs sampled was 23% [177]. It was isolated

from 50-66% of cases of pneumonia in feedlot cattle, a population that is highly susceptible to respiratory disease [2,31,136,139,172,199,246].

The incidence may be higher in individual feedlots. Hjerpe [108] isolated 86% mycoplasmas from 500 lungs examined from fatal cases of feedlot pneumonia in a feedlot in the USA. These lungs also contained 52% *M. haemolytica*, 26% *P. multocida*, 14% *E. coli* and 12% *A. pyogenes*. Of the mycoplasmas isolated, 76% were *M. bovis* [108]. Langford [139] examined samples from 26 feedlots and isolated 86% *M. bovis* from pneumonic lungs and 41% from the joints of these animals. Thirty two percent of these animals sampled yielded only *M. bovis*. In a survey of samples collected in the Netherlands, ter Laak *et al* [243] reported a higher incidence of *M. bovis* from the lungs of veal calves being fattened that had not been sourced from dairy herds. In this study the respiratory tracts of 75% of calves examined contained *Mycoplasma* spp.

An ELISA test was used to diagnose *M. bovis* infection by seroconversion in paired sera obtained for animals at entry into a feedlot and seven weeks later. The overall seroconversion rate was 54,7%. Significant risk factors for seroconversion were the mixing of fattening herds of different age groups (risk ratio (RR) 1,70 at 95% confidence interval (CI) 1,48 to 1,96) and the presence of at least one seropositive animal in the fattening herd (RR: 2,02; CI: 1,69 to 2,40). The proportion of clinical episodes of respiratory disease attributable to *M. bovis* infection was 50,3%. The average mass gain during the observation period was reduced by 7,6% in seroconverting calves and these animals had about two times more antibiotics prescribed than calves remaining negative for *M. bovis* (RR 1,83) [256].

Despite the fragility of most mycoplasmas in the environment, *M. bovis* can survive at 4°C for nearly two months in cleaning sponges and milk and for over two weeks in water and 8 months (236 days) in manure [246]. Survival drops considerably at higher temperatures[190]. The environment is, however, not the most important source of infection, as the infection is usually introduced by clinically healthy calves that are shedding the mycoplasma. Once established on multi-age sites it is very difficult to eradicate [179,190].

It has been shown that animals that become infected with *M. bovis* after arrival in the feedlot remained so for some period of time and that the prevalence of the organism increased over this period [5-8,293], indicating the successful colonisation of the respiratory tract by the organism. It appears that the organism is able to evade the normal lung clearance mechanisms and that a specific immune response capable of eliminating the organism from the respiratory tract may take some time to develop. Mycoplasmas avoid the pulmonary defences through a variety of mechanisms, including attachment to the ciliated epithelium, antigenic mimicry of host antigens and immunosuppression of host humoral and cell-mediated responses [6,9]. Although *M. bovis* has been isolated from the lungs of both sick and healthy animals in the feedlot situation, it was shown that the persistent increases in inflammatory cells associated with BRD suggested that the organism could be an important underlying factor increasing the risk of respiratory disease in the feedlot [7,42,155].

The virulence factors of *M. bovis* and mechanisms of pathogenicity are still largely unknown, but there are several membrane surface proteins (variable surface lipoproteins - *vsp*) that play a role, especially with regard to attachment of the organisms to the host cells and may also serve to aid the organism in diverting and evading the host immune system [42,190,205,217]. Mycoplasmas colonise the respiratory tract by attaching to the ciliated tracheobronchial epithelium, causing inhibition of ciliary activity and later cytopathic

effects. Mycoplasmas may therefore cause respiratory disease directly following the initial damage to the ciliated cells or may allow for the invasion by secondary pathogens. By attaching to the ciliated epithelium, they remain above the alveolar macrophages, thus avoiding phagocytosis [61,110,205,232]. *Mycoplasma bovis* has also been shown to be immunosuppressive for both humoral and cell-mediated responses, and this together with the antigenic mimicry of host antigens, forms part of the pathogenesis of mycoplasmal disease [28], as well as allowing persistence within the host[61,98,108,205,214]. The suppression of humoral and cell-mediated immune responses could serve to curtail the effectiveness of bacteriostatic agents, which depend on phagocytic cells to kill bacteria [108].

It has been reported that *M. bovis* possesses 13 *vsp* genes involved in antigenic variation which alters the antigenic character of its surface components and that may act to enhance colonisation and/or adherence or evade the host's immune defence systems [149,150,205,217]. These *vsp* genes operate by various mechanisms including DNA transposition and intrachromosomal recombination within the *vsp* locus. The extensive sequence variations could imply an expanded antigenic variation within populations of this organism and an important means of diverting and evading the host immune system [42,181].

A pro-inflammatory toxin has also been isolated from *M. bovis* that is a complex polysaccharide, which increases vascular permeability and is capable of activating complement. Infusion of 0,9 mg of this toxin into the bovine udder resulted in the characteristic eosinophilic mastitis produced by *M. bovis* [83,98]. It has been proposed that the lung lesions associated with *M. bovis* could be as a result of a release of toxin(s) by the organism [119,264]. All strains of *M. bovis* produce septal oedema, visceral pleuritis and interstitial pneumonitis. It is thought that this is in response to the strong tumour necrosis factor-alpha (TNF-α) production elicited by the infection [211].

The capacity to activate bovine alveolar macrophages has been shown to differ between pathogenic and non-pathogenic *Mycoplasma* spp. *M. mycoides mycoides* and *M. bovis* for instance activated procoagulant activity (PCA), tumour necrosis factor-α (TNF-α) and nitrogen monoxide (NO) to varying degrees [129,264], but the serogroup 7 *Mycoplasma* and the non-pathogenic *M. bovirhinis* and *Acheolplasma laidlawlii* did not induce these activities [129]. It has been shown that *M. bovis* induces lymphocyte death, that specific *M. bovis* protein production is necessary for the induction of lymphocyte death and that the death is not dependent upon the addition of apoptotic inducers as shown with other mycoplasmas [264] and that *M. bovis* has an immunosuppressive effect [263].

Mycoplasma bovis can penetrate between the cells of the respiratory epithelium into the lamina propria below and has invasive potential into the blood stream [24,170].

Pulmonary lesions in naturally infected calves comprise an exudative bronchopneumonia and extensive foci of coagulative necrosis surrounded by inflammatory cells [140,170]. In studies involving experimental infections in gnotobiotic calves with *M. bovis*, significant pneumonia was induced involving up to 30% of the lung surface and was of sufficient severity to cause clinical respiratory disease in some calves [119,253]. Distinctive areas of coagulative necrosis were prominent within the lesions. Chronic infections are often associated with lymphocytic "cuffing" pneumonia where there is marked hyperplasia of peribronchial lymphoid tissue causing stenosis of airway lumina and compression and collapse of adjacent pulmonary parenchyma and a mild exudation of neutrophils and macrophages especially into the cranioventral parts of the lungs [91,128,140,148]. *Mycoplasma bovis* antigen is mainly detected at the periphery of the areas of coagulative necrosis, in necrotic exudates and in close association with infiltrating macrophages and neutrophils [210].

There would however appear to be some differences in lesions between naturally infected calves and experimentally infected lungs, with the naturally infected calves showing an exudative bronchopneumonia and extensive foci of coagulative necrosis surrounded by inflammatory cells, while experimentally infected lungs showed suppurative bronchiolitis and varying degrees of peribronchiolar mononuclear cell cuffing [210].

Mycoplasma bovis has also been isolated from cases of mastitis as well as the genitalia, but it would appear that the respiratory tract is the natural reservoir [29]. It has been shown that calves coming from herds with a history of *M. bovis* mastitis had a considerably higher nasal prevalence of *M. bovis* than animals coming from non-problem herds, especially animals less than 12 months of age [29]. A 6% prevalence of *M. bovis* in the nares of calves from non-infected herds is considered to be the 'norm'. In this study, nasal colonisation in problem herds persisted for several months in the absence of feeding infected milk, which suggests that once nasal colonisation is established in a group of young calves, it tends to persist in that age group and is transmitted to younger stock by direct contact as they come along. Despite the large number of animals with nasal colonisation in this study, there appeared to be no evidence of respiratory disease in the calves. An interesting finding in this study was that the nasal prevalence decreased as the environment temperatures decreased, which is in contrast with the appearance of BRD, which increases as temperatures drop, or more particularly as the diurnal temperature variation widens.

Studies conducted in the 1980s suggest that the host's immune response to *M. bovis* may be contributory to lesion development. Based largely on the prominence of IgG1 and to a lesser extent IgG2-containing cells at the periphery of characteristic necrotising *M. bovis*-associated lesions, it was proposed that a specific immunoglobulin, rather than a cell-

mediated immune response, is at least partly responsible for the lesions seen in calves infected with *M. bovis*, although its specific role was not further identified [115,119]. Mycoplasmas have a particular affinity for the ciliated epithelial cells lining the respiratory tract and infection leads to the attraction into the lungs of cells that produce the characteristic peribronchiolar and perivascular accumulations including plasma cells actively synthesising immunoglobulins in the rat [128]. It is possible that these cells and lymphnodes draining the lungs are the major sites of production of the antibody to mycoplasma that is found in secretions and sera after infection. The IgG1 and G2 antibodies in the lung washings are derived from the plasma cells in the lung tissue surrounding the necrotic zones [111].

Vaccines have been used experimentally for *M. bovis*, using killed organisms. Animals were injected intramuscularly (*im*) twice, intratracheally (*it*) twice, *im* + *it* or subcutaneously (*sc*) three times. Only animals injected *im* + *it* or sc three times showed increased resistance to *M. bovis* respiratory infection. Antibody was detected in lung washings from the calves inoculated subcutaneously, but is thought to be derived from serum [111].

In a study by Allen *et al*, the only agent cultured from calves which were found to be non-responsive to treatment (other than the viral agents) was *M. bovis*. It was also found in all of the samples taken from relapsing calves[8]. Mycoplasmas have been reported to be extremely difficult to eliminate from infected animals and tissue, even when the MIC's have been as low as 0,25 – 0,5 µg/ml and fully susceptible to the antibiotic *in vitro* [94].

Since immunoprophylaxis and antibiotic treatment are known to be ineffective, appropriate control measures need to be implemented at the earliest possible stage. These

include the introduction of strict hygiene standards, the restriction of animal movements and the culling of clinically diseased animals [190]. These are clearly not feasible in the majority of feedlots, but need to be considered.

1.3.5.3.2. *Mycoplasma dispar*

The role of *M. dispar* is a bit more uncertain, due primarily to the difficulty in isolation using the normal culturing techniques, which means that many laboratories that test for mycoplasmas, overlook this species, leading to a considerable underestimate of the prevalence [108,111,166]. *Mycoplasma dispar* is highly fastidious requiring specialist media and long culture times [232]. Early passages do not exhibit typical colony morphology nor do they react with hyperimmune serum, making identification difficult [166,247]. They are also easily outgrown by species such as *M. bovirhinis* which also metabolise glucose [243].

Mycoplasma dispar, which has previously been shown to produce a "cuffing" pneumonia in calves with an exudative bronchitis and interstitial alveolitis, has been shown to cause ciliostatic and ciliotoxic alterations which impair the mucociliary clearance [9].

Mycoplasma dispar and *Ureaplasma diversum* have resulted in the production of pneumonia in calves experimentally. These were however considered subclinical, with limited lesions [42,114]. *Mycoplasma dispar* is more frequently associated with an alveolitis [133,140] in which neutrophils, macrophages and oedema fluid accumulate in the alveolar walls and spaces [140]. A chronic lymphocytic ("cuffing") pneumonia, where there is marked hyperplasia of peribronchial and peribronchiolar lymphoid tissue causing stenosis of the airway lumina and compression and collapse of adjacent pulmonary parenchyma has been described by some authors [16,42,108,18142,114,193]

Mycoplasma dispar lesions were found mainly in the medium and small airways, which contrasts to *M. hyopneumoniae* infections in pigs, that localise on the epithelial ciliated cells of the trachea and main bronchi. *Mycoplasma dispar* has been shown to cause degeneration of respiratory epithelial cells with loss of ciliary activity and ciliastasis following inoculation endotracheally [9,111,133]. It also produces a surface-active material that aids in the colonising of the bronchial epithelium [10,211]. Encapsulated *M. dispar* or purified capsule exerts have an inhibitory effect on the activity of bovine alveolar macrophages and prevents the activation of these cells [11,277].

Results of a study in dairy calves suggested a synergistic effect between *Mycoplasma* spp. and *P. multocida.* The *Mycoplasma* spp. isolated was most probably *M. dispar*, based on seroconversion data collected. Antibodies against *M. dispar* were significantly elevated in affected calves and could indicate a possible initiator role of *M. dispar* in the development of respiratory tract disease [274].

Mycoplasma dispar appears less immunogenic in calves than *M. bovis* and vaccine given either intramuscularly or intratracheally or both, did not produce a detectable protection from colonisation, and this may form part of its pathogenicity [111]. The difference in immunogenicity may be because *M. dispar* is less invasive [118].

1.4. SPECIMEN COLLECTION AND CLINICAL PARAMETERS

Because the aetiology of BRD is so complex and in order to study the flora of the respiratory tract, various collection methods have been used in the live animal. These include nasopharyngeal swabs (NPS), bronchoalveolar lavage (BAL) [76] and transtracheal washes or aspirates (TTW or TTA)[8,71]. The reliability with which upper airway cultures

(NPS) can be used to predict the presence of organisms in the lung has not been fully demonstrated in cattle and there has been much debate as to the suitability of the various swab collections used in the various studies reported on. Allen *et al* [5,6,8] and others [64,163] are of the opinion that bacterial pathogens isolated from the nasopharynx correlate well to those isolated from the lower airways at a group level, while in individual calves the NPS did not accurately predict BAL cultures. Other workers question these results [45-47,51,52,71,136,162,203,228,249,287]. Yates *et al* stated that no relationship was found between the presence of potential pathogens in nasal mucus and the occurrence of lesions in the lung [293]. This is of particular importance if sensitivity antimicrobial testing is to be done, as isolates cultured from nasal swabs may not represent sensitivities of organisms causing pneumonia [18,27,162,203,228,249]. Although the tonsillar crypts and the upper respiratory tract are the prime site for *M. haemolytica* multiplication, there have been discrepancies between sensitivities of the bacteria isolated and the clinical outcome[8,13,287]. Ideally specimens for sensitivity testing should be taken from the pneumonic lung, using tracheal swabs or tracheobronchial aspirates [14,51]. This reduces the chances of selecting organisms from the upper respiratory tract that may be commensals [45]. Bronchoalveolar lavage specimens are generally collected with either a laryngoscope, endoscope or a proprietary BAL catheter [162], which although allowing for accurate positioning in the bronchi, are expensive and easily damaged and difficult to keep sterile if many samples are to be collected [86]. The alternate is the use of a nasal tube which tends to be stressful to the animal, especially if dyspnoeic (personal experience) and also more difficult to prevent contamination. Contamination from the mucus and organisms in the upper respiratory tract are also an important consideration, as mucus may collect on the outer tube and contaminate the mouth of the inner tube as it is passed through [46]. Most researchers are of the opinion that transtracheal washes are better for bacterial isolations, while BAL is better for cytology and viral isolations (with the exception of BHV-1 which are more

readily isolated from nasopharyngeal samples) [46,286,287]. In a study, *M. bovis* was easily isolated from pneumonic lungs, but isolation from nasal samples was hampered by concomitant contaminants [221]. Isolation rates of mycoplasmas have been reported to be higher from lung lavage fluids than from nasal swab specimens [243,249]. Since pneumonia, rather than rhinitis, is the major cause of morbidity and mortality due to respiratory disease, transtracheal washes are the preferred specimens [228].

The selection criteria for sick or healthy animals has been a problem in the past and it is difficult to eliminate all false positives or negatives. In a North American study, 35% of cattle received treatment for respiratory disease, on the basis of their clinical appearance. When they were slaughtered, 78% of those treated had evidence of pulmonary lesions compared with 68% of the untreated cattle [25,40,288] (although these lesions were less extensive). This result suggests that the performance of ‘clinically healthy’ cattle may also have been reduced by subclinical disease. A similar study by Griffin [96], reported that 50% of cattle that had never been diagnosed as having respiratory disease during life had gross lung lesions at slaughter.

The clinical appearance of BRD syndrome depends directly on the relationships of the various causal factors and the host’s immune response. Lekeux [56,143] has proposed an interesting classification of clinical appearance according to the severity of the disease, the pathophysiologic mechanisms implicated and the level of reversibility. He has proposed four grades from Grade 1, which is subclinical where the animal succeeds in controlling the proliferation of the pathogens without obvious pulmonary dysfunction and without clinical signs. Treatment is unnecessary and uneconomical. Grade 2 is compensated clinical, where the inflammatory reactions to the pathogens are still at the beneficial level. Antibacterial cover will prevent further lesions. Grade 3 is non-compensated clinical

disease, with the lesions induced by the inflammatory responses being more severe than from the pathogens. The disequilibrium needs to be controlled by appropriate treatment to prevent an unfavourable outcome. Grade 4 which is irreversible clinical disease, whereby the pulmonary lesions generated either by the pathogens, proteolytic enzyme or oxygen radicals from the inflammatory cells, threatens the animals performance and survival. It is unfortunately not always possible to recognise this classification by clinical examination in the field and has lead to examination of more objective parameters to aid in the making a valid prognosis and thereby avoiding unnecessary treatment costs. Such studies have included looking into using plasma lactate measurements in BRD [56]. Plasma lactate concentrations were shown to increase with the severity of the disease process, and while the sensitivity and specificity of the test could not really distinguish the differences between the lower grades or the moderately diseased animals. (see Table 4). A plasma lactate level > 4 mmol/*l* was a reliable prognostic indicator for mortality within 24 hours.

Table 3. Critical plasma lactate levels to distinguish different levels of severity in cases of bovine bronchopneumonia[56].

	Grade 1 vs 2	Grade 2 vs 3	Grade 3 vs 4 (day 1)	Grade 3 vs 4 (day of mortality)
Lactate level	1.4 mmol/*l*	1.6 mmol/*l*	2.6 mmol/*l*	3.6 mmol/*l*
Sensitivity	0.67	0.57	0.95	0.95
Specificity	0.50	0.80	0.85	0.96

1.5. TREATMENT

The starting point for any control plan is to identify the following:

- the group(s) at risk and the risk period(s)
- the challenge organism(s)
- the possible control strategies (management, vaccination, treatment) [86,143].

To meet the criteria of efficacy, safety and minimization of residues, a therapeutic strategy must be applied early enough to prevent the development of irreversible lesions and must be adapted to the clinical grades of the disease. Strategies can include: suppression of infectious agents, modulation of the pulmonary inflammatory reaction and correction of the mechanical disorders. The aim of the latter is to improve pulmonary gas exchange by means of bronchodilators, vasodilators or stimulators of mucociliary clearance [143].

1.5.1. ANTIBIOTICS USED TO TREAT BRD[24,34,44,52,125,176,201,203]

The basic principle for antimicrobial therapy for BRD is to treat early enough, treat long enough and treat with the appropriate antimicrobial agent [14,16]. One of the major reasons for treatment failure is the presence of a lesion that is too far advanced for successful therapy. Most cattle will usually show some improvement within one to three days of initiating treatment and complete recovery may take four to seven days [16].

Response to antibiotic treatment of BRD may be variable because of the presence of mycoplasmas. It has been stated that diseases due to *M. bovis* are more resistant to chemotherapy [94,144,189,282]. Recent evidence suggests that *M. bovis* strains in Europe are becoming resistant to antibiotics traditionally used for the treatment of mycoplasma infections [19,177]. The minimum inhibitory concentrations and minimum mycoplasmacidal concentrations of danafloxacin, florfenicol, oxytetracycline, spectinomycin and tilmicosin were determined *in vitro* by a broth microdilution method. The results showed that all the strains tested had developed resistance to tilmicosin, most were resistant to tetracyclines and about 20% were resistant to spectinomycin. Florfenicol was slightly more effective and only danafloxacin was effective on all the strains tested.

The frequency of mycoplasma isolation from lung tissue and the severity of naturally occurring pneumonia was significantly reduced following tylosin treatment orally in

calves (from 71% to 39%), but not with ampicillins, which are ineffective against mycoplasmas [161].

BRD is not only costly to farmers, but also detrimental to animal welfare and is reported to result in the greatest use of antimicrobials in cattle husbandry[25,26,69]. There are also obvious animal welfare benefits from the early detection and effective treatment of respiratory disease [220]. There is also increasing concern about the use of antibiotics in food-producing animals and the contribution it may make to bacterial resistance in human pathogens[24,26,27,42,203,220], as well as increasing concerns about the costs of trimming tissue damaged by injection reactions from carcases at slaughter and about the detrimental effects of intramuscular injections on meat quality [25,84,268] The veterinary profession needs to rationalise the use of antibiotics in food animal production and the following questions need to be considered:

- are the probable bacterial and mycoplasmal pathogens likely to be sensitive to the antimicrobials *in vitro* and *in vivo*?
- can the product be expected to reach therapeutic concentrations in the infected tissues for a sufficient period of time?
- is this antimicrobial available in a preparation which is licensed for use in this class of animal?
- is the route of administration appropriate to the animal, and does the dosing interval suit the management situation?
- what is the required minimum withdrawal periods and are the meat residues likely to be a problem?
- are there any risks to human health in the use of these products?
- what previous success has been achieved by using these products on the farm or elsewhere?

- what is the cost of treatment?
- what is the likely cost-benefit of using this product over another [25,26]?

Reasons why treatments for bovine respiratory disease fail were proposed in the early 1970's and have not changed over the years. They are:

- Wrong diagnosis
- Lesions too far advanced
- Complicating causes
- Insufficient treatment
- Bacterial resistance
- Aberrant complications [122]

The cost of respiratory disease can be broken down into the following:

- prevention and treatment with drugs and vaccines
- veterinary professional fees
- farm labour, including opportunity costs
- mortalities, replacement costs
- loss of production from chronic cases and culls
- general production inefficiency, reduced livemass gains and reduced feed conversion rates, leading to extended feeding periods to finish
- reduced sale value of finished cattle, due to poorer carcass grading and possibly poorer marketing times [25,26]

The criteria for the selection of appropriate and cost-effective drugs, should therefore be based on good clinical practice and sound economic principles. These should also integrate quality assurance schemes and the monitoring of antimicrobial resistance patterns among known respiratory pathogens on an ongoing basis. Quality assurance

schemes must provide for better animal health monitoring, promoting preventive medicine programmes, while also considering animal welfare issues [25,220].

In a study to determine the frequency of pathogens persisting in tissues of animals that had failed to respond to antibiotic treatment, *M. bovis* and BVDV were the most common findings [100]. *Mycoplasma bovis* was isolated from 80% of the samples, 71% were from lung samples and 45% from joints. *Mannheimia haemolytica* was isolated from 23% of these samples and *H. somni* 14%. *Mycoplasma bovis* was the only bacterial pathogen identified in joints [100]. The frequent isolation of *M. bovis* is in agreement with other studies conducted in Canada [156,212]. Thirty two percent of the samples had two or more types of bacteria present, which suggests that any single bacterial pathogen may not always be the primary aetiologic agent that predisposes to unresponsive disease [100].

1.5.1.1. β-LACTAMS

- **Penicillins:**

Penicillins do not penetrate tissue well and are usually restricted to extracellular fluids and therefore need to be administered in higher doses [24]. They act on the bacterial cell wall and therefore mycoplasmas are not covered and the spectrum to Gram-negative organisms is limited. They are bactericidal and lipid-soluble. Long-acting preparations should not be used on their own in acute *Mannheimia* or *Pasteurella* pneumonias because of their lower tissue concentrations and limited spectrum. Secretion can be delayed by salicylates, phenylbutazone or sulphonamides, which are often used concurrently [34,125].

- **Aminopenicillins:**

(Amoxicillin and Ampicillin in particular) can penetrate the outer layer of Gram-negative bacteria more readily, giving the group a better spectrum, except for ß-lactamase producers,

unless the antibiotics are combined with clavulanic acid (amoxicillin) or sublactam (ampicillin) [88].

- **Cephalosporins:**

Especially the newer generations have a much broader spectrum against Gram-negative bacteria and *Mannheimia* or *Pasteurella* in particular. They are also highly resistant to ß-lactamase. Ceftiofur was specifically produced for the treatment of BRD and has a $MIC_{90} <$ 0,03 µg/ml for *M. haemolytica*, which is well below the recommended breakpoint of 2,0 µg/ml. In a survey of over 888 *M. haemolytica* post-mortem isolates, over the 1988 – 1992 period (Ceftiofur was launched into the USA feedlot industry in 1988), there was no evidence of resistance in the USA, Canada and Europe [67,257,258,280,290].

1.5.1.2. AMINOGLYCOCIDES / AMINOCYCLITOLS

The importance of the aminoglycosides in veterinary medicine is in the treatment of severe Gram-negative sepsis [44,200], although their highly cationic polar nature means that distribution across membranes may be limited[200]. The aminoglycocides are mostly bactericidal, water soluble and inhibit protein synthesis and effective primarily against aerobic Gram-negative bacteria with limited Gram-positive activity and are considered to be inactive against anaerobic bacteria [200]. Streptomycin and dihydrostreptomycin have narrow spectrum of activity and are considered by most workers to be ineffective in the treatment of BRD. Neomycin and kanamycin have a slightly more extended spectrum, while gentamicin and tobramycin are considered to be broad-spectrum. Spectinomycin is a related compound, with a medium-spectrum. The group does not penetrate the bronchial secretions well [24] and therapeutic concentrations are not attained in tracheobronchial secretions [125]. However, they are rapidly absorbed after parenteral administration and reach relatively high pleural fluid concentrations, particularly if inflammation is present [125]. Gentamicin should be administered

by intravenous route because of low cell penetration [24]. The group is better in alkaline (pH 8) than acidic environment, and are synergistic with ß-lactamase antibiotics. Excretion is via the kidneys and can be nephrotoxic. They generally have a relatively short plasma half-life and are often advised that they should be administered 2 to 3 times daily in cattle [125].

1.5.1.3. SULPHONAMIDES

Sulphonamides remain among the most widely used antibacterial agents in veterinary medicine, chiefly because of low cost and relative efficacy in some common bacterial diseases, having effects against both Gram-positive and Gram-negative organisms, but not against mycoplasmas [34,200]. The group are structural analogues of *p*-amino benzoic acid and inhibit bacterial folic acid synthesis. They are bacteriostatic and therefore host defense is important. They generally have a broad spectrum of activity, but must be administered early when organisms are rapidly multiplying [34]. They are well distributed with high lung concentrations (3-4x serum or plasma concentrations). The group can, however, depress normal cellulytic function in the ruminoreticulum and is incompatible with calcium.

- **Potentiated Sulphonamides:**

A group of diaminopyrimidines are also capable of inhibiting the dihydrofolate reductase in bacteria and are often added to sulphonamides to effect a double blockade of folic acid synthesis, with less chance of resistance development than either on their own [34]. Trimethoprim is the most commonly used diaminopyrimidine available and are usually combined with the sulphonamide in a 1:5 ratio. The potentiated sulphonamides generally have a good tissue penetration, particularly into the respiratory tract and secretions. The action of the combination is bactericidal, whereas the components separately are bacteriostatic[44,200].

1.5.1.4. TETRACYCLINES

Since they have a broad spectrum of activity (including mycoplasmas, chlamydia and rickettsia), they were the drug of choice for the treatment of BRD for many years. Tetracyclines readily penetrate most tissues, including lung and respiratory secretions, with higher concentrations in diseased tissue (probably the reason that *in vivo* efficacy is often better than *in vitro* testing [261]). Widespread increase in resistance has however been shown (have reduced uptake into bacterial cells and acquired ability of bacteria to "excrete" drug out of the cell, or lowered initial concentrations). Resistance is transferred by R-plasmids and mutant strains, especially with subtherapeutic or subinhibitory concentrations. Tetracyclines are active in both acidic and alkaline environments, but chelates with di- and trivalent cations, forming insoluble complexes. They affect the bacterial ribosomes, thereby impairing protein synthesis and are bactericidal in large doses, but otherwise bacteriostatic [24,34,44,125].

Products with a propylene glycol (PG) base causes tissue irritation and inflammation in cattle, while polyvinylpyrrolidine (PVP) base causes less pain and tissue damage and can therefore be administered in higher concentrations, thereby achieving higher blood and tissue concentrations. The newer 2-polyvinylpyrrolidone base causes even less damage and higher concentrations leading to smaller doses are now possible [24].

Long-acting formulations are often used as blanket prophylaxis on arrival / processing in feedlots in South Africa, against possible heartwater and anaplasmosis, which raises questions about effects on development of bacterial resistance and concurrent use of live bacterial vaccines. Long-acting formulations greatest value is when used as a follow up of final treatment of animals that have already undergone daily therapy [53], or prophylaxis when the morbidity rate reaches a predetermined level [124].

- **Doxycyclines:**

They have a greater tissue penetration than other tetracyclines, due to 5 to 10 times increased lipid solubility and increased protein binding, thereby enhancing tissue penetration and prolonging the biological half-life. They can, however, also have prolonged excretion and drug residues [24,34,44].

1.5.1.5. MACROLIDES:

These are basic drugs (most active pH 8), with high lipid solubility, reaching high concentrations in the respiratory tract, where they accumulate in the lungs with a lung:serum ratio of 3-5:1. Intracellular accumulation within phagocytes has also been shown and this may have an immunomodulatory effect, but the precise pharmacodynamic relationship between intracellular concentration and bacterial killing remains to be defined [200]. The tissue and intracellular accumulation may allow for effective use of doses that appear to produce suboptimal serum concentrations [24]. The group generally have a relatively broad spectrum of activity, being active against Gram-positive with some Gram-negative (including *Mannheimia* or *Pasteurella* and *Histophilus*) bacteria, a fair to good efficacy against anaerobic bacteria plus some mycoplasmas, rickettsia and *Chlamydophila*. They interfere with the bacterial protein synthesis at the ribosomal level and are generally bacteriostatic, but may be bactericidal at high concentrations [24,34,200].

- **Tylosin:**

Tylosin’s chemical structure and mechanism of action are similar to those of other macrolides and was developed exclusively for veterinary use. It is a weak base (pKa 7.1) and highly lipid soluble and also reaches high tissue concentrations, particularly in the lungs and udder. A partial cross resistance has been shown to other macrolides. The antibacterial

spectrum is mainly Gram-positive aerobes and anaerobes plus certain Gram-negative organisms such as *Pasteurella* and *Haemophilus*, but has an even greater activity against mycoplasmas [24,34,44,200].

- **Tilmicosin:**

Tilmicosin is a chemically modified macrolide derivative of tylosin that was originally developed as a single long-acting treatment for BRD. The product is characterized by low serum concentrations but large volumes of distribution with accumulation and persistence in tissues, including the lung, which may concentrate the drug 20-fold relative to the serum [200]. In addition to being bactericidal, tilmicosin is capable of modulating inflammation in the lung by reducing LPS-induced alveolar macrophage prostaglandin production [138]. The efficacy against *Pasteurella* is better than tylosin because the product produces therapeutic levels in the lungs for 3 to 4 days with a single subcutaneous injection of 300 mg/ml in a 25% propylene base (has important implications for self-injection by the lay-staff as the base is potentially toxic to the cardiovascular system). The product has been used with great success in Canadian feedlots, but showed resistance in *Pasteurella* in the USA and Europe before it was even launched (resistance increased from 4,5% to *M. haemolytica* and 8,3% to *P. multocida* in the 1989/90 BRD season to 9,5% and 22,5% respectively in the 1990/91 season [67,257,258,279,280,290]). This resistance pattern is probably due to the extensive use of erythromycin in the USA in particular, but also previous tylosin use [24].

Prophylactic use and single treatment has been compared to many of the normal treatments [99,165,192,219]. Prophylactic use on arrival at feedlots significantly reduced treatments during the first five days and the first month, with the average days to first treatment increasing from day 9 to day 21. The tilmicosin group had an increased average daily gain and feed efficiency over nonmedicated controls during the first month that was monitored [219]. Single

subcutaneous tilmicosin injection showed that colonization by *M. haemolytica* and *M. bovis*, the clinical scores and the extent of pneumonic consolidation were suppressed or greatly reduced in cases infected with the organisms by the intratracheal route [94].

- **Erythromycin:**

Erythromycin efficacy is predominantly Gram-positive, especially ß-lactamase, and reasonable to good against *Pasteurella*, but less effective against mycoplasmas than the other macrolides (Morter [169] found a high susceptibility but only a 60% response). Erythromycin was used extensively in feedlots in the USA, but less commonly used in the rest of the world.

1.5.1.6. FLUOROQUINOLONES:

They are related to nalidixic acid (are quinolone carboxylic acid derivatives [144]) and inhibit the bacterial enzyme DNA-gyrase and exhibit concentration-dependent killing [34,276]. Quinolones were initially primarily active against aerobic Gram-negative bacteria but the newer generations are active against both Gram-positive and Gram-negative bacteria, including *Mycoplasma* [34], but are ineffective against obligate anaerobic bacteria. They are bactericidal at twice the MIC. Enrofloxacin supposedly reaches a ratio of 2:1 [24] for lung: plasma and danofloxacin 4:1 [24,219] and penetrates intracellularly into phagocytic cells reaching concentrations of seven times greater than the extracellular fluid. The group has good pharmacokinetic properties and a low toxicity and with parenteral administration are rapidly absorbed. The rapid and extensive tissue distribution is because of their hydrophilic nature and low (<50%) protein binding [276].

The fluoroquinolones are part of the newer generation of antimicrobials for use against BRD. Although resistance develops quite rapidly to naladixic acid, it is not regarded as being a

significant problem with the newer generation products [34].Their unique action means a lesser chance of resistance developing, but a "reduced efficacy" was experienced in poultry in South Africa after extensive oral use (personal observation). This might be because of their peculiar biphasic effect, whereby they are not only less effective against susceptible organisms below MIC values, but are also substantially less active at concentrations higher than their optimal ranges. This is thought to be caused by the depression of RNA synthesis [44]. Perhaps more than any other class of antimicrobial agent, dosage of fluoroquinolones should be based on the susceptibility of the bacterial target, as clinical efficacy is dependent on dose and bacterial agent. The maximise clinical efficacy and decrease the selection of resistant bacteria, C_{max}: MIC ratio of $\geq$ 10:1 or AUC_{0-24}: MIC ratio $\geq$ 125:1 may be required [276].

1.5.1.7. AMPHENICOLS:

One of the few really effective antimicrobials against BRD over the years has been chloramphenicol. The product has however been banned from use in food producing animals, due to its effect on irreversible aplastic anaemia and other blood dyscrasias in man [34,167]. Chloramphenicol structural analogues, florphenicol and thiamphenicol, do not have the *p*-nitro group associated with idiosyncratic aplastic anaemia and are not banned in food producing animals. The group inhibit protein synthesis and are usually bacteriostatic, but may be bactericidal in some species. They have a wide range of bacterial activity against Gram-positive and many Gram-negative bacteria, but while mycoplasmas often show susceptibility *in vitro*, the outcome of therapy of pulmonary infections caused by this organism is often disappointing [24,34,53,167,200].

Florphenicol is a fluorinated derivative of thiamphenicol and has a wide tissue distribution and high bioavailability. It may be bactericidal against *Pasteurella* and *Haemophilus* at concentrations only one dilution above those which are bacteriostatic [200]. Florphenicol has

been approved for the treatment of BRD in feedlots, either at a dosage of 20 mg/kg intramuscularly, administered twice at forty eight hour intervals or once administered subcutaneously at 40 mg/kg. Both routes of administration have relatively long withdrawal periods.

1.5.2. ANTI-INFLAMMATORIES

Because of the important role of inflammatory processes in BRD from both the organisms involved and the host response, the use of anti-inflammatory drugs could be an important consideration as part of the therapy applied in the modulation of lung inflammation [145]. Corticosteroids lead to complete inhibition of arachidonate metabolites, but their use is considered by many to be counterproductive because of their possible effect on virus recrudesence, immunosuppression and aggravation of stress effects [16,136,164,199145,176,214]. Although corticosteroids do have a 'euphoric' and gluconeogenic effect on depressed cattle and may stimulate the calves to eat and otherwise speed up its recovery, the data on glucocorticoids for BRD is considered by some to be inconclusive and are not recommended until further research is conducted in large trials with naturally occurring disease [72,80].

Non-steroidal anti-inflammatory drugs (NSAID's) that block cyclo-oxygenase (COX) and lipoxygenase enzyme activity are therefore most commonly used. Although NSAID's are successful at blocking or reducing the production of prostaglandins, and in some cases, leukotrienes in treated cattle, the ability to suppress inflammation would appear to be very variable in bovines. Flunixin and ketoprofen effectively reduced fever in LPS infused calves, but were unable to prevent the associated leukopaenia, hypoglycaemia or hypotension [60,275]. In a study using oxytetracycline and flunixin meglumine, the treated

calves demonstrated a decreased respiratory rate, appeared less depressed and had a lower rectal temperature after the first day of treatment. There was however no statistical difference in lung pathology or mass gains [72,80]. The analgesic effect of NSAID's may improve the clinical status to the point where animals continue to eat and drink, which allows nutritional and fluid requirements if used early in the disease process [145].

The use of NSAID's in BRD are widespread, including extra-label and non-approved products, and although they have obvious anti-pyretic and analgesic activities in cattle, do not appear to have the same anti-inflammatory effects as experienced in some other species [17,18,24,60]. This might be partly as a result of the neutrophil-platelet interaction and the fact that the respiratory burst may occur in bovine neutrophils via cyclo-oxygenase independent pathways. The platelet effect is also partly COX-1 and the new generation of COX-2 anti-inflammatory drugs are likely to be even less responsive in cattle [60].

The reported efficacy of tilmicosin against BRD and mycoplasmas in spite of increasing resistance development [173], may partly be attributable to tilmicosin's ability to modulate inflammation in the lung, by reducing the alveolar macrophage-prostaglandin E_2 production induced by lipopolysaccharide (LPS), but not by altering the COX-2 expression [138].

1.5.3. OTHER SUPPORTIVE TREATMENTS

Because of the hypoxaemia caused by endotoxin release, consideration could be given to using an intravenous infusion of a small volume (5ml/kg bodymass) of hypertonic saline (7.2% NaCl) (HSS) for initial resuscitation of cattle with naturally developing BRD. Endotoxin administration causes a severe hypoxaemia accompanied by a significant

decrease in the partial pressure of oxygen (PaO_2) and an increase in the arterial-alveolar O_2 gradient. This was reversed in HSS treated animals, but not with isotonic saline or no treatment [240].

1.6. DISEASE PREVENTION AND CONTROL

1.6.1. PRECONDITIONING

When combined with good management practices, vaccination is considered an effective method of control of BRD [188,229]. The problem is identifying the pathogens to vaccinate against and when to administer the vaccine. Calves arriving at a feedlot are under considerable stress and therefore immunocompromised. They are possibly exposed to a massive challenge from new viral, bacterial and mycoplasmal pathogens, and the feedlot operators expect the animals to develop a solid immunity to up to fourteen vaccines administered on arrival. Long-term protection of animals offers important advantages in the long-term management of BRD with the reduction in the frequency of vaccine boosts and associated costs and indirectly with the reduction of economic losses associated with the treatment of sick animals and loss of performance [188]. Calves need to be prepared for where they are going and not where it has been, which entails a complete herd health and nutritional programme. Calves with inadequate passive transfer of maternal antibodies in the colostrum received, are at a greater risk of suffering BRD (odds ratio=3.1) than calves with adequate passive transfer [227,229,289]. Twenty seven percent of calves diagnosed with endemic pneumonia had inadequate levels of passively acquired antibodies [269]

The inability to adequately control BRD by means of treatments, vaccinations and other measures, means that other novel methods of controlling the disease have had to be

resorted to in order to reduce the number of high-risk calves coming into a feedlot [70,186,229]. Preconditioning calves prior to arrival at a feedyard remains a theoretically good approach [16,57,81,122,143,176,186,203,229]. The main aim of preconditioning is to ensure that calves have been weaned an adequate period before shipping – so-called "green calves" (the Alberta programme recommends at least 30 days); have received their primary vaccinations before being shipped off to the feedlots, so that the immune system has sufficient time to mount a response before any disease challenge. This means that the vaccinations at processing now become a booster and not the primary; and also ensure that the calves are "food bunk broke". These measures ensure that a calf will acclimatise quicker and get "on feed" [185,203]. In a vaccine study, cattle that were preconditioned before transport to the feedyard were 19.5 times less likely to become sick than cattle not previously vaccinated [186]. Nesbit *et al* [176] have however questioned the efficacy of early vaccination in South Africa based on a pilot trial where vaccinations a month prior to admission accrued minimal benefits. One of the problems with preconditioning is the attitude of the primary cow-calf producer who wants to reduce his input costs, rather than looking at the cost of a disease prevention programme or a value-added health programme [229].

1.6.2. VACCINES

The inability of chemotherapy to control *M. bovis* has focused some attention on vaccination. Surprisingly there are very few vaccines against mycoplasmas in cattle currently available in the world and none in Europe or South Africa. In Canada, over 80% of the biologics licensed for use in cattle are against agents associated with BRD and do not include any against mycoplasmas [35].

An inactivated vaccine containing BRSV, PI-3, *M. bovis* and *M. dispar*, showed some protection, with the death rate from pneumonia of 9% in unvaccinated controls, 3% for BRSV vaccinates and 2% for the quadrivalent vaccine ($p<0.001$). The proportion of calves receiving treatment for respiratory disease was 38% for the control group, 27% for the syncytial group and 25% for the quadrivalent group [116,119].

A vaccine prepared with formalin inactivated strains of *M. bovis* and *M. haemolytica* taken from the target herd reduced losses from pneumonia and the costs of treatments in newly introduced feedlot calves [260].

In another experiment to investigate colonisation of the lungs by *M. bovis*, calves were vaccinated with an inactivated vaccine intramuscularly, followed by a booster intratracheally. A second group of calves received two intramuscular injections of the vaccine. Calves vaccinated intramuscularly and intratracheally were better protected, as evidenced by fewer mycoplasma organisms being isolated from the lungs. This suggests that local immunity may be important in the protection of calves against *M. bovis* [113,234].

More recently a saponised inactivated vaccine was shown to be safe, highly immunogenic and protective against a strong experimental challenge of virulent *M. bovis* [180]. Vaccinated calves showed fewer respiratory signs while all unvaccinated calves developed signs of pneumonia. There was a statistically significant difference in body mass gain and lung lesions. The vaccine also reduced the spread of *M. bovis* to internal organs including joints. Saponins appear to have the ability to induce an isotype profile similar to that seen in natural immunity to bacterial infections. It has also been reported that saponins are able to inactivate mycoplasmas rapidly. Further research with and the development of saponin as an adjuvant for mycoplasmal vaccines need to be followed up [179,180].

Attempts to vaccinate against *M. bovis* arthritis have been less successful against experimental infection although high levels of antibodies were detected before challenge [179,198]. Chima however reported some success using both a live and a formalinised *M. bovis* vaccine [48]. Experimental vaccines against mastitis have also not been successful and in fact might have aggravated the condition [179].

It has been found that mycoplasmas are not very immunogenic [28] and that vaccines require an adjuvant such as Freund's complete adjuvant (FCA) to produce an immunity to infection. Adjuvants have been shown to increase the numbers and activities of macrophages at the site of inoculation and hence enhancement of B- and T-lymphocyte activities [28]. Possible explanations for the immunosuppressive actions of *M. bovis* inoculations include the failure of sufficient T-lymphocyte stimulation either by a lack of macrophage, mycoplasma, or T-lymphocyte interaction or by stimulating T-lymphocyte suppressor cells. Mycoplasma cell membrane morphology has been shown to be similar to that of mammalian cells and do not possess a cell wall or capsule. Mycoplasmas have also been shown to be able to incorporate environmental proteins, including antibodies, onto their membrane surface. Therefore it might well be that T-lymphocyte suppressor cells are functioning due to the similarity of mycoplasma membranes to self and/or to the camouflage of self antigens on mycoplasma [28].

Protective immunity against *M. haemolytica* antileukotoxin, *H. somni*, BHV-1 glycoprotein, BVDV and *Mycoplasma* spp. may be necessary to reduce the occurrence of BRD [32]. Vaccines have only been able to reduce the frequency and severity of BRD and does not eliminate it and therefore therapeutic measures remain indispensable to reduce the economic impact of the disease [143]. Booker *et al* [32] have proposed that a protective immunity against *M. haemolytica, H. somni,* BHV, BVD and certain *Mycoplasma* spp.

may be necessary to reduce the occurrence of BRD. This confirms work done by Martin [158] and others [1,48,93].

CHAPTER 2.

PROBLEM OR HYPOTHESIS

2.1. Problem statement:

Mycoplasma species, especially *M. bovis*, plays a role in BRD in feedlot cattle in South Africa.

2.2. Hypothesis:

Should *Mycoplasma* spp. play an important role in the development of BRD in feedlot cattle we would expect that there will be an increase in the isolation rate in transtracheal washes in animals showing clinical signs of respiratory disease, in comparison to the levels of isolation in calves with no evidence of respiratory disease.

2.3. Objectives of the study:

- To provide a literature review of the bovine respiratory disease complex on the role of the animal, its environment and the pathogens involved.
- To collect transtracheal aspirate samples from calves that are showing signs of respiratory disease and those that are not.
- To isolate and identify the *Mycoplasma* spp. and the primary bacteria in these samples.
- To determine the significance of the isolation rates of these microorganisms.
- To determine the antimicrobial sensitivity profiles of the bacterial isolates.

To the best of our knowledge the role of *Mycoplasma* spp. has not formally been studied in South Africa and if they have been studied, the results do not appear to have been published.

The project started several years ago when samples were collected to look at a possible link with *H. somni* and BRD in cattle dying towards the end of the feeding period. *Histophilus somni* was isolated then, but it was also suspected that mycoplasmas played a role. Results of further samples taken during the winters of the 2000 and 2001 were incorporated in this study. The interest at that stage was primarily of the bacteria involved and to monitor the resistance patterns to antibiotics commonly used as treatment, as a service to the feedlot operators. As the samples for the mycoplasmas and the bacteria are the same, the projects were combined.

CHAPTER 3.

MATERIALS AND METHODS

3.1 NUMBER OF CATTLE TO BE SAMPLED

Samples from sick and healthy animals were taken from feedlots that were experiencing problems with BRD. Samples were collected from feedlots throughout South Africa, other than the Western Cape. This ensured a widespread diversity of organisms and management practices. Samples were collected predominantly from May to September, which coincides with the highest BRD incidence period.

The number of samples to be included in the study was determined by the assumption that *Mycoplasma* plays an important role in the pathogenesis of BRD in South African feedlots. It was estimated that approximately 80 to 100 animals showing signs of BRD and 20 "healthy" animals will need to be sampled for the results to be statistically significant.

The sample size was determined using the following formula and calculated on two estimated prevalences [254]:

$$n = \frac{1.96^2 \times \hat{p} \times \hat{q}}{l^2}$$

where: $\hat{p}$ is the estimated prevalence

$\hat{q} = 1 - \hat{p}$, and

l^2 is the absolute allowable error

Table 4. Sample size of animals showing BRD

Estimated prevalence	50%	30%
Absolute allowable error	10	9-10
Sample size (95% confidence level)	97	81-100

The prevalence of mycoplasmas in lungs at necropsy varies considerably in the literature. *M. bovis* was isolated from 15% of pneumonia lungs studied in the UK[42] and 18% of lungs in Ireland [43], with Bryant *et al* reporting 37% mycoplasma-like lesions [40] and other workers isolating levels of 60 to 80%, with *M. bovis* predominant [108,230,232,248] (although some of these samples came from lungs that had not responded to treatment). Levels of 61 to 83% have been reported in naturally occurring outbreaks [192,242]. The impression gained in the field in South Africa, is that the level is probably somewhere between 30 and 50%

On the same basis, using an estimated prevalence of *Mycoplasma* in 5-10%[29,136,230,248] of animals not showing signs of BRD and again using an absolute allowable error of 10%, a sample size (at 95% confidence level) of 19 is required.

Consideration was given originally to collecting matched affected and healthy samples, but was discarded on the grounds of the reported low incidence in healthy lungs, the costs and the unnecessary handling of healthy animals.

3.2 DEFINITION OF CASES AND CONTROLS

Clinical signs of BRD in feedlots have been observed with the following frequencies: depression 96%, anorexia 76%, elevated respiratory rate 42%, nasal discharge 33% and cough 27%. The mean temperature measured was 40,8ºC with a range of 39,7 – 42,1ºC [8,186,203]. This formed the basis of the selection criteria for the selection of acutely ill animals in the study, and included a combination of the following clinical signs: febrile (T°≥40°C), depressed attitude, anorexia and/or a lack of rumen fill, the presence of a nasal discharge or obvious failure to clean the muzzle, the presence of a cough and an increased respiratory rate > 40 breaths/minute and a lack of abnormal clinical signs referring to organ systems other than the respiratory system, as well as having no prior treatment history for any other disease [32,228]. These animals were usually 'first pull' calves soon after arrival in the feedlots. Healthy animals were those that showed none of the abovementioned clinical signs and none pertaining to other organ systems.

Animals from the healthy or control group that showed clinical signs of BRD within a week of sampling were removed from the study.

3.3 TRANSTRACHEAL WASHES

Percutaneous transtracheal aspirates have been shown to be a practical and quick method of collecting lower respiratory tract samples [71,162,286]. With the animal adequately restrained in a stanchion or headgate, the head is secured in a slightly elevated position. An area of skin covering the ventral aspect of the middle to lower third of the trachea (the most distal part of the external trachea that is not covered by other tissue) was prepared by shaving and surgically disinfected. The trachea was grasped and the rings palpated. A trocar and cannula (angiocatheter) of suitable size was firmly pushed through the skin and

between two tracheal rings, perpendicular to the long axis of the trachea. A 'pop' is felt when going into the trachea and air can be heard flowing through the cannula. The cannula was advanced into the trachea and the catheter threaded through it. Initially 30 to 50ml of sterile physiologically balanced saline was instilled, but it was found that using 20 - 30ml was sufficient to obtain a good sample. The animal will invariably give a slight cough when the saline is instilled. The fluid was gently aspirated back into the syringe after about 20 seconds. Most of the fluid can be retrieved by altering the position of the distal end of the catheter while still in the bronchial region. A 30 - 50cm sterile polyethylene catheter in a 14 to 16 gauge x 6cm cannula or a sterile 14 gauge over-the needle catheter was found to be adequate (Bardicath, Bard Limited or Centracath, Vycon).

Transtracheal aspiration was found to be a quick and simple technique to perform in the field, and was physically innocuous to the calves. This technique has served us well in the past and very few complications have been encountered. These may include: coughing when instilling the saline; subcutaneous emphysema or cellulitis over the injection site; pneumomediastinum. Occasionally the needle may cut off portion of the catheter on removal from the trachea, but appears to be rapidly coughed up and does not appear to have caused any problems [162,286]. A bleb of local anaesthetic was initially used in the skin, but was found not to be necessary, as the animals tolerate the introduction of the catheter needle as well as they do an injection of lignocaine. Once the needle is in the trachea, the animals tend to remain still.

3.4 CULTURE OF THE MYCOPLASMAS

Samples collected were immediately put on ice before transportation to the laboratory. Hayflick's mycoplasma media with Synulox (amoxycillin + clavulanic acid, Pfizer) was used for the mycoplasma isolations and identification according to procedures that have been described previously [8,140,216]. Mycoplasmas that were isolated were stored at -86ºC, until species-specific antisera was available for identification. The immunofluorescent (IF) staining has been shown to be an easy and practical technique for the identification of mycoplasmas, as it is a species specific test and shown to be highly sensitive [89]. As mentioned previously, *M. dispar* could not be cultured using this media.

Most mycoplasmas grow poorly in ordinary aerobic incubators, as they require lowered oxygen tension and high humidity for growth. The ideal is an incubator set at 36-37°C with a 5-10% CO_2 atmosphere that is bubbled through water to maintain high humidity. Candle jars are not acceptable because there are probably some toxic products generated by the burning candle that inhibit growth of mycoplasmas. Once plated, mycoplasma colonies are usually seen after 48-72 hours incubation, however, they may require incubation up to 10-14 days for growth to be observed. Mycoplasma colonies were easily observed with the aid of a stereo dissecting microscope with a magnification of 40X.

3.5 IMMUNOLOGICAL TESTS

Since there are no diagnostic antibodies commercially available, it was necessary to produce polyclonal antibodies to selected mycoplasmas in rabbits. Species-specific antibodies were produced in rabbits by the laboratory to enable identification of the following mycoplasma species:

Mycoplasma bovis (indirect fluorescent antibody test - IFA)

Mycoplasma arginini (IFA)

Acholeplasma laidlawlii (IFA)

Mycoplasma alkalescens (direct fluorescent antibody test - FA)

Mycoplasma bovigenitalium (IFA)

Mycoplasma bovirhinis (FA)

Mycoplasma canadense (FA)

Mycoplasma spp. Group 7 (Leach) (IFA)

All the isolates were also tested to either antisera or conjugates that were already available in the laboratory against the following species:

Mycoplasma mycoides mycoides

Acholeplasma granularum

Mycoplasma verecundum

Mycoplasma fermentans

Mycoplasma flocculare

This was done using the following stepwise procedures:

- Antigen preparation
- Rabbit immunization protocol
- Antibody purification

3.5.1. *Mycoplasma* preparation for rabbit inoculation

Stored type strains at -86°C were defrosted and plated onto Hayflick's agar to check for growth and purity. The colonies were then placed in 500 *ml* sterile Hayflick's broth with 20% bovine serum and incubated at 37°C for 72 hours. The broth was then centrifuged at

15 000 g and 4°C for one hour. The sediment was washed twice in PBS (pH 7.2-7.5) and resuspended in 15-20 *ml* 0,1M PBS (pH 7,4). A portion was streaked onto Hayflick's agar to check growth and on blood agar to check for purity. The prepared antigen was stored in 2 *ml* volumes at -86°C.

3.5.2. Rabbit Immunization Procedure

Prior to immunization, the rabbit sera was tested for the presence of antibodies to mycoplasmas using the growth inhibition test [89]. Only those rabbits that were negative were used.

Two *ml* of the antigen suspension was emulsified with 2 *ml* Freund's complete adjuvant and 0.2 *ml* was injected sub-cutaneously into 8 sites along the back and 0.5 *ml* intramuscularly into each hip. From day 21, 1 *ml* antigen (without adjuvant) was injected intravenously 3 times per week for 6 injections.

At week 6, blood was collected and the serum tested for species-specific antibodies using the growth inhibition test. Once it was established that antibodies were present, the rabbit was anaesthetized and as much blood as possible was collected from the heart and the serum was drawn off.

3.5.3. Purification of antibodies

Precipitation of immunoglobulins for Indirect FA

Precipitated serum reduces background staining in the indirect method.

Ten *ml* of serum was added slowly, drop-wise, to 5*ml* $(NH_4)_2S0_4$ with continuous stirring and allowed to stir overnight at 4°C. The tube is centrifuged at 6000 rpm for 10 minutes at 4-10°C (Sorvall) and the supernatant discarded. The precipitate was resuspended in 10 *ml* of sterile deionized water and added to 5 *ml* $(NH_4)_2S0_4$. The suspension was then stirred for 1½ hours at room temperature. Thereafter it was centrifuged at 6000 rpm for 10 minutes at 4-10°C and the supernatant discarded. The precipitate was resuspended and dialyzed overnight in sterile normal saline. Phosphate buffered saline (pH 7.5) was added to make a 10 *ml* volume and the purified serum was stored at -20°C.

Immunofluorescence test

Approximately 5 mm blocks of agar that contain the colonies were placed on microscope slides. Six to eight blocks are fixed to the slide by embedding the squares in a mixture of 65% paraffin and 35% vaseline and rehydrated with a drop of PBS buffer (pH 7,4). The homologous rabbit antisera was diluted in PBS buffer (pH 7,2-7,4) in 2-fold dilutions from 1:5 and a drop of each dilution is placed on a block and incubated at room temperature in a moisture chamber for 30 minutes. They were then washed twice for 10 minutes in PBS and the excess buffer removed. A drop of fluorescein-conjugated anti-rabbit globulin was added on to each block and incubated at room temperature for 30 minutes in a moisture chamber. After washing twice for 10 minutes in PBS, the fluorescence was read with epi-fluorescent illumination using the 10x objective. The titre at which fluorescence was clear, with no background or non-specific fluorescence was selected. The IFA was also used with the best antibody dilution to detect the mycoplasmas listed.

Both the direct and indirect IF staining of colonies are of value for mycoplasma identification. The direct test requires a conjugated antiserum specific for each

mycoplasma to be tested. The conjugated fluorochrome is usually fluoroscein isothiocyanate [89]. The mycoplasma colonies on agar media are enclosed with a circular plastic ring to form a well, covered with a drop of the specific antibody conjugated with fluoroscein isothiocyanate, incubated at 37°C in a humified chamber for 30 minutes, and washed with phosphate-buffered saline (PBS) at pH 7,4. The reacted colonies are observed for fluorescence with a microscope using ultraviolet light [140]. Conjugating large numbers of antisera can however be very time-consuming.

The indirect test often requires only one conjugated reagent, an anti-rabbit globulin, since most antisera are produced in rabbits. This conjugate is available commercially. Both tests are specific, but the indirect test is more sensitive and tends to give less non-specific background fluorescence that might interfere with the interpretation of the test [89].

3.6 OTHER TESTS

Although not originally part of this particular study, aliquots of the samples were also used for aerobic culture on blood and MacConkey agar of other bacterial flora that might be present and antimicrobial susceptibility are included in the results. The disk diffusion test was done in accordance with the methods approved by NCCLS (National Committee for Clinical Laboratory Standards, 1994).

3.6.1. ANTIMICROBIAL DISK DIFFUSION TEST [174,202]

Introduction:

Although there are several laboratory methods for measuring the *in vitro* susceptibility of bacteria to anti-microbial drugs, the agar disc diffusion method is used most commonly against rapidly growing bacterial pathogens and reliable results can be obtained. Antibiotics usually diffuse in the media at a rate proportional to the original concentration and gives rise to the zone of inhibition.

Materials used include sterile cotton wool swabs (Adcock-Ingram Scientific), 5 *ml* saline tubes, McFarland 0,5 turbidity standard, 5% horseblood enriched Mueller-Hinton agar plates, plate with the test organism (24 – 48 hours old), disc dispenser with specified antibiotic discs (C A Milsch - Oxoid) and sliding calipers (Labretoria).

Method:

Using a loop or sterile cotton wool swab that has been moistened in saline, several colonies (4-5) from a pure culture are touched and suspended in 3 *ml* of saline solution. The turbidity of the bacterial-saline suspension was adjusted to a 0,5 McFarland turbidity standard. A sterile swab was dipped in the test suspension and the surplus liquid removed by rolling the swab against the side of the tube above the level of the fluid. The surface of a blood-enriched Mueller agar plate was evenly inoculated in three directions. The plate was allowed to dry a bit (3-5 minutes, but no longer than 15 minutes) and the antimicrobial disks applied by using an automatic disk dispenser. The plates were inverted and incubated at 37°C either in air or in 5% CO_2 for 18-24 hours. The zones of complete inhibition, including the diameter of the disk are measured using sliding calipers and compared with the sensitivity table for each antibiotic used in the laboratory for

interpretation (NCCLS, 1994). The antibiotic susceptibility of the bacterium is then recorded as “Susceptible”, “Intermediate” or “Resistant”.

The values in the table are based upon MIC breakpoints. These are concentrations that a specific antibiotic should achieve in the serum or tissues for the antibiotic to be effective against the target bacterium.

CHAPTER 4

RESULTS

The raw data for the mycoplasmal and primary respiratory bacterial pathogens isolated for the study period are included in Addendum A for both the clinically affected (sick) and the clinically normal (healthy) animals. A summary table of the findings are included in this section (Table 5 and Figure 2 for the clinically affected animals and Table 6 for the clinically normal animals).

Table 5. Summary of mycoplasmal and primary respiratory bacterial pathogens from clinically affected animals

YEAR	Number of samples submitted	Number of samples qualifying	*Mycoplasma*		*M. haemolytica*		*P. multocida*		*P. trehalosi*		*H. somni*		No Bacterial growth		*Mycoplasma* without primary bacteria.	
			n	%	n	%	n	%	n	%	n	%	n	%	n	%
2001	105	99	62	62.6	63	63.6	12	12.1	---	---	7	7.1	17	17.2	12	12.1
2002	124	111	49	44.1	32	28.8	40	36.0	5	4.5	5	4.5	23	20.7	11	9.9
2003	125	121	70	57.9	34	28.1	46	38.0	16	13.2	0	0	19	15.7	14	11.6
2004	92	88	41	46.6	30	34.1	20	22.7	5	5.7	1	1.1	15	17.0	10	11.4
AV.				52.8		38.7		27.2		7.8		3.2		16.9		11.3

Where:

n = Total number of isolates

% = Percentage of samples that yielded this species.

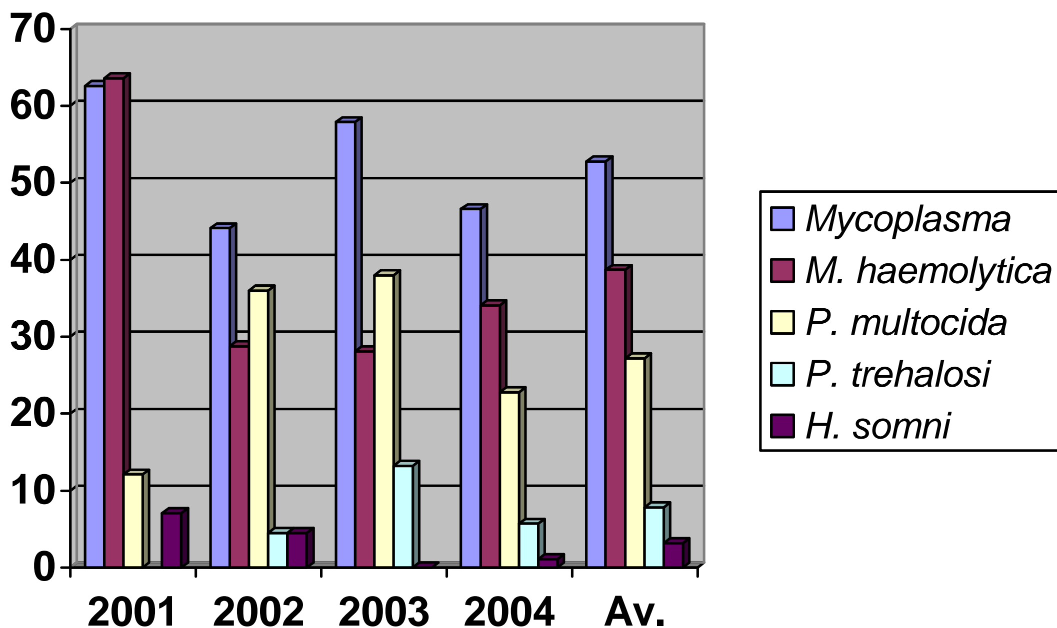
The difference in the numbers of samples submitted and qualifying for analysis is due mainly to contaminated plates making it difficult to determine whether mycoplasmas were present or not. These samples were therefore not included in the respective tallys nor in the calculations.

Table 6. Summary of mycoplasmal and primary respiratory bacterial pathogens from clinically normal animals.

YEAR	Number of samples submitted	Number of samples qualifying	*Mycoplasma*		*M. haemolytica*		*P. multocida*		*P. trehalosi*		*H. somni*		No Bacterial growth		*Mycoplasma* without primary bacteria.	
			n	%	n	%	n	%	n	%	n	%	n	%	n	%
2002	19	18	4	22.2	1	5.6	4	22.2	0	0	0	0	6	33.3	2	11.1
2003	14	13	3	23.1	1	7.7	4	30.8	2	15.4	0	0	7	53.8	1	7.7
AV.				22.7		6.7		26.5		7.7		0		43.6		9.4

A graphic representation of the mycoplasmal and bacterial isolates from clinically affected animals for the study period are given in Figure 2.

Figure 2. Percentage of Mycoplasmal and bacterial isolates from infected animals



The Fisher's exact test was used to determine the correlation between *Mycoplasma* spp. isolation and the primary respiratory bacterial pathogens and specifically to *M. haemolytica* and *P. multocida*. This test was applied because of the relatively small sample sizes [75]. The results are represented in Table 7.

The correlation between *Mycoplasma* spp. and primary respiratory bacteria for the study period was highly significant, p< 0,00001 and an odds ratio (OR) of 3,85. Animals with *Mycoplasma* are 3,85 times more likely to be associated with one of the primary respiratory bacterial pathogens. The correlation for *Mycoplasma* and *M. haemolytica* was p= 0,012 and an OR of 1,69, indicating a significant association, but a bigger sample size is needed to show a clear association. The correlation between *Mycoplasma* and *P. multocida*, however, was highly significant, p= 0,0008 and OR= 2,44.

Table 7. Summary of the correlations between *Mycoplasma* species and the primary respiratory bacterial pathogens isolated.

Year	Primary respiratory bacteria		*M. haemolytica*		*P. multocida*	
	p	OR	p	OR	p	OR
2001	0,0347	2,84	0,29	ns	0,53	ns
2002	0,0016	3,93	0,29	ns	0,0051	3,26
2003	<0,001	4,50	0,68	ns	0,013	2,90
2004	<0,01	3,55	0,186	ns	0,077	2,65
TOTAL	<0,00001	3,85	0,012	1,69	0,0008	2,44

ns = not significant

The study hypothesis was that *Mycoplasma* spp. play a role in the development of BRD in feedlot cattle in South Africa and that there would be an increase in the isolation rate of *Mycoplasma* spp. in transtracheal washes from animals showing clinical signs of

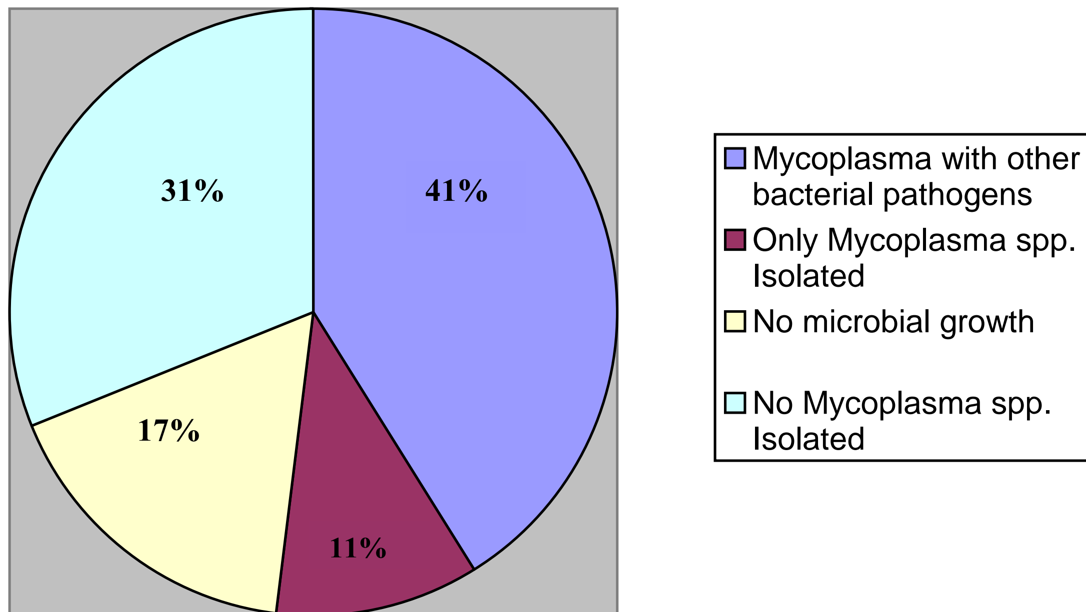
respiratory disease, in comparison to the levels of isolation in healthy calves. A summary table (Table 8) gives the percentage isolation of the *Mycoplasma* spp. and the primary respiratory bacterial pathogens isolated from the two groups. Using the Fisher's exact test, it was possible to show a statistically significant correlation of p= 0,001 and an OR of 3,75 between *Mycoplasma* spp. and the respiratory bacteria between the sick and healthy animals.

Table 8. Percentage isolations of mycoplasmal and primary respiratory bacterial pathogens from sick and healthy animals.

	Mycoplasma	*M.. haemolytica*	*P. multocida*	*P. trehalosi*	*H. somni*	No bacterial growth	*Mycoplasma* with no bacteria.
Sick	52.8	38.7	27.2	7.8	3.2	16.9	11.3
Healthy	22.7	6.7	26.5	7.7	0	43.6	9.4

Figure 3 represents a graphic presentation of the *Mycoplasma* spp. isolated in relation to the other bacterial pathogens.

Figure 3. Percentage mycoplasma isolations in relation to other bacterial pathogens from clinically affected animals.



Not all of the *Mycoplasma* isolates could be regrown after the initial storage of the samples, nor could all of the isolates be identified either after the storage or immediately in the case of the more recent samples. The identification findings are included in Table 9.

The isolation patterns of the *Mycoplasma* spp. and the combination of species found in the samples taken are given in Table 10.

Table 9. ***Mycoplasma*** **species that were able to be regrown and identified from TTA samples collected in South African cattle feedlots from clinically affected animals.**

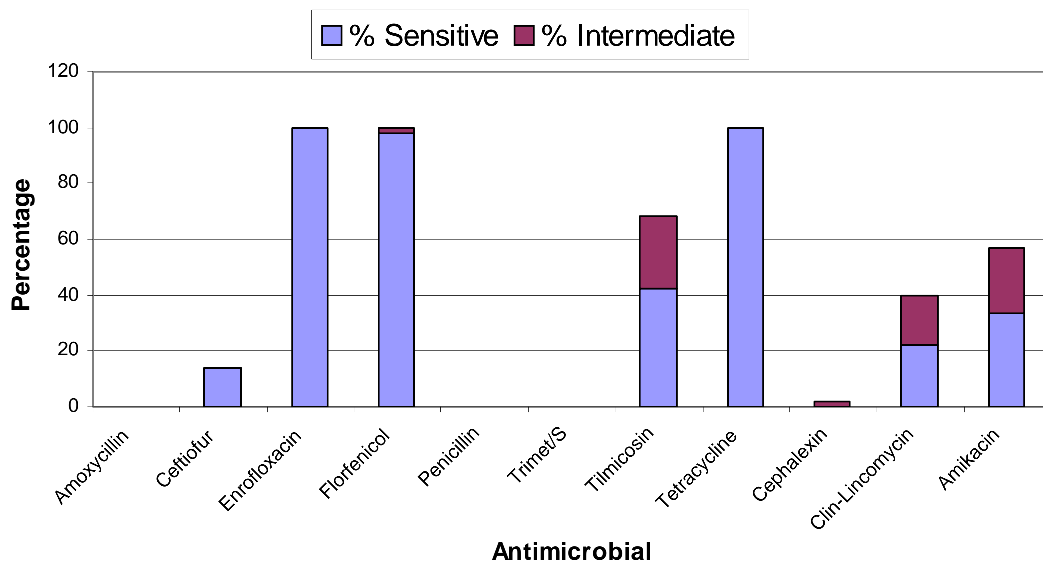
	Number	Percentage
M. bovis	**66**	**34.0**
M. bovirhinis	**7**	**3.6**
M. bovigenitalium	**8**	**4.1**
M. arginini	**35**	**18.0**
M. alkalescens	**4**	**2.1**
A. laidlawlii	**21**	**10.8**
Unidentified	**53**	**27.3**
Total	**194**	

Table 10 **Isolation patterns of** ***Mycoplasma*** **species isolated from TTA samples collected from clinically affected animals.**

Mycoplasma **species**	**Numbers**
M. bovis	**29**
M. bovirhinis	**5**
M. bovigenitalium	**8**
M. arginini	**7**
M. alkalescens	**4**
A. laidlawlii	**4**
M. arginini + M. bovirhinis	**1**
M. bovis + A. laidlawlii	**12**
M. bovis + M. arginini	**22**
M. bovis + M. bovirhinis	**1**
M. bovis + M. arginini + A. laidlawlii	**2**
M. arginini + A. laidlawlii	**3**

A representation of the *in vitro* sensitivity testing of the *Mycoplasma* spp. isolated in 2000 and 2001 using the disk diffusion test have been included (Figure 4). It must be pointed out that the methods used for this exercise have not been validated for mycoplasmas and have been included for interest more than anything else.

Figure 4. Average antimicrobial sensitivity of *Mycoplasma* spp. isolated from TTA samples 2000/2001 (n=62)



A summary of the results of the disk diffusion susceptibility testing for the primary respiratory bacterial pathogens isolated over the study period are given in Tables 11.1 to 11.4

Table 11. Percentage of antimicrobial susceptibilities of the primary respiratory bacterial pathogens isolated.

Table 11.1 ***Mannheimia haemolytica***

	Number of isolates	Amoxycillin	Enrofloxacin	Potentiated sulphonamide	Oxytetracycline	Ceftiofur	Penicillin	Florfenicol	Tilmicosin	Kanamycin
2000/1	73									
S		97.26	95.83	95.89	83.56	94.52	87.32	97.26	79.17	30.65
I		0	0	0.0	0.0	1.37	0.0	0	0.0	1.61
2002	49									
S		98	100	98	95.9	93.9	95.9	100	75.5	8.2
I		0	0	0	0	0	0	0	0	0
2003	46									
S		93.5	87	89.1	80.4	91.3	76.1	100	39.1	28.3
I		0	6.5	0	0	4.3	0	0	10.9	6.5
2004	36									
S		100	86.1	97.2	97.2	97.2	94.4	97.2	61	25
I		0	2.8	0	0	0	0	0	2.8	0

S = sensitive
I = intermediate sensitivity

Table 11.2. ***Pasteurella multocida***

	Number of isolates	Amoxycillin	Enrofloxacin	Potentiated Sulphonamide	Oxytetracycline	Ceftiofur	Penicillin	Florfenicol	Tilmicosin	Kanamycin
2000/1	17									
S		100	100	87.5	75	88.2	92.9	93.8	57.1	10
I		0	0	0	0	0	0	0	7.1	0
2002	71									
S		91.5	100	97.2	59.2	95.8	100	100	71.8	26.8
I		0	0	0	0	1.4	0	0	0	0
2003	62									
S		93.5	96.8	95.2	58.1	95.2	88.7	96.8	58.1	12.9
I		0	1.6	1.6	3.2	1.6	1.6	0	8.1	3.2
2004	27									
S		100	92.6	92.6	55.6	92.6	88.9	100	66.7	22.2
I		0	3.7	0	0	0	0	0	7.4	3.7

Table 11.3. ***Pasteurella trehalosi* 2002 – 2004**

	Number of isolates	Amoxycillin	Enrofloxacin	Potentiated Sulponamide	Oxytetracycline	Ceftiofur	Penicillin G	Florfenicol	Tilmicosin	Kanamycin
S	33	97	81.8	100	66.7	97	75.8	100	57.6	18.2
I		0	6.1	0	0	0	6.1	0	9.1	0

Table 11.4. ***Histophilus somni*** **2000 – 2004**

	Number of isolates	Amoxycillin	Enrofloxacin	Potentiated sulphonamide	Oxytetracycline	Ceftiofur	Penicillin G	Florfenicol	Tilmicosin	Kanamycin
S	15	93.3	100	100	100	100	100	100	80	60
I		0	0	0	0	0	0	0	0	6.7

The results for the study periods in Tables 11.3 and 11.4 have not been separated into the different years because of the small number of isolates.

5. DISCUSSION

5.1 *MYCOPLASMA*

The primary aim of the study was to show that *Mycoplasma* spp. play an important role in the development of BRD in feedlot cattle in South Africa and that there was an increase in the isolation rate in transtracheal washes in animals showing clinical signs of respiratory disease, in comparison to the levels of isolation in calves with no evidence of respiratory disease.

Although the samples from healthy animals was relatively small, it was possible to show that there was a statistically significant association between *Mycoplasma* isolation and respiratory disease, p = 0,001 and with an odds ratio (OR) of 3,75 in cattle from those feedlots included in the study and thereby proving the hypothesis put forward.

Mycoplasma spp. were consistently isolated from tracheal aspirates in this study for the period 2000 to 2004 from clinically affected animals. The rate of isolation was however very variable from year to year, with a high of 62.6% in 2000/1, dropping to 44.1% in 2002, rising again to 57.9% in 2003 and then decreasing to 46.6% in 2004, giving an average isolation rate of 52.8%, which is in line with the majority of overseas studies [13,108,172,173,177,192,242] (Table 5). The significance of this variation is uncertain and the intention is to monitor the isolation rates in future years as part of an ongoing study. The variation could be due to factors such as the effect of individual feedlots and/or weather patterns.

Another interesting and fairly consistent finding was the 11.3% of samples (range 9,9 to 12,1%) where *Mycoplasma* spp. was isolated without other primary respiratory bacteria being present. Of these, 27.7% (13 out of 47 isolates) were *M. bovis*. Although viral isolations were not part of this study and their role can therefore not be excluded, it adds to the debate of whether mycoplasmas should be considered as being primary or secondary pathogens in the whole complex. This finding also has possible implications on the antibiotic selection in treating BRD (9.4% of samples from healthy animals had *Mycoplasma* as the only isolate – Tables 6 and 8).

The distribution of the species identified was also significant (Table 9). *Mycoplasma bovis* is considered to be the most pathogenic of the species in the respiratory tract of cattle [2,42,69,98,177,234,246] and was isolated in 34.0% of the samples that could be regrown after storage and then identified (n=66 of the 194 isolates). This compares with the *M. bovis* isolation rates of 50-66% reported in the literature [2,31,136,139,172,199,246].

Of the 34.0%, *M. bovis* was isolated on its own in 20.6% (n=29) of the samples identified, in 15.6% (n=22) of the samples together with *M. arginini* and in 8.5% (n=12) of the samples with *A. laidlawlii* (Table 10). The isolation rates of the other *Mycoplasma* species as single isolates were all less than 6%. This coincides with the findings of other research that it is not uncommon to isolate two or more of the *Mycoplasma* spp. from a single incident of pneumonia [42,69,136,172,177,179,243]. As mentioned previously, the possible role of *M. dispar* in BRD in South African feedlots have not been considered, as this species cannot be identified given the techniques used in this study.

Drawing conclusions about the comparisons between *Mycoplasma* spp. isolated from infected and non-infected animals in this study is difficult because of the low number of

samples taken from non-infected animals. Thirty three samples were collected from healthy animals during 2002 and 2003, with seven of the 31 samples that qualified containing *Mycoplasma* spp. (Table 6). Three of these samples could either not be regrown or the specie could not be identified. *Mycoplasma bovis* was identified in three of the remaining samples, which is in contrast to the experience that this specie generally occurs in infected animals.

According to the literature, *Mycoplasma, M. haemolytica* or *P. multocida* are isolated from bronchial lavage fluids from healthy calves in only a few cases[283], with estimates being put at 5 – 10% levels for *Mycoplasma* [29,136,230,248]. Isolation rates of *Mycoplasma* spp. from healthy animals in this study were 22.7%, compared to 52.8% in sick animals (Tables 6 and 9). This level in healthy animals was considerably higher than anticipated. The statistical model used in the study required the collection of samples from at least 19 healthy animals, while 31 samples were finally included in this study from animals sampled according to the criteria for clinically normal as defined under **Methods and materials** (Table 4). Some difficulty was however experienced in identifying such healthy animals or excluding animals in the subclinical stages of respiratory disease. This is borne out by the finding that 16.9% of samples from affected animals had no microbial growth and yet were clinically sick according to the definition. This does not preclude the possibility of such animals being affected by some viral agent, as virus isolations were not included in this study. The samples were also collected during the period of highest prevalence of the disease, which might have had an effect on the results, as these animals could have been incubating the disease, but not showing any clinical signs. Such animals should have been excluded from the study according to the definition, but very few of the feedlot operators were able to give feedback on the fate of the animals from whom

samples were collected from. With hindsight, samples from healthy animals should probably have been collected during a period of lower disease incidence.

5.2 OTHER PRIMARY BACTERIAL PATHOGENS

The ratio of *M. haemolytica* to *P. multocida* in the study also showed interesting trends (Table 6 and Figure 2). Samples taken in 2000/1 had a *M. haemolytica* isolation rate of 63.6%, which is considerably higher than previous findings in South Africa [176,261]. These dropped to 28.8% in 2002, 28.1% in 2003 and then increased again to 34.1% in 2004, with an average of 38.7% for the study period. Other laboratory identification techniques for this species were however used from 2002 onwards and some of the previously identified *Mannheimia* were subsequently identified as *P. trehalosi*. The average isolation rate for the latter was however only 7.8% and would not have made much difference to the *M. haemolytica* levels. On the other hand, the isolation rates for *P. multocida* increased from 12.1% in 2000/1 to 36% in 2002, 38% in 2003 and 22.7% in 2004, with an average of 27.2% for the study period. The dramatic increase in 2002 probably coincided with the use of a new *M. haemolytica* antileukotoxin vaccine, which includes the previously mentioned strain 17. This period also coincided with the withdrawal of the previously used *P. multocida* bacterin vaccine. Bacterial isolations are to be continued for a period in order to monitor trend changes.

The low isolation rate of 6.7% for *M. haemolytica* from 'healthy' calves (vs. 38.7% in sick animals) is expected, being the most likely cause of pneumonia [16,59,61,170,186,203] (Tables 6 and 8). *Pasteurella multocida* isolation on the other hand was much the same for healthy and sick animals at 26.5% and 27.2% respectively, which shows that the organism is possibly more of a commensal or opportunistic pathogen. The finding that 43.6% of

samples from healthy animals showed no microbial growth is probably to be expected (compared to the 16.9% of samples that had no microbial growth from affected animals).

Histophilus somni has become one of the most significant causes of mortality in beef calves in western Canada [32,32,33,207,207,266,266,267,267], but the isolation rate in this study was very low at 3.2% in sick animals (range 0 to 7.1%) and 0 in healthy animals. This could possibly be due to the age differences between feedlot cattle in Canada and South Africa, with cattle in South Africa being finished off at a younger age or a lighter mass. The samples included in this study were also from 'first pull' cases, which is usually within the first three weeks of arrival in the feedlots. From personal experience, this pathogen would appear to be more prevalent in older cattle towards the end of the feeding period [98].

5.3 ANTIMICROBIAL SUSCEPTIBILITY TESTING

Antimicrobial susceptibility testing was included in this study as an extension to the microbial isolation rates that were generated from a wide range of feedlot management systems and a wide geographical area of the country. As mentioned in the literature review, the decision to use a particular antibiotic to treat BRD depends on a range of factors, not least of which include the bacteria isolated and their susceptibility patterns. The data presented will provide some additional information that can be used to reach a more informed decision on appropriate antibiotic selections.

Antibiotics selected for the antibiograms used in the study were determined by the antibiotic groups that were in relatively common usage in the majority of feedlots when the study was started in 2000, as well as those products that have been specifically registered for use in BRD.

Over the survey period, very few multiple resistant organisms were isolated, but there was a trend towards more of these pathogens being identified. This is probably to be expected as the primary thrust in BRD management has up until now been the use of antimicrobials. The few cases of multiple resistant organisms encountered could in the majority of cases also be correlated to feedlot operations with poorer management and antibiotic usage protocols.

The antibiogram for *Mycoplasma* spp. isolated in 2000/1 (Figure 4) has been included for interest more than anything else. The techniques used had at that time not been validated worldwide. For instance, ceftiofur was shown to have some effect against *Mycoplasma*, which clearly is not correct, as cephalosporins are only effective against micro-organisms with a cell wall. The exercise however gives some indication of the probable sensitivity patterns that could be expected should treatment be aimed at *Mycoplasma* as well as against the bacterial pathogens, which has been the trend up until now. One of the needs identified by the mycoplasma interest group is to develop a validated MIC laboratory testing technique for mycoplasmas.

Mannheimia haemolytica (Table 11.1)

There were 204 *M. haemolytica* isolates for the study period and were found to be susceptible to the majority of antibiotics tested other than kanamycin which was below 31% sensitivity (selected as a representative of the aminoglycoside group because they were previously used extensively in USA feedlots) and possibly tilmicosin. Interestingly, all sensitivities dropped for 2003 samples other than florfenicol.

Sensitivities for amoxicillin showed very little variation of between 94 and 100% for the study period. Initial results for enrofloxacin were between 96 and 100%, but then dropped to 87% and 86% in 2003 and 2004 respectively. Increased resistance can be expected as the group has been on the market for many years and from experience has shown 'a reduced efficacy' in other intensive production systems. The sensitivity for potentiated sulphonamides was above 95% for all years other than a decrease to 89% in 2003.

Sensitivity results for oxytetracycline were very variable, ranging from 80% in 2003, 84% in 2000/1, to 96% in 2002 and 97% in 2004. These results are important as many feedlots use tetracyclines routinely as metaphylaxis for anaplasmosis and heartwater, especially in the summer months and chlortetracyclines in the feed are also commonly used during the winter months when BRD is most prevalent. The use of a combination of oxytetracycline and potentiated sulphas was also a common practice for the treatment of BRD about ten years ago.

Sensitivity for ceftiofur varied from a low of 91% (plus 4% intermediate) in 2003 to a high of 97% in 2004. It was one of the first 'big gun'antibiotics registered exclusively for BRD initially, but has now been used extensively in feedlots for at least fifteen years and a degree of resistance would be expected.

The sensitivity for penicillin against *M. haemolytica* was surprisingly high other than in 2003, which was only 76%. Other results were 87% in 2000/1, with 94% and 96% in 2004 and 2002 respectively.

The sensitivities for florphenicol against *M. haemolytica* were very good and ranged from 97% to 100% over the study period.

The sensitivities for tilmicosin were very interesting and variable. They showed a dramatic decrease in sensitivity to 39% (plus an additional 11% intermediate sensitivity) in 2003 and only 61% in 2004. 2000/1 was at 79% and 2002 at 76%. The product is frequently advocated for metaphylactic use, especially during the colder months when the BRD incidence is highest. This is based primarily on improved mass gains following treatment, probably as a result of reducing subclinical BRD cases. This practice should probably be reinvestigated, based on the sensitivity results against *M. haemolytica* as well as *P. multocida* determined in this study. One possible reason put forward for the efficacy of this metaphylaxis is that tilmicosin and related antibiotics are more effective against mycoplasmas than against many of the other pathogens involved [108]. Efficacy results with the product might however be completely different as it is known that tissue concentrations are considerably higher than that of serum and persist for a relatively long period [200].

Pasteurella multocida (Table11.2)

There were 177 *P. multocida* isolates identified over the study period and the sensitivity patterns were more variable than for *M. haemolytica*. Again the aminoglycosides and tilmicosin showed poor sensitivities.

The sensitivities for amoxicillin remained high and ranged from 91 to 100% during the survey period, making this a viable option for bacteria in BRD, but not for mycoplasmas. Enrofloxacin sensitivity was initially 100% in 2000/1 and 2002, but this gradually dropped to 97% in 2003 and 93% in 2004. Sensitivity for the potentiated sulphonamides was

initially a low of 88% in 2000/1, but ranged from 93% to 97% for the other years, with surprisingly little variation.

Pasteurella multocida is known to be more resistant to the tetracyclines and the sensitivities of oxytetracycline against this bacteria was generally poor (less than 60%), other than the 75% in 2000/1. As expected, these figures are considerably lower than for *M. haemolytica* and reiterate the concerns given under *M. haemolytica.*

The sensitivity of ceftiofur was slightly lower than for *M. haemolytica* with a low of 88% initially in 2000/1, but this increased to 96% in 2002, 95% in 2003 before dropping to 93% in 2004, making it a viable proposition for treating BRD. The sensitivity of penicillin against *P. multocida* was surprisingly high and varied from 93% in 2000/1, 100% in 2002 before decreasing to 88% in 2003 and 2004, while the sensitivities for florphenicol were again at a relatively high level of 94% in 2000/1 and 97% in 2003 and at 100% for the other two years.

The sensitivity of tilmicosin against *P. multocida* needs to be considered as being poor, with a low of 57% in 2000/1(plus 7% intermediate sensitivity) and 58% in 2003 (plus 8% intermediate), and 72% in 2002 and 67%(plus 7% intermediate) in 2004.

Pasteurella trehalosi (Table 11.3)

Only 33 isolates were identified and the results have not been differentiated into years because of the low number of isolates per year during the study period. There was a 100% sensitivity for the period for potentiated sulphas and florfenicol, 97% for amoxicillin and ceftiofur, 82% plus 6% intermediate for enrofloxacin, 76% plus 6% intermediate for

penicillin G, 67% for oxytetracycline, 56% plus 9% intermediate for tilmicosin and 18% for kanamycin.

Histophilus somni (table 11.4)

Only 15 isolates were identified for the period and the results have not been differentiated into years because of the low numbers. The old adage that this pathogen is sensitive to just about any antibiotic thrown at it was borne out in this survey, with 100% sensitivity for enrofloxacin, potentiated sulphas, oxytetracyclines, ceftiofur, penicillin G and florfenicol. Amoxicillin was 93%, tilmicosin 80% and even kanamycin had a 60% (plus 7% intermediate) efficacy rate. Sensitivity of *H. somni* might however not relate to clinical efficacy as this organism is known to cause severe tissue damage during the acute phase of the disease [95,203].

5.4 FURTHER STUDIES

A *Mycoplasma* interest group has been formed as a result of this study. The group at this stage is made up of the consultants to the feedlots, the laboratories involved in the analysis of the samples, the study team, pathologists as well as many of the pharmaceutical companies supplying the feedlots. This is based primarily on the realisation as to how important mycoplasmas could be to the feedlot industry and possibly other branches of bovine practice. One of the aims of the group is to continue the study, so as to increase the database in order to decide whether it is a primary pathogen or not and which animal groups are most affected.

Some of the needs identified by the group include further study possibilities, including:

5.4.1 Antibiotic MIC techniques specifically for mycoplasmas need to be validated.

5.4.2 Possible vaccine development.

As mentioned elsewhere, there are no commercially available vaccines against mycoplasmas and *M. bovis* in particular for use in feedlots. Vaccines are available in pigs and poultry, but vaccine development for use in feedlots needs to be investigated further.

5.4.3. Establish the role of *M. dispar* in BRD in feedlots

To expand the capabilities of the laboratory used in the study to grow and identify *M. dispar* that might have been in the samples collected during the study.

5.4.4. Development of easier detection methodologies.

As indicated elsewhere, mycoplasmas require special growth media and conditions in the laboratory and a special request for isolation is usually required [234].

5.4.5. Possible role in other bovine disease conditions.

Mycoplasma mastitis is also receiving much attention [121,135,211,218,277], for similar reasons as proposed for BRD. Synergism of *M. bovis* with other potential udder pathogens may be linked to the immunosuppressive effects of *M. bovis* [28,277] and in the respiratory tract such as *M. hyopneumoniae* in pigs [49]. *Mycoplasma bovis* is able to inhibit the *in vitro* killing of bacteria [117] and reduce neutrophil activity [250], thereby compromising the local non-specific udder defenses and allowing secondary infections [85]. Synergism has been shown between mycoplasmas and *Streptococcus dysgalactiae*. Both agents as mono-infections cause only

a mild mastitis, but *S. dysgalactiae* provoked a severe mastitis in udders previously infected with mycoplasma [194].

In a study to investigate possible causes of persistently high somatic cell counts (SCC) in dairy cattle in Victoria and North Queensland, Australia, 43% and 62% of herds were positive for *M. bovis* respectively. *M. bovis* was detected in 77% of cows of which 19% alone had *M. bovis* without any other bacteria, 17% had *M. bovis* in combination with major mastitis pathogens (MMP) and 40% had *M. bovis* in combination with non-major mastitis pathogens. *Mycoplasma bovis* had previously not been considered to be a major cause of mastitis in Australia, possibly because of the poor sensitivity for the detection of *Mycoplasma* in milk using the normal culture methods. The development of new PCR techniques has alerted us to the important role that *Mycoplasma* may play in subacute and/or chronic mastitis and its effect on milk quality [218]. Further research is required in this field, but it is possible that bacteria such as *Streptococcus agalactiae, Staphylococcus aureus* and other species could be secondary pathogens acting synergistically with *M. bovis*. In 36% of milk samples from cows with persistently high SCC, *M.bovis* was detected alone or together with other bacteria major mastitis pathogens (MMP) alone were detected in only 9% of quarters [85,218].

Mycoplasma bovis mastitis responds poorly to antibiotics and it is therefore best to segregate or cull carrier cows and to instigate rigid sanitation procedures to prevent transmission from infected to non-infected cows [137,189,190,277]. There may be a relatively high percentage of mycoplasma shedders amongst infected cows and this can be an important source of infection among dairy calves [211,277].

5.4.5. A study to examine the correlation between *Mycoplasma* and viral infections.

Some studies have been conducted in feedlot cattle in South Africa regarding the role of viral infections, but there does not appear to have been any studies done that are similar to the present study, but looking into *Mycoplasma* and the viruses.

CHAPTER 6.

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Addendum A

Table 12. Bacterial and Mycoplasmal isolates from TTA samples from clinically affected animals

Table 12.1 2000 TTA's from clinically affected animals

	Growth	*M.haemolytica*	*P.multocida*	*P.trehalosi*	*H.somni*	*Mycoplasma*	Mycoplasma spp.	Other bacteria
1	NG	-	-	-	-	-		
2	NG	-	-	-	-	-		
3	NG	-	-	-	-	-		
4		-	++	-	-	+		
5		++	-	-	-	-		
6		+	-	-	-	+		
7		+	-	-	-	+		
8		-	-	-	-	+		
9		-	-	-	-	+		
10		+	-	-	-	+		*Arcanobacterium*
11		-	-	-	-	+		
12		+	-	-	-	-		
13		+	-	-	-	+		
14		+	-	-	-	+		
15		+	-	-	-	-		
16		+	-	-	-	-		
17		-	+	-	-	ND		
18		-	+	-	-	ND		
19		+	-	-	-	ND		
20	NG	-	-	-	-	ND		
21	NG	-	-	-	-	ND		
22		+	-	-	-	ND		

Table 12.2 2001 TTA's from clinically affected animals

1		++	-	-	-	+		
2		+	-	-	-	+		
3		+	-	-	-	+		
4		+	-	-	-	+		
5		+	-	-	-	+		
6		++	-	-	-	+		
7		+	-	-	-	+		
8		+	-	-	-	+		
9		+	-	-	-	+		
10		+	-	-	-	+		
11		+	-	-	-	+		
12		++	-	-	-	+		

Addendum A

	Growth	*M.haemolytica*	*P.multocida*	*P.trehalosi*	*H.somni*	*Mycoplasma*	Mycoplasma spp.	Other bacteria
13		++	-	-	-	+		
14		-	-	-	-	+		
15		-	-	-	-	+		
16		+	-	-	-	+		
17		+	-	-	-	+		
18		+	-	-	-	+		
19		-	-	-	-	+		
20		+	-	-	+	+		
21		+	-	-	-	+		
22		+	-	-	+	+		
23		-	-	-	+	+		
24		+	-	-	+	+		
25	NG	-	-	-	-	-		
26		-	-	-	-	+		
27	NG	-	-	-	-	-		
28		-	-	-	-	+		
29		-	-	-	-	+		
30		-	-	-	-	+		
31		+	-	-	-	+		
32		-	-	-	+	+		
33	NG	-	-	-	-	-		
34		-	-	-	-	+		
35		+	-	-	-	+		
36		+	-	-	-	-		
37		+	-	-	-	-		
38		+	-	-	-	-		
39		+	-	-	-	-		
40	NG	-	-	-	-	-		
41	NG	-	-	-	-	-		
42	NG	-	-	-	-	-		
43		+	-	-	-	-		
44		+	-	-	-	-		
45		+	-	-	-	-		
46		++	-	-	-	-		
47		+	-	-	-	+		
48		+	-	-	-	-		
49		+	-	-	-	-		
50		+	-	-	-	+		
51		+	+	-	+	+		
52		+	-	-	-	-		

Addendum A

	Growth	*M.haemolytica*	*P.multocida*	*P.trehalosi*	*H.somni*	*Mycoplasma*	**Mycoplasma spp.**	**Other bacteria**
53		-	+	-	-	+		
54	NG	-	-	-	-	-		
55	NG	+	-	-	-	-		
56	NG	-	-	-	-	-		
57		-	+	-	-	+		
58		+	-	-	-	-		
59	NG	-	-	-	-	-		
60		-	+	-	-	-		
61		+	-	-	-	-		
62		+	+	-	-	+		
63		-	+	-	-	-		
64		-	-	-	-	+		
65		+	-	-	-	+		
66		++	-	-	-	+		
67		+	+	-	-	+		
68		+	-	-	-	+		
69		++	-	-	-	+		
70		+	-	-	-	+		
71		-	+	-	-	+		
72		++	-	-	+	+		
73		+	-	-	-	+		
74		+	-	-	-	+		
75		+	-	-	-	-		
76	NG	-	-	-	-	-		
77		+	-	-	-	-		
78		+	+	-	-	-		
79		+	-	-	-	+		
80		+	-	-	-	+		
81		-	+	-	-	+		
82	NG	-	-	-	-	-		
83		-	+	-	-	+		

NG = No bacterial or Mycoplasmal growth on culture
ND = Not able to determine from samples submitted
Note: *Mycoplasma* spp. not identified as samples were not stored at this stage of the study.

Addendum A

Table 12.3 2002 TTA's from clinically affected animals

	Growth	*M.haemolytica*	*P.multocida*	*P.trehalosi*	*H.somni*	*Mycoplasma*	Mycoplasma spp.	Other bacteria
1		++	+	-	-	+		
2		+	-	-	-	-		
3		-	+	-	-	+		
4		++	-	-	-	+		
5		-	+	-	-	+		
6		+	++	-	-	+		*Arcanobacterium pyogenes*
7		-	+	-	-	+		
8		-	+	-	-	+		
9		+	-	-	-	+		
10	NG	-	-	-	-	-		
11		+	-	-	-	+		
12		+	-	-	-	-		
13		+	-	-	-	-		
14		+	-	-	-	+	*M.bovigenitalium*	
15		+	-	-	-	-		
16		++	+	-	-	-		
17		+	-	-	-	+	*M.bovirhinis*	
18	NG	-	-	-	-	-		
19		+	-	-	-	+	*M. alkalescens*	
20		+	-	-	-	+	No regrowth	
21		-	+	-	-	-		
22		-	+	-	-	+	*M.bovis*	*Arcanobacterium pyogenes*
23		+	-	-	-	-		
24		+	-	-	+	-		
25		-	-	-	-	+	*M.arginini*	
26	NG	-	-	-	-	-		
27		+	-	-	-	-		
28		-	-	-	-	-		*Moraxella* sp[2]
29		+	+	-	-	+	*M.arginini* *M.bovirhinis*	
30	NG	-	-	-	-			
31		-	+	-	+	+	No regrowth	

Addendum A

	Growth	*M.haemolytica*	*P.multocida*	*P.trehalosi*	*H.somni*	*Mycoplasma*	**Mycoplasma spp.**	**Other bacteria**
32		-	-	-	-	-		*Neisseria* sp[2] Non-groupable *Streptococcus*[2]
33	NG	-	-	-	-	-		
34		++	-	-	-	-		*Streptococcus dysgalactiae*
35		-	-	-	-	+	No regrowth	*Moraxella sp*[2]
36		+	-	+	-	-		
37		+	++	+	-	-		
38		-	-	-	-	-		*Streptococcus* sp[2] *Escherichia coli*[2]
39		-	-	-	-	-		*Streptococcus* sp[2] *Escherichia coli*[2]
40	NG	-	-	-	-	-		
41		-	+	-	-	+	No regrowth	
42	NG	-	-	-	-	-		
43		-	-	-	-	+	No regrowth	No significant bacterial growth
44		-	+	-	-	+	*M.bovirhinis*	
45		-	+	-	-	-		
46		-	-	-	-	+	*M.bovigenitalium*	*Moraxella* sp[2]
47	NG	-	-	-	-	-		
48		-	+	-	-	-		
49		-	-	-	-	-		*Moraxella* sp[2]
50		-	+	-	-	+	No identification	
51		-	-	+	-	+	No regrowth	
52	NG	-	-	-	-	-		
53		-	+	-	-	+	No regrowth	
54		-	+	-	-	-		
55		-	+	-	-	+	*M.bovis* *A.laidlawlii*	
56		-	+	-	-	+	*M.bovis* *A.laidlawlii*	
57		-	+	-	-	-		
58		-	-	-	-	+	*M.bovis* *M.arginini*	No bacterial growth

Addendum A

	Growth	*M.haemolytica*	*P.multocida*	*P.trehalosi*	*H.somni*	*Mycoplasma*	**Mycoplasma spp.**	**Other bacteria**
59		-	++	-	-	-		
60	NG	-	-	-	-	-		
61	NG	-	-	-	-	-		
62		-	-	-	-	-		*Streptococcus* sp
63	NG	-	-	-	-	-		
64		-	-	-	-	-		*Moraxella* sp[2]
65		-	-	-	-	-		*Moraxella* sp[2]
66	NG	-	-	-	-	-		
67	NG	-	-	-	-	-		
68	NG	-	-	-	-	-		
69	NG	-	-	-	-	-		
70	NG	-	-	-	-	-		
71		-	-	-	-	+	*M.bovis*	
72	NG	-	-	-	-	-		
73		-	-	-	-	+	*M.bovigenitalium*	*Arcanobacterium pyogenes*
74		+	++	-	-	+	*M.bovigenitalium*	
75		-	-	-	-	+	No regrowth	No bacterial growth
76		-	-	-	-	+	*M.bovigenitalium*	No bacterial growth
77		-	+	-	-	+	*M.bovigenitalium*	
78	NG	-	-	-	-	-		
79		-	-	-	-	-		Beta-haemolytic bacteria
80		-	++	-	-	+	No identification	
81		-	+	-	-	-		
82		-	++	-	-	+	No regrowth	
83		+	-	-	-	-		
84		-	+	-	-	+	No regrowth	
85		-	++	-	-	-		
86		+	-	-	-	-		
87		-	+	-	-	-		
88		-	-	+	-	-		
89		-	++	-	-	+	No regrowth	
90		+	-	-	-	-		
91		+	-	-	-	-		
92		-	+	-	-	-		
93		-	+	-	-		Contaminated	
94		+	+	-	-		Contaminated	
95	NG	-	-	-	-	-		

Addendum A

	Growth	*M.haemolytica*	*P.multocida*	*P.trehalosi*	*H.somni*	*Mycoplasma*	Mycoplasma spp.	Other bacteria
96		-	-	-	-	-		Contaminants only
97		-	+	-	-	-		
98		-	+	-	-		Contaminated	
99		-	+	-	-		Contaminated	*Arcanobacterium pyogenes*
100		+	-	-	-	+	No regrowth	
101		-	+	-	-		Contaminated	
102		-	-	-	+	+	*M.bovis*	
103		+	-	-	+	+	*M.bovis* *M.arginini*	
104		-	+	-	-	-		
105		-	+	-	-	-		
106		+	+	-	-		Contaminated	
107		-	-	-	-		Contaminated	*Moraxella* sp[2]
108		-	+	-	-		Contaminated	
109		-	+	-	-		Contaminated	
110		-	+	-	-		Contaminated	
111		+	-	-	-	+	*M.bovis*	
112		-	+	+	-	+	No regrowth	
113		-	-	-	-	+	No regrowth	Beta-haemolytic *E.coli*
114		-	+	-	+	+	*M.bovis* *A.laidlawlii*	
115		-	-	-	-	+	*M.bovirhinis*	Contaminants only
116		+	+	-	-	+	*M.bovis*	
117		+	-	-	-	+	No regrowth	
118		+	+	-	-		Contaminated	
119		-	-	+	-		Contaminated	
120		++	-	-	-		Contaminated	
121	NG	-	-	-	-	-		
122	NG	-	-	-	-	-		
123	NG	-	-	-	-	-		
124		+	+	-	-	+	*M. alkalescens*	

Addendum A

NG = no bacterial or Mycoplasmal growth on culture

++ = more than one specie of organism isolated

[1] = other pathogenic bacteria isolated

[2] = other bacteria isolated, usually considered non-pathogenic

No regrowth = Mycoplasmas that were previously isolated and stored at -86°C but could not be regrown after storage

No ID / identification = Mycoplasmas cultured, but could not be identified using the current set of antisera

Mycoplasmas identified:

Mycoplasma bovis indirect fluorescent antibody test (IFA)

M.arginini (IFA)

Acholeplasma laidlawlii (IFA)

M.alkalescens direct fluorescent antibody test (FA)

M.bovigenitalium (IFA)

M.bovirhinis (FA)

M.canadense (FA)

Mycoplasma spp.*Group 7* (Leach) (IFA)

All the isolates were also tested to either antisera or conjugates against the following species, but no positive results were obtained:

Mycoplasma mycoides mycoides

Acholeplasma granularum

M.verecundum

M.fermentans

M.flocculare

Addendum A

Table 12.4 2003 TTA's from clinically affected animals

		M.haemolytica	*P.multocida*	*P.trehalosi*	*H.somni*	*Mycoplasma*	Mycoplasma spp.	Other bacteria
1	NG	-	-	-	-	-		
2		-	-	+	-	+	No ID	
3	NG	-	-	-	-	-		
4		-	+	-	-	+	No ID	
5		+	-	-	-	-		
6		-	-	+	-	+	No regrowth	*Moraxella* sp[2]
7	NG	-	-	-	-	-		
8		-	-	-	-	+	No regrowth	
9	NG	-	-	-	-	-		
10	NG	-	-	-	-	-		
11	NG	-	-	-	-	-		
12	NG	-	-	-	-	-		
13		-	+	-	-	+	*M.bovigenitalium*	
14		-	+	-	-	+	*M.bovis* *M.arginini*	
15		-	+	-	-	+	*M.bovis*	
16		-	-	+	-	+	*M.bovis* *A.laidlawlii*	
17		+	-	-	-	+	*M.bovis* *A.laidlawlii*	
18		-	+	-	-	+	*M.bovis* *A.laidlawlii*	
19	NG	-	-	-	-	-		
20		-	-	-	-	-		*Streptococcus equisimilis*[1] *Moraxella* sp[2]
21		-	-	-	-	+	*M.alkalescens*	*Moraxella* sp.[2] *Streptococcus* sp.[2]
22		-	-	+	-	+	No ID	*Streptococcus agalactiae*[1]
23	NG	-	-	-	-	-		
24	NG	-	-	-	-	-		
25		-	-	-	-	+	*M.bovis* *M.arginini*	*Moraxella* sp[2] *Streptococcus* sp[2]
26		-	-	-	-	+	*M.bovis* *M.arginini*	*Moraxella* sp.[2]
27		-	-	-	-	+	No ID	*Moraxella* sp.[2]
28		-	+	-	-	-		*Moraxella* sp[2]
29		-	+	-	-	-		

Addendum A

	Growth	*M.haemolytica*	*P.multocida*	*P.trehalosi*	*H.somni*	*Mycoplasma*	**Mycoplasma spp.**	**Other bacteria**
30		+	++	-	-	-		*Moraxella* sp[2]
31		-	+	-	-	+	*M.bovis* *A.laidlawlii*	*Moraxella* sp[2]
32		-	-	-	-	+	*M.bovis* *M.arginini*	*Streptococcus agalactiae*[1]
33		-	-	-	-	+	*M.arginini*	No bacterial growth
34		-	++	-	-	+	*A.laidlawlii*	
35		+	-	-	-	+	No ID	
36		-	-	-	-	-		*Moraxella* sp[2]
37		-	-	-	-	-		*Streptomyces* sp[2]
38		-	-	-	-	-		*Streptococcus bovis*[1]
39		+	-	-	-	+	Contaminated	
40		-	+	-	-	+	*M.bovis* *M.arginini*	
41	NG	-	-	-	-	+	Contaminated	
42		-	++	-	-	+	No ID	*Mannheimia langua*[1]
43		+	-	-	-	+	*M.bovis* *M.arginini* *A.laidlawlii*	*Moraxella* sp[2] *Enterococci* [2] *Staphylococcus* sp[2]
44		+	-	-	-	+	*M.bovis* *M.arginini*	*Moraxella* sp[2]
45		-	-	-	-	-		*Bacillus* sp[2] *Streptococcus* sp.[2]
46		+	-	-	-	+	*M.bovis*	
47		-	-	-	-	+	Contaminated	*Bacillus* sp[2] *Moraxella* sp[2] *Staphylococcus* sp[2]
48		-	+	-	-	+	*M.arginini*	*Moraxella* sp[2]
49	NG	-	-	-	-	-		
50		-	+	-	-	-		
51	NG	-	-	-	-	-		
52		-	+	-	-	+	No ID	*Bacillus* sp[2] *Moraxella* sp[2]
53		-	+	-	-	-		*Moraxella* sp[2]
54		+	-	-	-	+	*M.bovis*	No bacterial growth
55		-	-	-	-	+	No ID	

Addendum A

	Growth	*M.haemolytica*	*P.multocida*	*P.trehalosi*	*H.somni*	*Mycoplasma*	Mycoplasma spp.	Other bacteria
56		-	+	+	-	-		*Moraxella* sp[2]
57		-	+	+	-	-		*Moraxella* sp[2]
58		-	+	-	-	+	No ID	*Moraxella* sp[2]
59		+	+	-	-	+	*M.bovis* *M.arginini*	*Moraxella* sp[2] *Bacillus cereus*[2]
60		-	+	-	-	+	*M.bovis*	
61		-	+	-	-	+	*M.arginini*	
62		+	-	+++	-	-		*Enterococci*[2]
63		++	-	+	-	+	*A.laidlawlii*	
64	NG	-	-	-	-	-		
65		-	-	+	-	-		
66		++	-	-	-	-		*Moraxella* sp[2]
67		+	-	-	-	-		*Moraxella* sp[2]
68		+	-	+	-	+	No regrowth	*Enterococci*[2]
69		+	-	++	-	+	No regrowth	*Actinobacillus capsulatus*[2]
70		+	-	-	-	+	*M.alkalescens*	*Moraxella* sp[2]
71		-	-	-	-	+	No ID	*Moraxella* sp[2]
72		-	-	-	-	+	*M.bovigenitalium*	*Moraxella* sp[2]
73		+	+	-	-	-		*Moraxella* sp[2]
74		-	+	-	-	+	*M.bovirhinis*	*Moraxella* sp[2]
75		+	+	-	-	+	*M.bovirhinis*	*Moraxella* sp[2]
76		-	+	-	-	+	*A.laidlawlii* *M.arginini*	
77	NG	-	-	-	-	-		
78		-	++	-	-	+	*A.laidlawlii* *M.arginini*	
79		-	+	-	-	+	*A.laidlawlii* *M.arginini*	

Addendum A

	Growth	*M.haemolytica*	*P.multocida*	*P.trehalosi*	*H.somni*	*Mycoplasma*	**Mycoplasma spp.**	**Other bacteria**
80		-	+	-	-	+	*M.bovis* *M.arginini*	
81	NG	-	-	-	-	-		
82	NG	-	-	-	-	-		
83		+	-	+	-	+	*M.bovis* *M.arginini*	
84		-	+	-	-	+	*M.bovis* *M.arginini*	
85		-	+	-	-	+	*M.bovis* *M.arginini* *A.laidlawlii*	
86		-	+	-	-	+	*M.bovis*	
87		-	+	-	-	+	*M.bovis* *M.arginini*	*Moraxella* sp[2]
88		-	-	-	-	+	*M.bovis* *M.arginini*	*Pasteurella* sp[1] x2 *Moraxella* sp[2]
89		-	-	-	-	+	*M.bovis* *A.laidlawlii*	*Moraxella* sp[2]
90		-	+	-	-	+	Contaminated	*Moraxella* sp[2]
91		-	+	-	-	+	*M.bovis* *M.bovirhinis*	
92		-	+	-	-	+	*M.bovis* *M.arginini*	
93		+	-	-	-	-		
94		-	-	-	-	+	No regrowth	*Corynebacterium* sp.
95		-	+	-	-	+	*M.bovis* *A.laidlawlii*	*Moraxella* sp[2]
96		-	++	-	-	+	*M.bovis* *M.arginini*	*Moraxella* sp[2]
97		-	+	-	-	+	*M.bovis* *A.laidlawli*	
98		+	-	-	-	+	*M.bovis* *A.laidlawlii*	*Moraxella* sp[2]

Addendum A

	Growth	*M.haemolytica*	*P.multocida*	*P.trehalosi*	*H.somni*	*Mycoplasma*	Mycoplasma spp.	Other bacteria
99		-	-	-	-	-		*Bacillus cereus*[2] *Enterococci* sp[2]
100		-	+	-	-	+	*M.arginini*	*Moraxella* sp[2]
101		+	-	-	-	+	*A.laidlawlii*	*Moraxella* sp[2] *Streptococcus* sp[2]
102		+	-	-	-	-		*Bacillus cereus*[2] *Moraxella* sp[2]
103		-	-	-	-	-		*Bacillus cereus*[2] *Moraxella sp*[2]
104		-	-	-	-	-		*Pseudomonas aeruginosa*[2]
105		-	+	-	-	-		*Bacillus cereus*[2]
106		+	-	-	-	+	*M.bovis* *A.laidlawlii*	
107	NG	-	-	-	-	-		
108		+	-	-	-	+	*M.bovis*	*Moraxella* sp[2]
109		-	-	-	-	+	*M.bovis* *M.arginini*	*Bacillus* sp[2] *Moraxella* sp[2] CDC group M6[2]
110		+++	-	-	-	+	*M.arginini*	
111	NG	-	-	-	-	-		
112		-	+	-	-	+	*M.bovis* *M.arginini*	*Haemophilus agni*[1] *Moraxella* sp[2]
113		+	-	-	-	-		
114		-	+	-	-	+	*M.bovis*	*Moraxella* sp[2]
115		-	+	-	-	-		
116		+	-	-	-	+	*M.arginini*	*Moraxella* sp[2]
117		+	-	-	-	-		*Bacillus* sp[2]
118		-	++	+	-	-		
119		+	-	-	-	-		*Bacillus* sp[2]
120		+	-	-	-	+	*M.bovis*	
121		+	-	+	-	-		
122		-	+	+	-	-		*Enterococci* sp[2] *Moraxella* sp[2]
123		-	-	-	-	-		*Bacillus* sp[2] *Staphylococcus* sp[2]
124		+	-	-	-	+	No ID	
125		+	-	+	-	-		*Bacillus* sp[2]

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Addendum A

Table 12.5 2004 TTA's from clinically affected animals

	Growth	*M.haemolytica*	*P.multocida*	*P.trehalosi*	*H.somni*	*Mycoplasma*	Mycoplasma spp.	Other bacteria
1		-	+	-	-	-		
2		-	+	-	-	+	*M.bovis*	*Moraxella* sp[2]
3	NG	-	-	-	-	-		
4		-	-	-	-	-		*Moraxella* sp[2] *Bacillus* sp[2]
5		-	-	-	-	-		*Bacillus* sp[2] *Pseudomonas* sp[2]
6		+	-	-	-	-		*Bacillus* sp[2] Some fungal growth
7		-	+	-	-	-		*Moraxella* sp[2] *Bacillus* sp[2]
8		-	+	-	-	-		
9		-	+	-	-	+	*M.bovis*	*Moraxella* sp[2] *Bacillus* sp[2]
10		-	++	-	-	+	Contaminated	
11		-	-	-	-	+	*A.laidlawlii*	
12		+	-	-	-	-		*Staphylococcus* sp[2]
13		+	-	-	-	+	*M.bovis* *M.arginini*	
14	NG	-	-	-	-	-		
15	NG	-	-	-	-	-		
16		+	-	-	-	+	*M.bovis* *M.arginini*	
17	NG	-	-	-	-	-		
18		+	-	-	-	-		
19		-	-	-	-	-		*Bacillus* sp[2] *E.coli*[2]
20		+	-	-	-	-		*Aeromona hydrophilia*[2] *Acinetobacter* sp[2]
21		+	-	-	-	-		*Moraxella* sp[2]
22		-	-	-	-	-		
23		-	-	-	-	-		*Enterobacter* sp[2]
24		-	-	-	-	-		*Bacillus* [2] *Moraxella* sp[2]
25		+	-	-	-	-		
26		+	-	-	-	-		
27		-	+	-	-	-		
28		-	-	-	-	-		*E.coli*[2] *Bacillus cereus*[2]
29		+	-	-	-	+	*M.bovis*	
30		-	++	-	-	+	*M.bovis*	
31		-	-	-	-	+	Contaminated	*Moraxella* sp[2]
32		+	-	-	-	+	*M.bovis*	*Bacillus cereus*[2]
33		-	+	-	-	+	*M.bovis*	*Moraxella* sp[2]

Addendum A

	Growth	*M.haemolytica*	*P.multocida*	*P.trehalosi*	*H.somni*	*Mycoplasma*	**Mycoplasma spp.**	**Other bacteria**
34		+	+	-	-	+	*M.bovis*	
35		+	-	-	-	+	*M.bovis*	*Moraxella* sp[2]
36		+	-	-	-	-		*Moraxella* sp[2]
37		-	+	-	-	-		*Moraxella* sp[2]
38		++	-	-	-	-		
39	NG	-	-	-	-	-		
40		-	-	-	-	+	*M.bovis* *M.arginini*	
41		-	-	-	-	+	Not identified	
42	NG	-	-	-	-	-		
43		-	-	-	-	+	*M.bovis*	
44		-	-	-	-	+	Contaminated	
45		-	-	-	-	+	*M.bovis*	*Moraxella* sp[2]
46		-	-	-	-	-		*Bacillus* sp[2]
47	NG	-	-	-	-	-		
48		-	-	-	-	+	*M.bovis*	*Moraxella* sp[2]
49		-	-	-	-	+	*M.bovis*	*Moraxella* sp[2]
50		-	+	-	-	+	*M.bovis*	
51		-	+	-	-	+	*M.bovis* *M.arginini*	*Enterococcus* sp[2]
52		-	+	-	-	+	*M.bovis*	*Moraxella* sp[2]
53		-	-	-	-	+	Contaminated	*Moraxella* sp[2]
54	NG	-	-	-	-	-		
55		+	-	-	-	+	Not identified	*Enterococcus*[2] *Moraxella* sp[2] Some fungal growth
56		-	+	-	-	+	*M.bovis*	*Streptococcus agalactiae*[1]
57	NG	-	-	-	-	-		
58		+	-	-	-	-		
59	NG	-	-	-	-	-		
60	NG	-	-	-	-	-		
61		-	-	-	-	-		*Bacillus cereus*[2] *Moraxella* sp[2] a rough *E.coli* Some fungal growth
62		+	-	-	-	+	*M.bovis*	Some fungal growth
63		+	-	-	-	+	Not identified	*Bacillus cereus*[2]
64		+	+	+	-	+	Not identified	*Moraxella* sp[2]
65		++	-	-	-	+	Not identified	
66		+	+	-	-	+	Not identified	
67		+	-	-	-	-		
68		-	-	-	-	+	Not identified	*Moraxella* sp[2]

Addendum A

	Growth	*M.haemolytica*	*P.multocida*	*P.trehalosi*	*H.somni*	*Mycoplasma*	Mycoplasma spp.	Other bacteria
69	NG	-	-	-	-	-		
70	NG	-	-	-	-	-		
71		-	-	-	-	+	Not identified	*Streptococcus* sp[2]
72		-	-	-	-	-		*Flavobacterium* sp[2]
73		-	+	-	-	-		
74		+	-	-	-	-		
75		+	-	-	-	+	Not identified	
76		+	-	-	-	+	Not identified	
77		+	-	-	-	+	Not identified	*Moraxella* sp[2]
78		+	-	-	-	+	Not identified	
79		-	+	-	-	+	Not identified	*Moraxella* sp[2]
80		-	-	+	-	+	Not identified	*Moraxella* sp[2]
81		-	-	+	-	+	Not identified	
82		-	-	+	-	+	Not identified	
83		-	-	+	-	-		*Enterococcus*[2]
84		-	-	-	+	+	Not identified	
85	NG	-	-	-	-	-		
86		+	-	-	-	-		*Moraxella* sp[2]
87		-	-	-	-	+	Not identified	*Enterococcus* sp[2]
88		-	-	-	-	-		*Moraxella* sp[2]
89		+	-	-	-	+	Not identified	
90	NG	-	-	-	-	-		
91		-	+	-	-	-		
92		-	+	-	-	+	Not identified	

Table 13. Bacterial and Mycoplasmal isolates from clinically normal animals

Table 13.1 2002

	Growth	*M.haemolytica*	*P.multocida*	*P.trehalosi*	*H.somnus*	*Mycoplasma*	Mycoplasma spp.	Other bacteria
1		-	-	-	-	-		*Enterococcus* sp[2]
2	NG	-	-	-	-	-		
3	NG	-	-	-	-	-		
4	NG	-	-	-	-	-		
5		-	-	-	-	-		*Staphylococcus aureus*
6		-	-	-	-	+	*M.bovis*	*Enterococcus* sp[2]
7		-	-	-	-	-		*Arcanobacterium pyogenes*
8		-	+	-	-	+	*M.bovis*	
9		-	+	-	-	+	*M.bovirhinis* *M.canadense*	
10		-	+	-	-	-		
11		-	+	-	-	-		
12		-	-	-	-	-		Beta-haemolytic *E.coli*
13		-	-	-	-	-		*Moraxella* sp[2] *Enterococcus* sp[2]
14	NG	-	-	-	-	-		
15		-	-	-	-	-		Contaminants only
16	NG	-	-	-	-	-		
17	NG	-	-	-	-	-		
18		-	-	-	-	+	No regrowth	
19		+	-	-	-	-	Contaminated	

Table 13.2 2003

1		-	-	-	-	+	*M.bovis.* *M.laidlawlii*	
2	NG	-	-	-	-	-		
3	NG	-	-	-	-	-		
4		-	+	-	-	+	Contaminated	
5		-	+	-	-	-		
6	NG	-	-	-	-	-		
7	NG	-	-	-	-	-		
8	NG	-	-	-	-	-		
9		-	-	+	-	+	No ID	
10	NG	-	-	-	-	-		
11		-	-	+	-	+	No regrowth	
12		-	+	-	-	-		
13	NG	-	-	-	-	-		
14		+	+	-	-	-		*Moraxella* sp[2]