INFECTIOUS DISEASES OF THE UPPER AND LOWER BOVINE RESPIRATORY TRACT

by

ABDULSALAM BAKHSH ALHENDI, B.V.M.& A.R.

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in the Faculty of Veterinary Medicine,
University of Glasgow, Scotland, U.K.

Department of Veterinary Medicine
University of Glasgow.
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Infection must be considered as a struggle between two organisms ... the parasite and its host. This brings about adaptation on both sides.

METCHNIKOFF, 1891*

(* cited by Linton (1982) IN Microbes, Man and Animals, John Wiley and Sons, Chichester, New York, Brisbane, Toronto, Singapore)

To my father Abdullah, mother Zainub
and in the memory of my late brother,
Dr. Sirajuddin

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DECLARATION

I declare that the work presented in this thesis has been carried out by myself, albeit with collaboration and assistance from a number of other colleagues or departments.

Initially, certain of the microbiological and serological procedures were conducted in conjunction with Dr. E.M. Allan, then of the Department of Veterinary Pathology who also performed the various pathological examinations. Members of the Department of Veterinary Parasitology carried out certain of the parasitological procedures reported in Chapter 4 while the haematological investigations that formed a part of the case studies presented in Chapter 6 were conducted by the Department of Veterinary Pathology.

Certain aspects of the investigations described in Chapter 3 have been presented in the following publication:

> Selman, I.E., Allan, E.M., Gibbs, H.A., Wiseman, A. and Young, W.B. (1984) Effect of anti-prostaglandin therapy in experimental parainfluenza type 3 pneumonia in weaned, conventional calves. Vet.Rec., 115, 101-105.

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SUMMARY

In Chapter 1, the literature relating to bovine respiratory disease is discussed with particular emphasis on infections, infectious diseases and other disorders of the bovine upper respiratory tract. Chapter 2 describes the materials and methods used as a general basis for the studies presented in later parts of the thesis. Minor variations to these techniques or additional procedures carried out for specific investigations are presented in their appropriate chapter.

Chapter 3 describes a study that was originally designed to assess the effects of the antiprostaglandin compound, flunixin meglumine, under controlled studies in calves experimentally infected with parainfluenze type 3 (PI3) virus. Throughout the course of this study, nasopharyngeal swabs were collected for mycoplasmal and bacterial investigations and tissues from various sites in the upper and lower respiratory tract were similarly examined. Flunixin meglumine significantly limited the extent of pulmonary consolidation in the test (treated) calves and thereby conferred clinically obvious therapeutic advantages. In the first group of calves subjected to detailed microbiological study, the diminished level of pulmonary consolidation correlated with lower numbers of mycoplasmal and bacterial isolations although the range of species isolated was unaffected. In the second group of calves, the beneficial effects of flunixin meglumine were less obvious probably

as the result of (unsuspected) pre-existing chronic pneumonia throughout the group. In these same calves, the total "load" of both mycoplasmas and bacteria was far greater than in the first group. Mycoplasmal isolations were far more numerous in the untreated controls although the reverse was true for bacterial isolations. As with the first group, the range of species detected was unaffected by anti-inflammatory therapy.

In Chapter 4, the mycoplasmal and bacterial population within the upper and lower respiratory tract of five calves with experimentally-induced parasitic bronchitis were compared with findings in uninfected control animals. The clinical and pathological effects of infection with the cattle lungworm, D.viviparus, were also studied. A wide range of bacteria were found at all levels of the tract in the control calves. Little difference was found between lower tract infections of the two groups of calves slaughtered during the prepatent phase of the lungworm infection. However, a far wider range of bacterial species were found and far more numerous isolations were made from the calves slaughtered during the patent phase of the disease. Results relating to mycoplasmal isolations were similar but less impressive due to the smaller numbers of organisms and isolations involved.

A natural outbreak of bovine pneumonic pasteurellosis was studied and described in Chapter 5. The dynamics of infection, the clinical and pathological consequences of that infection and the sensitivity

profile of the organism involved (P.haemolytica A1) were investigated. While nasopharyngeal swabs were certainly found to be positive during the course of the incident, their value as diagnostic aids is questioned mainly on the basis of the ready availability of other material for diagnosis, including dead calves, on the one hand, and the fact that nasopharyngeal swabs may give rise to misleading results on the other.

Finally, the mycoplasmal and bacterial flora of a number of cattle with chronic respiratory disease were studied and reported in Chapter 6. Cases of diffuse fibrosing alveolitis did not harbour many bacterial species within their respiratory tracts. In contrast, a wide range of species were found to be present at all levels of the tract in cattle suffering from chronic suppurative or chronic non-suppurative pneumonia.

In each of the investigations reported in Chapters 3-6, careful note is taken of the species of mycoplasmas and bacteria isolated from the total of 54 cattle studied. In fact, apart from the calves that underwent a "natural" outbreak of pneumonic pasteurellosis, the presence of recognised pulmonary pathogens was not widespread. Mycoplasma bovis was detected on a number of occasions (particularly in the calves studied in the second experiment reported in Chapter 3) as was P.haemolytica. One organism that was isolated very frequently from all levels of the tract in a great number of the cattle studied was Acinetobacter calcoaceticus and it is suggested that further work

should be carried out in order to establish whether this organism is truly pathogenic for the bovine respiratory tract.

The fact that very many lower tract infections were made - even in clinically normal, non-pneumonic cattle - is discussed in relation to the findings of other workers. On the basis of this work it is suggested that lower tract infections of a mostly transient nature is normal in cattle and possibly other ruminants. It is further suggested that these infections are very probably associated with the inhalation of soil or pasture-borne bacteria along with eructated ruminal gases.

Clinical terms

The terms used are those advocated by Selman and Wiseman (197). Each one used is defined in detail in General Materials and Methods (Chapter 2).

Clinical and other abbreviations used in the various tables are explained as and when necessary on each table as asterisked footnotes.

Microbiology

The names of the bacteria are those used in Bergey's Manual of Determinative Bacteriology (21). Where the name of an organism occurs in the text for the first time or at the beginning of a sentence it is written out in full.

Pathology

The term upper respiratory tract includes the nasal passages, the nasopharynx and the trachea, caudally to the bifurcation. The lower respiratory tract is everything caudal to the bifurcation. Occasionally reference is made to "associated lymphatic tissue". This, in relation to the upper tract refers to the tonsils and the retropharyngeal lymph nodes; when used in relation to the lower tract this means the bronchial lymph nodes. In certain of the sections distinction is also drawn between cranial (a) and caudal (p) sections of the tract, for example the nasal conchus and the trachea.

Pharmaceutical preparations

Therapeutic agents are referred to by their generic name.

References

In the reference section, the contractions for the various journals quoted are those given in Serial Sources for the Biosis Data Base published by the Biosciences Information Service, Philadelphia.

GENERAL INTRODUCTION

GENERAL INTRODUCTION

While an enormous amount of effort and expense has been directed towards the greater understanding of the cattle pneumonias it must be said that with certain notable exceptions, the knowledge that has been acquired has not greatly contributed to their prevention.

In many instances, particularly in respect of the non-infectious pneumonias such as fog fever (acute bovine pulmonary oedema, ABPE) and the bovine form of farmer's lung disease, prevention rests upon the avoidance of those circumstances that are now recognised as initiating the problems; unfortunately, under normal practical conditions, this is often not possible on a long-term basis. Without doubt though, the most costly pneumonias that affect the cattle industry are the infectious pneumonias which are usually (but certainly not exclusively) problems that affect immature, intensively managed stock. Of the wide range of infectious pneumonias that are now known to exist, effective prevention tools, in the form of vaccines, are available for two major problems only in the western world, namely infectious bovine rhinotracheitis (IBR) and bovine parasitic bronchitis (hoose, husk) and a third condition contagious bovine pleuropneumonia (CBPP) which has traditionally been a problem of more extensively managed cattle in less well developed regions. Certainly numerous other vaccines exist but their efficacy would appear to be, at best, doubtful and, at least, non-existent.

Progress in the development of effective vaccines or other preventative regimes for the other infections is hampered, not least by the very large number of viruses, mycoplasmas and bacteria that are to be found in the upper and lower respiratory tracts of cattle. This situation is compounded by the fact that, apparently, many such infections exist in these sites just as commonly in non-pneumonic calves as they do in cases of pneumonia.

For many years, (mainly because of the frequent lack of success of experimental infection models that have incorporated the use of microbial isolates that have appeared, on the basis of field evidence, to be aetiologically involved in outbreaks of calf pneumonia), it has been fashionable to view even prime suspects, for example Pasteurella haemolytica, as being only really pathogenic when other factors such as prior viral infections and "stress" are involved. Recent experimental and field evidence would, however, raise doubts about the frequency with which viral-bacterial interactions are necessary before pneumonic incidents arise. Specifically, certain strains of P.haemolytica are now identified as being primary pathogens for the bovine respiratory tract in their own right.

Further confusion regarding the significance of many respiratory infections in calves has been caused by the extrapolation of information across species barriers. In this respect, it would seem particularly unfortunate that at a recent symposium on feedlot pneumonias, one

eminent authority stated that "the distal portions of the lung are usually sterile" and then went on to say that certain post-viral bacterial pneumonias usually involved single specific organisms that were common in the population of a particular area at that particular time. The fact that such statements were based upon field studies in humans and experimental studies on mice have been largely overlooked as was the work of several different research groups over the last 20 years that has shown quite clearly that the lower airways of normal, non-pneumonic, cattle very commonly, perhaps "normally", may carry a variety of infections.

With these factors and views in mind, the following series of studies was planned with the aim of reviewing the literature relating to upper and lower respiratory tract infections with particular emphasis on mycoplasmas and bacteria and then to investigate this same situation in a variety of experimental and "naturally-occurring" bovine respiratory disorders. Furthermore, it was planned that throughout the studies particular emphasis should be given to upper tract infections and disorders since these would appear to have been largely neglected in favour of "the pneumonias" by the majority of earlier workers.

CHAPTER 1

REVIEW OF THE LITERATURE

CHAPTER 1. REVIEW OF THE LITERATURE

In the following pages, only brief mention will be made of the structure, function and defence mechanisms of the normal bovine respiratory system. Thereafter, the various views as to what constitutes the upper and lower respiratory tract will be discussed. In view of their overwhelming importance, the bovine pneumonias will then be reviewed. However, the main thrust of this chapter will be aimed at reviewing and discussing infections, infectious diseases and other problems that may affect the upper bovine tract.

The respiratory system incorporates nostrils, nasal cavities and sinuses, pharynx, larynx, trachea, bronchi, lungs, pleura and thoracic cavity.

The bovine nose is like many other animals, embedded in the skeleton of the face unlike the human where it projects distinctly from the face. The nasal cavities of the bovine animal is relatively long (191). Most of the middle part and the septum of the nasal cavities is lined by pseudo-stratified columnar epithelial cells which contains both ciliated and goblet cells and which is covered by a layer of mucus (74). The mucous membrane of the nasal cavities is highly vascular and the temperature within the cavities is significantly lower than that of the deeper parts of the respiratory tract. The paranasal sinuses are air-filled cavities, lined by ciliated columnar epithelial cells, that communicate either directly or indirectly with the nasal cavities. Various theories exist as to their function but

this is still somewhat speculative.

The pharynx is divided by the soft palate into an upper nasopharyngeal and a lower oropharyngeal portions (62;74). The tonsils, are situated within the pharynx and are organised into discrete masses - the palatine, lingual and pharyngeal tonsils which in fact form a ring around the pharynx (126). The palatine tonsils in the bovine animal are paired oval bodies and are located in pockets in the lateral walls of the pharynx, ventral to the soft palate and lateral to the base of the tongue. They are embedded in the submucosa and are completely covered by mucous membrane except in the region of the tonsillar crypts or fissures. They do not project into the pharynx and cannot be seen during normal clinical examination (23). The lingual tonsils are bean-shaped, about 10 cm long and are located on the base of the tongue. The pharyngeal tonsils are located at the caudal end of the median septum, medial to the opening of the auditory tube, that is in the median posterior walls of the nasopharynx (78). Their surface is covered by stratified squamous epithelium, without cilia.

The tonsils generally are covered by epithelium which rests on a basement membrane under which is a well-developed vascular plexus in a connective matrix of cells containing submucosal glands, lymphocytes, mast cells, polymorphonuclear cells and macrophages as well as plasma cells (28).

The larynx is situated at the caudal aspect of the pharynx, ventral to the cranial opening of the

oesophagus. The larynx comprises a number of articulated cartilages, the vocal cords, and the various laryngeal muscles and ligaments. It is relatively short and wide. The larynx is lined by stratified squamous non-keratinized epithelium extending from the entrance into the larynx to the caudal edge of the vocal folds, where it changes gradually to typical respiratory epithelium (62).

The trachea is a non-collapsible, cartilagenous and membranous tube which, in the adult bovine animal, is about 65 cm long. Approximately 50 oval rings are present in the bovine trachea which is lined by a ciliated epithelium with numerous secretory glands lying in the mucous membrane (191). Caudally, the trachea bifurcates into right and left major bronchi which are structurally similar to the trachea and thence to the smaller bronchi serving the various lung lobes.

The lungs, together, form a cone-shaped structure which is really divided into left and right lungs; the right having four lobes in the bovine animal and the left having three. The lungs contain respiratory bronchioles, alveolar sacs, alveolar ducts and alveoli together with vascular and supportive tissue. The lungs are covered by a visceral pleura while the lining of the thoracic cavity, which opposes this, is called the parietal pleura.

The main activity of the respiratory system in any species is, of course, respiration which has been defined as the process or processes by which the actual

exchange of gases (that is oxygen and carbon dioxide) takes place, beginning with the respiratory bronchioles and terminating with the alveoli (78). However, the respiratory system has numerous other important functions which include, in the upper airways, olfaction, phonation and warming, moistening and regulating the airflow into the lungs and in the lower tract and tissues, the regulation of acid-base balance of the extracellular fluids of the body and the removal of foreign airborne particles (62;78). The lungs are also involved in many enzymatic and detoxification processes including the neutralisation of xenobiotic compounds (30,193).

This important and far-reaching subject of pulmonary defence mechanisms has been addressed in a variety of ways depending on the interests of individual authors (51,88,116,128,175,186,224). However, the mechanisms detailed by Crofton and Douglas (51) would appear to cover most of the important non-specific activities including the gag and cough reflexes, the normal movement of the air within the bronchi during respiration, mucociliary action and the role of alveolar macrophages. To these functions must be added the various, more specific, immune responses that may occur in both the upper and lower airways; at the same time it must be recognised that upper and lower tract components may differ in immunological competence with the lung having a greater inventory of protective mechanisms than the trachea or the nose (224). It must also be recognised that the above phenomena may act either independently, or

in concert, to prevent or curtail damage by physical, chemical or biological agents.

The subject has also been studied by Pirie (165,167) who emphasised the importance of mucociliary activity, humoral factors (e.g. lysozyme, lactoferrin, interferon and complement components) in the fluid mileu of the respiratory tract which may adversely affect the ability of organisms to colonise or invade the surfaces of the tract, alveolar macrophages, the cough reflex and alveolar clearance. It was suggested that microorganisms that are not removed or inactivated by immune or other mechanisms could become established in the lungs and cause pneumonia. In further discussion, the same author (165,167) also emphasised the importance of specific antibodies in nasal and/or tracheobronchial secretions (particularly IgA produced locally and secreted into the tract), immune phagocytosis by macrophages and also specific, cell-mediated immune reactions initiated by immunologically activated cells within the lungs. It was felt that the effective resistance to respiratory tract infections depended principally on local specific defence mechanisms.

Secretory IgA is the predominant immunoglobulin in respiratory secretions cranial to the larynx. Biologically, IgA antibodies may act as an initial immunologic barrier at the portal of entry to invasive viral infection, for example, influenza and respiratory syncytial viruses, and rhinoviruses. It may also play a more definitive role in the determination of infection

and immunity (88). Other functions of IgA include agglutination (and hence, it may enhance mucociliary transport of bacterial particles), neutralization of toxins and inhibition of attachment of bacteria to epithelial cells. However, the major biologic function of IgA would appear to be to regulate microbial entry.

In contrast, IgG is far more important in the lower respiratory tract. It efficiently agglutinates particles, opsonizes bacteria, activates complement, neutralizes bacterial toxins and viruses and lyses gram-negative bacteria in the presence of complement. Similarly, the role of IgM would include agglutination, complement fixation, and lysis of gram-negative bacteria. Although IgE antibody is well recognised in terms of its role in immediate-type hypersensitivity (type 1) reactions, its role in defence of the lung remains obscure.

In one relatively recent study, IgA producing cells were found to be by far the most numerous of antibody producing cells in the lungs of healthy calves although in pneumonic calves and in mature cattle, IgG producing cells were more common (9).

The tonsils also serve as a local and initial immune ~~response~~ defence against infection (70;222). IgA antibody were produced by plasma cells in the tonsils as a result of antigenic stimulation by infection (163).

The pulmonary airways of normal calves contain large numbers of macrophages and lymphocytes, but only relatively few neutrophils, eosinophils, basophils, and

epithelial cells (224).

Both T and B lymphocytes can operate in the respiratory tract and the former types are commonly found in bronchial secretions. Antigen-stimulated T lymphocytes can, by secreted lymphokines recruit additional lymphocytes and macrophages to the lungs (217). T lymphocytes are stimulated by antigens, and transformed to 'killer cells' which are cytotoxic for antigen-containing cells (51).

Alveolar macrophages in an 'activated state' show an enhanced capacity to ingest opsonised microorganisms; moreover, phagocytosis of macrophages may be enhanced by specific antibody (180). The function of alveolar macrophages is either stimulatory to the respiratory tract immune response and activative of the lymphocytes or suppressive of these. To some extent, the net stimulatory or suppressor effect of alveolar macrophages is species-dependent. The lung macrophages of rats, dogs, rabbit and calves are inhibitory. However, while alveolar macrophages are a vital component in terms of lung defence mechanism, their antibacterial abilities are depressed by agents such as Parainfluenza 3 (PI3) virus (20;93).

In brief, cell-mediated immunity and antibody interact with non-specific respiratory mechanisms to enhance protection against microbial pathogens. Marked resistance without immune response is provided by ciliary and phagocytic clearance of inhaled particles (101). Antimicrobial activities include: lysosomal action

against gram-positive bacteria; lactoferrin inhibition of bacterial iron metabolism; interferon inhibition of viral replication; activation of the alternate complement pathway with enhanced phagocytosis and lysis of microbial pathogens; non-specific opsonization by IgG and IgM; enhanced leukocyte phagocytosis and anti-microbial activity in the presence of alveolar lining material (88, 218).

Considerable variations exist as to what is deemed to constitute the upper and the lower parts of the respiratory tract. Basically, it would appear that such variations in view reflect the interests and disciplines of different authors, consequently schemes that have been advocated have been based on physiological, anatomical, histopathological and clinical criteria.

In the medical literature, the upper respiratory tract has generally been viewed as being that part between the external nares to the caudal end of the larynx (19,51,103,116,214). However, one author at least (18) has preferred to base his division on the direction of mucociliary action.

Further controversy exists within the veterinary literature. Certain authors (29,30,84,104) have advocated that the larynx should be considered as part of the upper tract in keeping with the general view of human physicians while others (11,25) have suggested that, on clinical grounds, the upper tract should also include the trachea. However, one worker (128) has recently stated that in his opinion, the upper respiratory tract should

be viewed as extending down to the level of the start of the respiratory bronchioles; in other words the decision should be based upon histopathological criteria.

1. INFECTIONS, INFECTIOUS DISORDERS AND OTHER DISEASE PROBLEMS OF THE BOVINE RESPIRATORY TRACT

For the purposes of the following review and discussion, the bovine respiratory tract will be divided on the basis of the criteria suggested by earlier, clinical workers (11,25). Thus, the division between upper and lower tract will be taken as being at the level of the bifurcation of the trachea. Since the major discussion will revolve around problems of the upper bovine respiratory tract, these will be dealt with after a shorter review of pulmonary disorders - the pneumonias - of cattle.

The pneumonias of cattle

In any thesis dealing with respiratory problems - even when major emphasis is to be placed upon upper tract infections and disorders - reference must be made to the pneumonias because of their widespread prevalence and enormous economic impact. This is particularly true of the pneumonias of immature and intensively-managed stock, although a wide variety of interesting and important pneumonias may also arise in adult cattle.

In the following section, an attempt will be made to briefly comment upon the problems that may affect the different ages and types of cattle livestock and material from a number of recent major review articles will be drawn upon.

- acute and chronic pneumonias of young, housed calves

In Britain, many calves are bought by specialist calf rearers at around one or two weeks of age and reared in intensive veal and beef units (13). The difficulties that these units face due to the prevalence, pattern and severity of the various calf pneumonias are enormous and pose very significant economic effects (13,159).

Major damage and a wide range of infections are often to be found in calves from such units (34,159) suggesting that many microbiological studies might best have been conducted on the pneumonias of "home-reared" calves in which the situation often seems somewhat less complicated (159). In such calves more specific respiratory syndromes may be recognisable and may often be linked with specific infectious agents (159). The subject has been reviewed in detail recently (159).

Of the numerous viruses that have been found to be associated with pneumonic calves (159,201), two (bovine respiratory syncytial virus and parainfluenza type 3 virus) are now widely accepted as of major importance, often giving rise to acute, high morbidity pneumonic incidents with high mortality (34). Three mycoplasmas (Mycoplasma bovis, M.dispar and Ureaplasma diversum) are recognised pathogens for the bovine respiratory tract (4,5,86). In dairy calves, these organisms are commonly associated with chronic, high morbidity pneumonias (4,5) although M.bovis may sometimes appear to be slightly more inclined to give rise to a somewhat more acute syndrome (7,86). In contrast to the

above viral infections, the mycoplasmal pneumonias are not usually accompanied by high mortality rates (4,86). Vast numbers of different bacteria have been found in the lungs of both pneumonic and non-pneumonic calves (5,49,148) but only a small proportion have come to be regarded as anything other than transient residents or, perhaps, secondary/opportunistic invaders (5,49). The subject is dealt with at length in a later chapter. Indeed, many workers take the view that few bacteria have pathogenic capabilities in their own right, that is, in the absence of viruses or adverse environmental circumstances (201,234). However, Pasteurella haemolytica biotype A, serotype 1 (A1) has, after many years of controversy (234) now been shown to be pathogenic per se (80,81).

Bovine pneumonic pasteurellosis (transit/shipping fever) is an acute febrile pneumonia that usually arises in calves that have been range-reared, weaned at eight or nine months of age and then promptly housed - often for the first time in their lives (80). Most outbreaks occur within two weeks of housing and management and marketing practices dictate that such outbreaks are most common during the months of October and November (80). Weaning, transport, marketing and housing induce stress reactions in these young ("single-suckled") calves, a situation that is also thought to be of major importance in the pathogenesis of the disease (234) as is prior, or current, viral respiratory disease (234). However, as already stated, field and experimental evidence now exists to support the view that at least one strain of

P.haemolytica is a primary respiratory pathogen for cattle (81).

While the mortality rate is usually not very high in pneumonic pasteurellosis (80,108) its production effects and attempts to avoid it may cost a great deal. In the feedlots of the USA, for example, its impact is such that it costs $US 250-750 million annually and in "bad years" may cause the death of 750,000 cattle (80).

- parasitic bronchitis

This disease is caused by the cattle lungworm, Dictyocaulus viviparus (102,220). It is mainly a hazard in artificially-reared calves, although given special circumstances, single-suckled calves and even adult cattle may become severely affected (193,195). Infection is acquired by eating herbage contaminated by cattle that have excreted lungworm larvae in their faeces either in previous years or earlier, during the same grazing season. There are numerous inter-related factors including grazing management and behaviour; larval survival, development and activity; weight of larval challenge and subsequent activity within the final host (220). Outbreaks usually arise in calves grazing permanent pastures during the second half of their first grazing season (102,195,220).

Once ingested, lungworm larvae migrate from the intestine to the lungs where they develop to adulthood in the larger airways, the whole process taking almost four weeks (102). There are several clinical forms of the

disease depending on parasite numbers, stage of infection and degree of host resistance (220). Clinical signs are characteristic dominated as they are by varying degrees of respiratory distress and coughing (102. Both clinical signs and pathological changes are directly related to the presence of lungworm and their products in the airways (102). Parasitic bronchitis may be prevented by the administration of a vaccine consisting of live (X-ray attenuated) lungworm larvae before the start of the first grazing season (220). Treatment with modern anthelmintics is often not totally successful (194).

- allergic pneumonias

This subject has been reviewed relatively recently (193,226).

Some cattle may become allergic to drugs, vaccines or sera, a situation that occasionally results in respiratory distress and death soon after the administration of the compound in question. Rupturing the subcutaneous larvae of the cattle warble fly (Hypoderma bovis or H.lineatum) may provoke a similar reaction. Some cattle may even become allergic to the milk proteins they have synthesised in their udders. The following brief discussion, however, is limited to only one of the relatively few naturally-occurring allergic pneumonias described in recent years (226).

The human disease known as 'farmer's lung' is caused by the thermophilic actinomycete Micropolyspora faeni, which abounds in hay that has overheated as a result of having been baled damp. Because of their very

small size its spores, if inhaled, penetrate the lungs to the terminal levels of the respiratory tract. Repeated incidents result in the formation of specific antibodies to the spores and/or their metabolic products (226).

Eventually, a Type III (Arthus') hypersensitivity reaction occurs, which may result in severe and irreversible, progressive and fatal allergic pneumonia. Given multiple daily exposures to mouldy hay over several winter-feeding periods, cattle may become severely affected with a respiratory problem that is clinically, pathologically and immunologically identical to the human disease (226).

- chemically-induced pneumonias

Inhaled toxic compounds would appear not to be of major importance in cattle (193,195). However, acute pneumonic incidents have been reported following the inhalation of nitrogen dioxide (that is similar to "Silo-filler's disease" in man), chlorine and zinc oxide fumes (193,195). Certain situations are, however, now well-documented wherein ingested toxic compounds may give rise to severe respiratory incidents which are often associated with significant mortality (193). Adult cattle feeding on mouldy sweet potatoes (Ipomoea batatus) may develop an acute, often fatal, respiratory distress syndrome through ingesting "stress metabolites" synthesised by the potato as the result of infection with the fungus Fusarium salani. These metabolites are structurally similar to perilla ketone, a toxin present

in the wild mint (Perilla frutescens). Ingestion of this plant results in clinical signs and in pathology identical to that seen in mouldy sweet potato poisoning, and also in cattle that have eaten mouldy corn stalks and Tasmanian stinkwood (Ziera arborescens) (30,193).

Fog fever (acute bovine pulmonary emphysema, ABPE) is a sudden, often fatal, respiratory disease of adult beef cattle (30,32,193). In Britain it occurs in the autumn, soon after cattle are introduced to 'foggage' (lush aftermath pasture) or, far less frequently, to Brassicae. In the former case, the grass contains high levels of the naturally-occurring amino acid, L.tryptophan. This compound is converted in the rumen by bacterial action to 3,methylindole (3MI) which is then changed within the lung tissue to a further, as yet unidentified, pneumotoxic substance (30,193).

Fog fever is as dramatic pathologically as it is clinically. The lesions that occur are pulmonary congestion, oedema, hyaline membranes, alveolar epithelial hyperplasia and interstitial emphysema, precisely those that occur in mouldy sweet potato and wild mint poisoning. It is therefore interesting to note that the family of compounds (3-substituted furans) that are known to give rise to the latter syndromes are structurally similar to 3MI (30,193).

It is important to emphasise that the above account of the cattle pneumonias is far from comprehensive and, quite apart from other high morbidity conditions that exist, the importance of the more

"individual" pneumonias of cattle should be noted, not least because they would appear to be so prevalent (195,197). For example, pulmonary thromboembolism is relatively common arising, as it does, from a thrombosis of the caudal vena cava (31,193). There is also a condition of elderly cows; diffuse fibrosing alveolitis, that is virtually identical to the problem as it occurs in man (30,31,193). Lung cancers are rare but have nevertheless been described (31,32).

Perhaps the most economically significant 'individual' pneumonias of all, however, are those suffered by the incurable 'respiratory cripples' that emerge some weeks or months after some of the major outbreaks. Such cases affect all ages of stock, causing either chronic suppurative or non-suppurative lesions, usually in the cranial (dependent) lung lobes (197,198). Quite apart from the loss of production and, finally, of the animal itself, considerable time and money is often wasted in unsuccessful attempts to treat such cases - often for want of a sound clinical diagnosis.

2. INFECTIONS, INFECTIOUS DISORDERS AND OTHER DISEASE PROBLEMS OF THE UPPER BOVINE RESPIRATORY TRACT

For convenience, the traditional sub-division of diseases into congenital and acquired problems will be adhered to in the following section.

Congenital problems of the upper tract

It would appear from an examination of the avaible literature that such problems are far less common

(or, perhaps, have been far less commonly documented) than is (or has been) the case in humans, horses and dogs (51,56,134).

Chronic stertor in a two-month-old Friesian bull calf was found to be due to a congenital absence of the naso-maxillary aperture (136), and a nasolachrymal duct anomaly has been described in a seven-month-old Brown Swiss calf (92). Of perhaps greater significance in terms of possible predisposition to the development of laryngeal abscessation was the description of what appeared to be a congenital malformation of the arytenoid cartilages (125).

Acquired problems of the upper tract: specific infections

As already stated, the upper airways of cattle may be infected by a wide range of viruses, mycoplasmas, bacteria and other agents. Frequently, such infections are either totally asymptomatic or else merely reflect a more significant degree of involvement elsewhere (that is usually in the lower parts) in the respiratory tract and/or in other parts of the body. The situation regarding virus infections in cattle has been discussed in detail elsewhere, particularly in relation to the so-called "respiratory viruses", of cattle but also to other, more systemic, infections such as bovine virus diarrhoea/ mucosal disease and malignant catarrhal fever (114). The type-example of a viral infection that is very often apparently limited to the upper respiratory tract is infectious bovine rhinotracheitis (IBR) and this disease will be discussed in detail.

- Infectious bovine rhinotracheitis (IBR)

Infectious bovine rhinotracheitis is a highly acute, contagious and febrile disease which occurs most commonly in feeding or fattening cattle. It is characterised by a variable degree of inflammation of the upper respiratory tract and, less frequently, conjunctivitis. The course of the illness is usually less than one week and the mortality rate is usually low (138,231).

The disease was first described as affecting dairy and beef cattle in California in 1953 (140,190). It had earlier been seen in beef cattle in Colorado where it had been called "red nose", "dust pneumonia", "necrotic rhinotracheitis" and "necrotic rhinitis" (150). In 1955, the disease was designed "infectious bovine rhinotracheitis" (140). The causal virus was first isolated in tissue culture one year later (143). Further work, demonstrated that serum obtained from cattle originating from New Jersey and New York in 1947 contained antibodies to the virus (82).

In 1958, it was reported that the virus causing "infectious pustular vulvovaginitis (IPV)" was identical to that which caused IBR (118). Since the virus had growth and morphological characteristics indistinguishable from those of herpes simplex virus, it was then classified as a herpesvirus (15).

More recently, all herpesviruses for which the usual natural hosts are members of the family Bovidae have been designated bovid herpesviruses 1,2,3 etc.

(185). Under this system, the causal agent of IBR has been termed bovid herpesvirus 1 (BHV1) a name which has been applied to all virus isolates serologically related to the virus of IBR.

Bovid herpesvirus 1, the causal agent of IBR, has the physical, biochemical, immunological and epidemiological properties of the herpesviruses, which are DNA viruses (79). The virion consists of an enveloped icosahedral nucleocapsid which surrounds the nucleic acid; the diameter of the enveloped particle ranges from 120-180 nm. The envelope, which is acquired as the nucleocapsid buds through the nuclear membrane, is important for infectivity and confers upon the virus the physical properties of ether, chloroform, acid and disinfectant sensitivity (15,53).

The virus is stable between ph 6.0-9.0 but labile at 4.5-5.0 (87) and has a buoyant density ranging from 1.249-1.254 cm^{-3} (17). The growth cycle is relatively short, with progeny completed within 12 hours (133,151). The virus replicates readily in a wide variety of cell cultures from cattle and other species, producing distinctive cytopathic effects which serve as a basis for virus isolation and neutralisation tests for serum antibody (40).

Since BHV1 infection may cause clinical manifestations other than respiratory diseases (79) and the severity of the clinical signs vary greatly (150) it has been suggested that different isolates differ antigenically (83). However, comparisons of the neutral-

isation kinetics of four BHV1 isolates have revealed only minor antigenic differences and the behaviour of the four viruses in tissue culture was identical. On the other hand, restriction endonuclease techniques have revealed differences between BHV1 isolates from respiratory tract and aborted foetus origin (154).

Following the discovery of IBR in the USA in 1953 (190) it has been reported from most parts of the world with the possible exception of South America (25,151,235). The relatively high prevalence of IBR in Australia, Europe, New Zealand and the USA possibly reflects the intensive nature of their cattle enterprises (25).

Respiratory diseases are often cited as the most significant cause of feedlot loss, perhaps even more costly than all other problems combined (109,121). However, losses due to IBR are rarely quoted separately since only a small proportion of cattle infected with IBR die of "secondary bacterial pneumonia" (227,231). In one survey of 15 incidents in fattening units carried out in Scotland (233) it was found that the average cost was £5600/farm (or £36/animal at risk). This proved to be six times higher than average losses on dairy units (£800/farm, £6/animal at risk).

Regular seasonal appearances of IBR have been recorded, usually in autumn and winter (53,150,190). On the other hand, some outbreaks have also been described as a summer event (141).

In fact, it is not clear whether seasonal trends reveal any true seasonal prevalence or merely the effects of different management practices at different times of the year; it has been suggested that such practices may exert "stress factors" that induce re-excretion of virus from latent carriers (138).

It would seem that certain of the views that exist in veterinary epidemiology are simply extrapolations of what has been demonstrated in relation to respiratory diseases of humans (14,35,59,63,127,223). Thus, while no serious studies would appear to have been instigated into the relationships, if any, between IBR and weather, various reports have suggested that temperature fluctuations are of major epidemiological importance (90,216).

In experimental studies, results have been inconsistent with some workers (110) claiming that no relationship exists between temperature and humidity and the response of the bovine respiratory tract to aerosols containing BHV1, while others have at least shown better survival of BHV1 at low humidity (35). Still others have claimed BHV1 survives best at low temperatures and high humidity (66).

In short, it would seem that the situation in cattle with BHV1 infection is rather like that in humans where "we do not yet understand how seasonal and other factors affect the incidence of colds and influenza" (14).

Numerous outbreaks of IBR have involved closely assembled cattle of different ages in intensive systems. It is generally agreed that transmission of BHV1 under natural circumstances is by close contact between susceptible animals and the infected ocular/nasal discharges from sick animals (25). Transmission by genito-muzzle contact has also been reported among cattle (250). However, IPV/IBP has been confirmed as being normally transmitted by coitus (168). Experimentally, transmission has been carried out by various routes, with various ages of cattle (16,47,82,118,208).

Generally, the disease starts in feedlots after a new batch of cattle have been brought in. Such cattle may have been sub-clinically infected, underlying re-excretion of latent virus or else convalescent. The disease then spreads slowly within the group from two to five weeks until all adjacent pens become infected (166).

Since IBR was first reported in the USA in the 1950's (190), it has been recorded as occurring in all ages of cattle,albeit with variation in the severity of the disease (25). The virus has been isolated from a one-week-old calf (53,179) a ten-day-old calf (123), and in a calf of three weeks of age (179).

Experimentally, calves ranging from two to 18 weeks of age have been found to be susceptible (156). Experimentally-induced (156) and naturally occurring IBR (123) have proved fatal in newborn calves. Moreover, the pathological lesions and clinical signs have been seen to be severe in very young animals, both experimentally

(16,156) and naturally (123). On the other hand differences in age susceptibility to IBR virus infection have also been demonstrated (223).

Apparently such variations may have been due to an age-related difference in the capacity to initiate cell-mediated immune responses which, it has been suggested, are mainly responsible for the recovery from BHV1 infection (187). Similarly, it has been suggested that maternal-acquired antibody in calves that have suckled immune dams may well change the age distribution of infected cattle (124).

Infectious bovine rhinotracheitis may affect both female and male cattle. In most field outbreaks it has been noted that both sexes were affected under the same conditions (8,61,150,184,230,231). However, in a few instances it has been reported as occurring in only one sex of cattle (71,115,123). Both sexes have been found to be equally susceptible to experimental exposure (16,157). In summary, it can be said that there is no indication that preferential sex susceptibility exists (138).

Variations in type susceptibility very probably reflects variation in local management and types. Accumulated evidence reveals that all types of cattle are probably of equal susceptibility (75,160,138).

Most outbreaks of IBR have occurred in intensive systems where groups of mixed breeds of cattle have been housed together (8,190,230,231). Moreover, several different dairy breeds such as Jersey (223), Holstein

(71) and Holstein-Friesian (123) have been noted as exhibiting classic clinical and pathological features of IBR as have Belgian Blue and White cattle (129). Other beef breeds such as Aberdeen Angus (61) and Hereford (141,184) have also been found to be similarly affected.

It can therefore be concluded that susceptibility to IBR virus does not differ between different breeds of cattle.

In most natural outbreaks of IBR the morbidity rate has been very much higher than the mortality rate, although substantial variability can occur (138,227). The morbidity rate has been recorded as ranging from 20-100% in severe outbreaks (25,202). On the other hand, the mortality rate is usually considerably lower than 10% and often nil (53,138).

The disease is not highly fatal but both mortality and morbidity have been generally found to be higher in feedlots than in dairy cattle probably because of the close contact between individuals as well as the frequent introduction of susceptible animals into enzootic situations with the former type of stock (79).

The main factor in initiating outbreaks of IBR has been stated as being due to the introduction of new groups of replacement stock in feedlots (79). This had led to speculation that the virus has been introduced in latently infected animals in which it has become re-activated as a result of stress associated with transport and adjustment to feedlot conditions (113). Latent IBR infections have been demonstrated in cattle after

clinical recovery from the disease. Such infections have been activated in convalescent or recovered cattle by the administration of corticosteroid or adreno-corticotropic hormone (ACTH) (79,154). It has been shown that calves, vaccinated with inactivated IBR virus and later challenged with virulent virus, excreted virus when injected subsequently with ACTH (200). It is conceivable, therefore, that stress equivalent to, or greater than, that induced experimentally, may occur under natural conditions resulting in activation of latent infection and the spread of the disease (138).

The great variation in the severity of clinical signs following natural BHV1 infection appears to be largely dependent upon strain and dose of virus, age-susceptibility and environmental factors (202). In North America, where IBR is endemic in many regions, the clinical disease is usually mild in dairy cattle (53,138) and in range cattle (46,142). A more severe form of the disease is usually seen in feedlot cattle (106,138,150) and it may be fatal in young calves (16,123,179). In Britain, a mild form of the disease has been reported in dairy cows and in beef (range) cattle while the syndrome in fattening cattle was considered to be somewhat more severe (227). The very severe syndrome described more recently in Scotland has been shown to be due to another virus strain (227).

Examination of the literature reveals a range of incubation periods. Chow and other (47) stated that the incubation period for IBR under field conditions was

unknown but that 82% of the affected cattle in their study showed clinical signs after they had been in feedlots for 20-60 days. Kahrs (113) stated that the range was 2-6 days, depending on dose, route of infection and other factors. However, other workers (25) have indicated that most natural infection occurs from 10-20 days after new cattle are introduced.

The course of the illness is variable among individual animals (190) and, apparently, among outbreaks but is usually less than one week (230).

The clinical conditions which were described by Schroeder and Moys (190) and Miller (150), have been recognised in natural outbreaks of IBR with minor differences over the intervening years (79). The main clinical signs of IBR have been recorded as being characterised by a sudden drop in milk production, pyrexia (104-108^{o}F), tachypnoea (30-60/min), drooling of saliva, mucoid to mucopurulent nasal discharge, explosive coughing, dullness and anorexia.

The most commonly recorded presenting sign has been bilateral nasal discharge (serous initially but increasingly mucopurulent as the illness progressed). In the early stages of the disease, examination of the nostrils usually reveals an acute inflammation with ulceration of the mucous membrane. In severe cases, yellowish-brown diphtheritic lesions (plaques) are often seen. Tachypnoea develops as well as sporadic coughing, sometimes with the expulsion of tracheal cast. Halitosis and mouth-breathing have been stated as being features of

both early and late stages of the disease (53,150,230). On auscultation of the chest, the typical harsh respiratory sounds were considered to have been originating from the trachea (53).

Not uncommonly, there is also conjunctivitis with profuse lachrymation resulting in the matting of the hair on the cheeks. In severe cases of IBR associated with conjunctivitis, marked oedema of the conjunctiva and punctate haemorrhages may be found (61).

Prolonged IBR usually results in irreversible lobular pneumonia.

A large number of other syndromes have been associated with BHV1 infection. The virus responsible for infectious pustular vulvovaginitis (IPV) was isolated and identified as a herpesvirus in 1958 (118) and that of infectious balanoposthitis (IPB) in 1960 (27). Serological studies (138) carried out in 1959 had suggested a close identity between the causal agents of these two diseases and IBR in 1959 and these agents were finally confirmed as identical in 1963 (114). Signs of vaginal irritation are evident in affected females, often with vaginal lesions (plaques) and discharge; in the affected bulls erection and ejaculation prove to be painful (79) and preputial haemorrhages and discharge may occur (96).

Abortions have been widely reported as a sequel to both clinical and subclinical respiratory tract infections with BHV1 (113). Usually abortions occur between 4-7 months of gestation (79) and retention of the

placenta is a common sequel in clinically-affected individuals (53). While semen quality does not appear to be affected when contaminated by BHV1 (96) its use may cause endometritis. The use of contaminated semen has been reported to diminish conception rates (119,168) although such effects are less obvious when natural breeding is practised (168).

In Kenya and South Africa a condition called "epivag" (epididymitis/vaginitis) was first described in 1938 (58) and has recently been ascribed to BHV1 infection (117).

Conjunctivitis is a common feature in outbreaks of IBR with photophobia, lachrymation and conjunctival punctate haemorrhages and localised granular lesions (1,138,231). Keratitis is not a feature except when the problem is complicated by M.(M.)bovis infection (231); there is evidence that BHV1 infection enhances the pathogenicity of this bacterium (174).

Certain strains of BHV1 have been shown to be capable of causing encephalitis in young calves (76). The causal agent of IBR has also been suggested as being capable of causing mastitis (181), diarrhoea in young calves (53,123,129,179) and chronic dermatitis, in one instance, of the perineal areas of bulls (36).

Infectious bovine rhinotracheitis is not normally a fatal disease, but the lesions may intensify as the disease progresses and/or the animal's condition deteriorates as the result of secondary bacterial complications. The severity of the disease has been

suggested to be dependent on age-susceptibility, the strain of virus and environmental factors (202).

The gross findings of IBR may be summarised as acute necrotic rhinotracheitis (206) or necrotising rhinitis, pharyngitis and laryngotracheo-bronchitis (8).

In early and mild cases, there is a moderate degree of hyperaemia, oedema and several small ulcers with slight serous rhinitis in the nasal cavity. In more severe cases, the inflammation is widespread and intense involving the pharynx, larynx and trachea. The exudate may then become copious and increasingly catarrhal in nature; later, it may become fibrinous and adherent to the wall of the nasal passages, accumulating on the larynx and the trachea, forming a yellowish-brown pseudo-membrane of variable thickness. This pseudomembrane may partially block the nasal meati. Petechiation and echymosis have been described as commonly occurring in the frontal sinuses and trachea. In advanced cases, necrosis of the respiratory tract mucosa, which appears as a diphtheritic membrane, may occur (8,115,141,150,231). In addition, the palatine and maxillary sinuses may become partially filled with a mucopurulent exudate. Obstruction of the larynx was frequently seen in studies on severe cases (8). The lymph nodes may become swollen, reddened and oedematous (8,138,141). In addition to the above, lesions were also described by Miller (150) which included disseminated purulent pneumonia, alveolar emphysema, minute caseation and necrosis in the sinuses. Finally

acute inflammation and frequent deep ulceration of the mucosa of the abomasum, severe enteritis in the small intestine, mild inflammation in large intestine have been reported (150,190).

Microscopically, acute catarrhal inflammation has been described with excessive amounts of mucus, and haemorrhages present on the epithelium of the upper respiratory tract of early or mildly affected cases. The mucosa may become oedematous and infiltrated with dense fibrinous exudate containing neutrophils, while the submucosa becomes infiltrated with lymphocytes, macrophages and plasma cells. In more advanced and severe cases, the epithelium has been found to be undergoing degeneration and desquamation (8,138).

In most fatal cases severe bronchopneumonia occurs (8,123). The bronchioli become filled with purulent exudate, while the peribronchiolar alveoli may be found to be oedematous, containing fibrin, leukocytes, and erythrocytes. Lymph nodes draining the inflamed areas usually exhibit oedema, hyperaemia and diffuse lymphoid hyperplasia (138). Eosinophilic (Cowdry type A) inclusion bodies may be identified in infected cells (42) and in islands of intact epithelial cells (184). However, infections with certain isolates appear to lack this feature (143,235).

Since IBR has come to be considered as a highly important disease which causes economic loss in cattle operations (138), laborious efforts resulted in a number of modified live IBR virus vaccines being developed

relatively soon after the disease was first described. However, many such vaccines were found to be causing abortions when used in pregnant cattle and consequently their usage was later restricted to feedlot cattle. Later, inactivated IBR virus vaccines were introduced albeit with somewhat disappointing results (189). More recently, an intranasal, temperature-specific, live virus vaccine has been produced which only replicates in the cooler upper respiratory tract and is therefore incapable of producing clinical disease (236). This vaccine is known to initially stimulate interferon production and then both systemic and local cell-mediated immunity and antibody.

The extensive use of modified live virus vaccines has reduced the frequency of IBR, but reports have mentioned the failure of certain vaccines to prevent IBR-induced respiratory tract disease and conjunctivitis in cattle (52). The intranasal vaccine has resulted in the shedding of virus from vaccinated animals and subsequent transmission of BHV1 to non-vaccinated animals (139); corticosteroid treatment of animals vaccinated with modified live vaccine has also resulted in reactivation of latent virus infection (236). Reactivation and shedding has been found following Dictyocaulus viviparus infection in animals that had recovered from IBR (155). Similarly, 3-methylindole has been shown to reactivate and reexcrete IBR virus in the nasal mucus (67).

Finally, a subunit vaccine consisting of viral protein has been claimed to show great promise (132).

IBR can be diagnosed by the history of the disease, onset pattern, clinical signs and course of the disease in endemic areas. In the case of new outbreaks, IBR can be confirmed from laboratory diagnostic findings such as virus isolation, serology, histopathology, electron microscopy, skin reactions to viral antigen and direct immunofluorescence (25).

The differential diagnosis should be considered carefully because of similarities and inter-related symptoms with other diseases. However, pneumonic pasteurellosis and parainfluenza 3 (PI3) infection have been given special significance in the diagnosis of IBR by one worker (113).

- fungal infections of the upper bovine respiratory tract

While note has already been made of the large number of viral, mycoplasmal and bacterial infections that may be found in the upper respiratory tracts of cattle - usually it would seem unassociated, at least in terms of mycoplasmas and bacteria, with clinical disease - the question as to whether or not a similar situation exists with regard to fungal infections appears to have been largely unexplored. However, two specific fungal infections have been described, maduromycosis and rhinosporidiosis, both of which induce proliferative rhinitis. Each of these conditions will now be briefly dealt with.

Maduromycosis has been defined as a chronic proliferative rhinitis of cattle, characterised by a variable degree of stertor. Bilateral nodular growths may

be found in the nasal cavities which do not induce distortion of the turbinate cartilage (182). The condition is accepted as being caused by the fungus Hilminthosporium sp. (33,60,182) although other fungi have also been incriminated as causative agents.

Clinical signs initially include sneezing and mucopurulent nasal discharge; later, as the problem progresses, dyspnoea arises as the result of the large nodules obstructing the nasal cavities. Loud snoring sounds (stertor) may be heard from a distance (182). Large, raised tumourous nodules may be found on both sides of the nasal septum which, if they become sufficiently large, often may obstruct nasal respiration.

The histological appearance of these lesions is of a very fibrous connective tissue containing an intense eosinophilic infiltration, with some foreign body (giant) and mononuclear cells (33,60,182). This marked eosinophilic infiltration has been a feature of all reported cases, thus suggesting an associated allergic reaction (33,60,182).

Surgery has been the only recommended method of management to date, although it would appear to be unsuccessful relatively frequently (182).

Rhinosporidiosis is a chronic, non-contagious and non-fatal infection of the nasal cavities characterised by the development of inflammatory granulomatous masses which eventually become so large as to eventually result in mouth breathing and stertor.

The disease was reported first in man (130),

later horses, cattle, mules, dogs, goats, geese and ducks. The infection has been reported as a sporadic problem from U.S.A, South America, South Africa and Australia (25) while it is endemic in Argentina, India and Sri Lanka (112). In general the disease in man is more severe than in animals; moreover, disseminated lesions have only been reported in man (112).

The infection is caused by a fungus, Rhinosporidium seeberi. Recently it has been suggested that the organism might better be classified as an algea (221). The organism has so far resisted attempts at in vitro cultivation. The mode of transmission and source of the infection is unknown (221). The disease in man has been associated with swimming and working in stagnant water, suggesting that water is the organisms' natural habitat (221).

- parasitic infections of the upper bovine respiratory tract

Although the cattle lungworm, D.viviparus, is commonly found in the trachea of heavily infested cattle, this parasite is best viewed, given the nature and pathogenesis of the disease, a parasitic pneumonia and bronchitis. It will therefore not be discussed under this heading but rather in the section that briefly deals with the cattle pneumonias. However, one other helminth infection, that which involves the parasite, Schistosoma nasalis, will be described below.

Nasal schistosomiasis is defined as a chronic infection of the nasal cavities of cattle, characterised by mucopurulent nasal discharge and obstruction, leading

to respiratory stertor. The disease is caused by the parasite Schistosoma nasalis (57,176). Experimental infection with this parasite has reproduced a syndrome identical to that seen in the naturally occurring disease (177,178). Animals other than cattle have been reported infected, and the condition is endemic in India, Burma and Sri Lanka (12,144). The initial presenting signs are characterised by bilateral nasal discharge associated with mouth breathing and, less occasionally, sneezing. At later stages mucopurulent nasal discharge, marked stertor and dyspnoea develop (37).

The lesions are localised in the anterior third of the nasal cavities and are usually bilateral. The presence of the parasites and their eggs cause marked irritation and the development of often large, multiple granulomatous lesions. Microscopically, the mucous membrane is seen to be oedematous, and the nodules contain tiny abscesses which contain the parasitic eggs (38,39).

Although similar in many ways to other forms of proliferative rhinitis, bovine nasal schistosomiasis may be differentiated on the basis of its characteristic granulomatous reaction, the very marked eosinophilic infiltration that arises and, most particularly, the presence of schistosome eggs within the lesions.

Treatment with tartar emetic, antimosan and anthiomaline is said to be effective, though not without risk (57,146).

Acquired problems of the upper tract: Abscessation

Abscesses may, of course, occur anywhere in the upper respiratory tract and localised infections or abscesses outwith the tract may exert pressures that may result in an occluded lumen under certain circumstances. Perhaps the best example of this situation is when retro-pharyngeal lymph nodes become abscessed or, for that matter, infected and enlarged as the result of specific infections such as A.lignieresii or M.tuberculosis. However, there is one condition which appears to be a specific disease entity although its aetiology is still to some extent in doubt. That syndrome is laryngeal abscessation or laryngeal necrosis which very commonly is found in association with so-called calf diphtheria. This condition will now be reviewed in detail.

Laryngeal abscess (often also called laryngeal necrosis) has been described as an acute or chronic infectious, non-contagious, disease characterised clinically by fever, dyspnoea and stertor, and pathologically by necrosis of the mucous membranes over the arytenoid cartilages (107). It has been said to be a highly fatal infection that involves the throat and the upper respiratory tract in feeder cattle (108) although, at least under British conditions, cases are usually culled because of the irreversible nature of the problem rather than die.

The syndrome was first reported in Germany in 1877 in young dairy cattle (98). Later, other workers recorded having observed a similar problem. Soon after,

Loeffler in 1884 (209) isolated a bacterium which he named Bacillus necrophorus. A similar organism was found in various lesions in man over the following years and was given the name Bacterium fundiforme (209). It is now known as Fusobacterium necrophorum but had been previously known by a variety of other names including Actinomyces necrophorus, Bacteroides necrophorus, Spherophorus necrophorus and Fusiformis necrophorus (209). The same organism has been identified as the cause of necrotic stomatitis so for reasons of simplification, the respiratory syndrome has been termed "laryngeal diphtheria" (107) although this name was soon changed to "laryngeal necrobacillosis" by other workers (25). The name calf diphtheria was stated as being misleading because the disease had been encountered in two-year-old cattle (69).

A bacterium "Fusobacterium necrophorum" has been regularly isolated from clinical laryngeal infection or necrosis (2,68,120). This has been considered the main constant causative agent. However, other bacteria have also been isolated, either with F.necrophorum or alone, and they, too, were sometimes considered to be causing necrotic laryngitis or laryngeal abscess. In one report (44) A.pyogenes was the only isolate from such cases whereas in another (125) both F.necrophorum and A.pyogenes were found. Fischer (72) claimed to have isolated P.haemolytica, P.aeruginosa, E.coli and A.pyogenes but never F.necrophorum. Fusobacterium necrophorum has also frequently been associated with

liver abscesses and foot rot in cattle as well as in sheep (84). Experimental attempts to induce laryngeal lesions with the organism have not been successful (73) and this lack of success, even with the presence of the bacteria in the lesion, has suggested the possibility of an additional or alternative aetiological agent presently unknown (73). It has been suggested by one worker (Selman - unpublished observations) that calf diphtheria and laryngeal abscessation and/or necrosis are the result of F.necrophorum infection superimposed upon lesions of bovine papular stomatitis (BPS).

The bacterium F.necrophorum is anaerobic and grows well at 37°C in cooked meat medium. It is present commonly in animal environment. It has the ability to produce soluble exotoxin and a necrotising endotoxin in its host tissue (209).

Laryngeal abscessation has been reported from many countries. However, it is most prevalent in areas where animals are housed during the cold season (25,108) although it has also been observed in running cattle on the pastures (91,131,212). It has been frequently reported in Australia (212), Belgium (152), Britain (188) and the U.S.A. (108).

Calf diphtheria and laryngeal abscessation/ necrosis appear sporadically in a cattle population and may cause heavy economic loss if left untreated. Various surveys have been carried out especially in the U.S.A. which are reported here. Jensen and MacKey (108) have presented numerical data about the disease in its early

existence. The incidence of calf diphtheria was 0.17% (total 55,000) in 1955 but had become 0.5% (total 77,000) in 1960. Furthermore, 114 (6%) of 1988 animals had lesions of laryngeal diphtheria (109).

This infection is one of "a triumvirate" of respiratory diseases of feedlot cattle (the other two being IBR and shipping fever) which cause extensive economic loss through mortality, loss of physical condition, inefficient feed utilisation and expensive treatment (2).

Like IBR and shipping fever, calf diphtheria and laryngeal abscessation occur mainly in the late autumn and winter in feedlot cattle (2,91,109) but no indication of climatic involvement in the spread or occurrence of the disease has been reported to date.

It has been stated that the licking of nasal and/or mouth secretions of an affected animal by a healthy one can lead to the establishment of the infection (98). Recent theories emphasise the contamination of milk buckets and other feeding utensils as vectors of spread of the disease since the causative agent is a common inhabitant of the animal environment (25). As stated earlier, experimental infection has, so far, proved unsuccessful (98).

There is no satisfactory explanation for factors predisposing to the disease. Hungerford (97) has claimed that filthy conditions and injuries from rough feeds, thorns, etc. as well as vitamin A deficiency are predisposing factors. Others (108) have stated that

predisposing factors to calf diphtheria are not known. It is hypothesised that a contact ulcer is caused by repeated closure of the larynx. This may facilitate the entering of the organism to the larynx (109). Note has already been made of the possibility of lesions arising from earlier erosions of BPS.

Laryngeal necrosis is most common in cattle 1-2 years of age (108), and particularly in yearling feeders. However, a single case has been reported in a nine-year-old animal (44).

It has been stated that both sexes may equally contract the infection (97,108,219) and both dairy and beef cattle may succumb. An outbreak of calf diphtheria in nearly 3000 calves and feeder cattle has been reported (91). All breeds are susceptible to laryngeal necrosis (108) and it has been reported in Aberdeen Angus crosses, Charolais, Friesians, Herefords and Holsteins (68,109, 125,188).

It has been stated that morbidity rate ranges from 0.2 to 0.4%, with case fatality rates ranging from 15-20% (108). Clinical studies in the U.S.A. involving 2,785 animals revealed a morbidity rate of 66% (91). In one earlier report (69) that is prior to the time of antibiotic usage, the case fatality rate was reckoned to be around 90%.

The course of calf diphtheria was stated by Jensen and MacKey (108) to last from 7-10 days, and from 2-3 months in more chronic cases of laryngeal abscessation.

The disease starts with sudden onset, increase in temperature up to 105°F (106°F), dyspnoea and decline in body condition. Acute dyspnoea was prominent in all clinical cases of severe acute calf diphtheria with laryngeal complications (44,69,125,188,210). Other symptoms reported have been inappetence, coughing and noisy respiration. In severe cases, salivation, mouth breathing, neck and head extending have been noticed. Also, a mucopurulent nasal discharge has been observed (98).

Bronchopneumonia and pulmonary abscesses may develop and chronic cases often develop ruminal tympany according to one group of workers (108). Death is likely to occur from toxaemia or obstruction to the respiratory tract between the second to seventh days (25).

The lesions would appear to start with an acute mucositis throughout the larynx, pharynx and nose caused by pen dust, pasteurellosis, mycoplasmas, haemophiluses, and viruses. This is followed by bilateral erosions of the inflamed mucosa over the rima glottidis, especially the vocal processes and medial angles of the arytenoid cartilage. Progressive colonisation of the erosions and invasion of deep tissues by the causative organism then ensues (108). Udall (219) indicated that stomatitis and pneumonia may accompany necrotic laryngitis. The disease has been classified into acute and chronic types (109). In the acute form, necrosis and accumulated exudate may be seen on the mucous membranes over the vocal processes and medial angles of arytenoid cartilages. The infection

may also spread into the vocal cords and cricoarytenoidus dorsalis muscles. In chronic cases, lesions are located in the same areas as in the acute condition, are frequently bilateral and are most commonly localized over the vocal processes. The lesions consisted of necrotic cartilage surrounded by purulent exudate, a tract extending from the infection to a mucosal surface with granulation tissue projecting from the tract orifice into the airways. Regional lymph nodes may be swollen and hyperaemic (108). Papillomatosis has been reported in association with chronic diphtheria (109).

Microscopically, colonies of F.necrophorum may be seen in necrotic tissues usually with little evidence of other species of bacteria. Exudate and granulation tissues are abundant.

The disease is said by some workers to respond well to antibacterial drugs, such as sulphapyridine (69,91). Local treatments have involved tracheal intubation, particularly when dyspnoea is acute (68). Currently, broad-spectrum antibiotics are used to combat the infection initially and some workers (108) also recommend the use of dexamethazone and a topical spray of proteolytic enzymes. Surgical treatment of laryngeal necrosis has been claimed to be successful. Dutch workers (152) have performed arytenoidectomy and created a permanent tracheal fistula in the anterior cervical part of the trachea following which 21 out of 30 cases healed completely. Surgery was also reported by Fischer (72) who carried out laryngofissure to relieve dyspnoea and

laryngeal stertor.

Due to inadequate understanding of the cause of calf diphtheria and absence of positive predisposing factors, it has been stated (108) that preventive measures cannot possibly be developed. However, other workers (25) have emphasised the value of proper hygienic precautions in calf pens or feeding and drinking places with avoidance of rough feed.

Acquired problems of the upper tract: Allergy

Three specific infections (maduromycosis, rhinosporidiosis and nasal shistosomiasis) of the nasal passages of cattle have already been described. Each produces granulomatous lesions or polyps within the nasal passages. Not surprisingly before these conditions were properly defined, when the generic term "bovine nasal granulomatosis" was generally applied, considerable confusion and controversy arose. In this section, another cause of nasal granulomatosis - the only defined allergic disorder of the bovine upper respiratory tract - will be discussed in detail; that condition is bovine atopic rhinitis.

- bovine atopic rhinitis

The form of bovine nasal granuloma that is now known as atopic rhinitis has been defined as a non-infectious disease of cattle caused by an unknown allergen or allergens in the environment. It is characterised clinically by loud nasal sounds, thick mucopurulent nasal discharge and nasal pruritis, and

pathologically by small multiple nasal granulomata.

Bovine nasal granuloma was first recognised in Australia 50 years ago (85). The disease was called the dairy-cattle type of nasal granuloma and referred to by laymen as "snoring" or "snuffling" disease. In New Zealand it was termed summer catarrh (137,223), Jersey catarrh (77), and chronic granular rhinitis (54,77). The condition has been likened to "hay fever" following successful reproduction of the disease (170). On the basis of epidemiology and clinical features it has also been called "summer sniff", summer sniffle, summer snuffles and enzootic nasal granuloma (25). Since the problem was eventually defined experimentally as an allergic phenomenon (170) the term "bovine atopic rhinitis" appears to be the most appropriate.

Although allergic in origin, specific allergens have not, as yet, been identified (169). Recent studies have led to the suggestion that fungal spores, or other aerogenous particles, produced by microorganisms in pastures, are the major allergens involved (39). Certainly, no microorganisms have been consistently found in association with clinical field cases of bovine nasal granuloma in Britain (229). Nevertheless, as already stated, it has been confirmed that the disease is allergic in origin following the successful reproduction of the disease by repeated acute episodes of immediate (type 1) hypersensitivity reactions (170). The experiment in which ovalbumen and incomplete adjuvant was used over a period of several months resulted in clinical and

pathological signs similar to field cases of bovine nasal granuloma.

The disease has been repeatedly reported from Australia, Fiji and New Zealand (39). In Britain it was first reported under the name chronic nasal catarrh (225). Recently, in Scotland, a typical case of bovine nasal granuloma was described as typical of a number of cases in a problem herd (229).

Because atopic rhinitis is neither infectious nor fatal, its incidence seems to have been largely neglected. In Australia where it is common, it has been found that 22.1% of the 8,077 cows have shown lesions (77), whereas in New Zealand, where it is also prevalent, 3.6% of 1000 cattle had lesions (95). However, the consequences of the disease are rarely considered important, especially as most of them do not usually lose condition or milk. The economic impact has been attributed mainly to culling, and occasionally to reduction in milk production and loss of condition (162).

General reports have indicated that seasonal appearance is a characteristic of bovine nasal granuloma. It has been observed as being particularly common in areas with high summer rain fall (95). In natural clinical cases, the problem increases in intensity as the summer progresses and then goes into a stage of remission in the winter (162,225,229). Histological studies in clinically normal Jersey cattle has revealed that the nasal mucosa has shown "greater reactivity" in summer (169).

No specific climatic factors have been identified save that the problem occurs in warm, humid conditions where grass and meadow flowers are in bloom (6,39,162).

In its early occurrence in Australia atopic rhinitis was rarely seen in animals aged less than nine months (162). although, experimentally, the syndrome has been produced in a five-month-old heifer. In general, field cases are usually over two years of age (171). In Scotland, the syndrome was diagnosed in Friesian cows between the ages of one and four years (229). Above all, it has been concluded that animals between six months and four years are most commonly affected (25). Both sexes have been reported to be susceptible (25).

Atopic rhinitis has been shown to affect all types of cattle. In New Zealand young beef cattle have shown lesions (77) while in Australia dairy heifers and older dairy cows have often been reported as being susceptible (95). Long before these latter reports, two British studies (225), carried out in 1927 and 1937 mentioned its occurrence in dairy cows.

One extensive survey of dairying in Australia revealed that 30% of Jersey and Guernsey cattle and 10% of Friesian, Shorthorn and Ayrshire cattle, were affected while only small numbers of other breeds were affected (95). In another survey, 36.3% of Jersey cows were found to have lesions (77). One group of workers has concluded that the Channel Island breeds (Jerseys and Guernseys) are most susceptible to the disease (95).

Hore and others (95) surveyed 8,077 cows in Australia, and found 22.1% to have lesions although the incidence varied from 3.7-48.1% according to rainfall rate in different areas. Similarly in New Zealand, a minimum of 40% of farms and 36.3% of culled cattle were reported to have been affected whereas only 3.6 of young beef cattle showed bovine nasal granuloma (77). Also clinical cases were found in 50% of the herds studied.

Although the respiratory distress may be sufficiently severe to cause loss of condition and reduction in milk yield, affected animals do not usually die (25). However, a report by one worker (162) stated that extension of secondary nasal infection to the para-nasal sinuses and thence to the brain may cause death in occasional cases.

Since certain breeds may be more susceptible, especially Jersey, to nasal granuloma, "atopic rhinitis" has been suggested as having a genetic factor to predisposition (169), a feature which is shared by allergic diseases of man (51). A similar suggestion was made when nasal eosinophils were detected in some Jersey and Friesian cattle whose parents had suffered from atopic rhinitis (39).

The disease has been classified into acute and chronic (77,162) and mild and severe forms (77,229). The "incubation" period apparently varies between 1-4 weeks (162,225,229). The disease is generally mild initially and only progresses to the chronic form over a period of years with remission and exacerbations according to

season and weather. The symptoms in mild cases were serous nasal discharge (162,225) and ocular discharge (229). In acute and advanced cases fevers of up to 105°C have been reported (162) along with profuse mucopurulent nasal discharge, nasal pruritis, ulceration of the nasal mucosa, swelling of the face, obstruction of the nasal passages leading to mouth breathing and stertor, were found. Small (1-4mm), hard, white nodules in the nasal vestibules were present in most clinical cases. Other reported signs have included slight tachypnoea (40/min.), moderate hyperpnoea (229), epistaxis (162,229) and halitosis (162). Another worker (85) had stated that there was distortion and/or atrophy of the turbinate together with a mucoid exudate in the maxillary sinuses. The rubbing of the nose by cattle against walls and occasionally sticking their heads into bushes has been described by some farmers (162,229).

Macroscopically, the lesions are confined to the anterior third of the nasal passages or vestibules (6,39,85,169) and variations in their appearance are considered to reflect the severity of the disease (6,39). Generally, the mucosal lesions are bilateral and, frequently, symmetrical (6,85) with a tenacious mucopurulent exudate overlying the mucosa. Most commonly, the lesions consist of multiple nodular mucosal elevations of various sizes covered by intact epithelium. However, recently there has been described two types of granuloma found in a clinical case of atopic rhinitis (171). Type 1 lesions were flat, fairly smooth, pale pink

in colour and had coalesced to form raised areas up to 15mm in diameter. In contrast, Type 2 lesions were discrete, firm, raised, nodular areas a few 1-3mm in height and diameter, frequently haemorrhagic and reddish-orange in colour. Such nodules were also found scattered over the mucosa of the pharynx, larynx and trachea after successful reproduction of the disease (170,171). More widespread lesions have also been seen in field cases (39) along with congestion and haemorrhage of retropharyngeal lymph nodes (6). Distortion of the ventral turbinate bone was reported in clinical cases in Australia (85,169). A rare case was reported in the USSR (39) in which there was necrotic rhinitis and atrophy of the turbinate bones.

Histological changes have consisted of squamous metaplasia and goblet cell hyperplasia of both the surface epithelium and the terminal gland ducts. These changes are frequently accompanied by dense eosinophil infiltration of the epithelium and adjacent lamina propria (169). Other changes in the lamina propria include oedema and, in addition, infiltration by eosinophils, plasma cells and lymphocytes. In well-advanced cases, nodules consist of granulomatous inflammatory tissues composed principally of newly formed muscular arterioles, fibroblasts, collagen and intermingled mast cells, plasma cells, lymphocytes and variable numbers of granulocytes. Most of these microscopical changes have also been described in experimentally-induced cases (170,171).

Since the allergen or allergens responsible for atopic rhinitis have still to be defined, preventive procedures cannot be applied. Attempts to control the disease by vaccination of cattle with formalin-treated lesion material has been tried but found to be unsuccessful (85). Culling is usually only applied in cases where significant loss of milk production or condition occurs.

There is no curative treatment for bovine nasal granuloma (atopic rhinitis) although every conceivable drug would appear to have been tried (162).

Acquired problems of the upper tract: neoplasia

- bovine nasal adenocarcinoma

The fifth condition that has been defined in cattle in which proliferative lesions arise in the nasal cavities is a specific malignant neoplasmic process - bovine nasal adenocarcinoma.

In general, neoplasmas of the nasal cavities or paranasal sinuses in cattle are rare. However, ethmoidal carcinomas or adenocarcinomas have been reported as occurring in cattle from Brazil, The Dominican Republic, Hong Kong, Portugal, Scandinavia and South Africa (172). Nasal adenocarcinoma has been described as a specific, "clustering", cancer of high-yielding dairy cattle of Dutch extraction in Brazil (158,153,172).

Successful transmissions of adenocarcinoma using cell-free material has been carried out at least in sheep with resulting tumour formation in the ethmoidal sinuses

(48). The pathological appearance of the resulting tumours closely resembled the tumour cells of human nasopharyngeal carcinomas which are associated with the Epstein-Bar virus thus suggesting that these tumours may be of viral origin (48,153).

Initially, cases of bovine nasal adenocarcinoma have presented with lachrymation and a mucous nasal discharge (158). Later, respiratory sounds emanating from the nasal cavities have been a marked feature of the disease along with sudden episodes of respiratory distress (usually when eating), nasal discharge, epistaxis and halitosis (158). At an advanced stage, marked loss of condition and dyspnoea have been noted with death occurring some ten to 90 days after the condition was first noticed.

In one report (158), the lesions were said to be confined to the cranial ethmoidal sinus, the turbinates and the nasal septum. In another, later, report (172), the lesions were described as being soft and tumourous, limited to the ethmoidal sinus and accompanied by marked secondary inflammatory changes and necrosis.

On histological examination, most of the tumours that have been examined have been of the mucous type (172). Retention of mucus has led to dilatation of the glands, the cells of the glandular epithelium have increased in size and in extreme cases, the tumours have been found to form a mucinous mass. In the serous type of tumour, the glandular lumina has been seen to contain proteinacious material but no mucus (211).

The prognosis for such cases would appear to be hopeless.

CHAPTER 2

GENERAL MATERIALS AND METHODS

CHAPTER 2. GENERAL MATERIALS AND METHODS

Animals

Cattle of various ages, breeds and types were admitted to the Department of Veterinary Medicine of the University of Glasgow Veterinary School either for experimental purposes or for the purposes of clinical teaching. A detailed description of them and their management following admission is given in each of the chapters dealing with specific investigations.

Clinical examinations

Every animal that was admitted for experimental studies was considered to be clinically normal at the time of admission and had no history of significant clinical disease prior to purchase. Additional screening procedures are described in their appropriate chapters. The cattle that formed the basis of the studies carried out in Chapter 6 of this study were admitted for clinical teaching purposes and were therefore affected with a variety of respiratory disease prior to, at and following admission. Clinical examinations were not limited to the respiratory system although only findings relevant to that system are cited in the following studies. In fact, none of the cattle that were used were suffering from other significant clinical problems.

Clinical terms were as advocated elsewhere (197, 198). Briefly, clinical definitions were as follows:

- presenting signs

obvious abnormalities clearly visible on arrival.

- demeanour

  limited to "dull" or "bright.

- appetite

  limited to stockman's assessment except in the study described in Chapter 3 where group intake was measured as Kg (concentrate) ingested/50 kg of calf to allow for sequential slaughterings.

- fever

  rectal temperature in excess of 102^{o}F or as otherwise stated.

- tachypnoea

  a respiratory rate in excess of 30/minute.

- hyperpnoea

  deeper-than-normal breathing.

- dyspnoea

  difficulty in breathing.

- auscultation (respiratory sounds)

  the boundaries for auscultation were lines drawn from the posterior angle of the scapula to the point of the elbow and from here to the uppermost (palpable) part of the second-last rib and over the chest wall medial to the elbow. Sounds heard during the respiration cycle were described as either soft, harsh or very harsh.

- auscultation (adventitious sounds)

  additional sounds, usually heard at the end of inspiration were described as either "crackles" or "squeaks".

- percussion

  this was carried out over the area denoted under "auscultation" and changes in tone were noted along with whether pain was evident or coughing occurred during, or after, the procedure.

- other findings such as nasal discharge, cough etc. were described as they occurred in terms of degree, frequency etc.

Pathological examination

Pathological examinations were carried out by Dr. E.M. Allan, initially of the Department of Veterinary Pathology, latterly of the Department of Veterinary Medicine of the University of Glasgow Veterinary School. Procedures were as described elsewhere (4).

Sampling procedures

- blood samples

  These were collected by puncture of the jugular vein in all cases.

- nasopharyngeal swabs

  These were obtained from Exogen Ltd., Clydebank Industrial Estate, Beardmore Street, Clydebank, G81 4SA, Scotland) and consisted of a 30cm nylon stem, a cottonwool bud and a handle which acted as stopper of the tube in which the swab rested prior to, and after, use. Swabbing frequencies varied according to the needs of each. Study and respective frequencies are cited for each particular study.

- tissues

After macroscopic examination, samples from the respiratory tract were removed aseptically into 1.8ml sterile phosphate-buffered saline. The standard sampling sites varied from one study to the next but basically the following sites were examined:

(a) cranial nasal conchus (NCa)

(b) caudal nasal conchus (NCp)

(c) nasopharynx (NP)

(d) larynx (L)

(e) upper trachea (TRU)

(f) lower trachea (TRL)

(g) right cranial bronchus (RCB)

(h) right middle bronchus (RMB)

(i) right caudal bronchus (RCB)

(j) right cranial lobe (RC)

Microbiological procedures (nasopharyngeal swabs)

- mycoplasmas

Swabs were inserted for their full length into the nasal cavity, rotated several times, then withdrawn. The tip of the swab was then snipped off into mycoplasma transport medium. The sample was then stored at -70^{o}C until all samples could be examined together.

Culture and isolation procedures followed the steps outlined below:

(a) Three bottles were set up each of which contained 1 ml of glucose-serum broth.

(b) the nasopharyngeal swab was placed in the first bottle.

(c) the culture was then incubated at 37°C for 30 minutes.

(d) the second and third bottles were filled by a process of sequential double dilution.

(e) both the first and the second diluted samples were cultured on a glucose serum agar plate and incubated for 3-4 days in a 5% CO_2 chamber.

(f) the plates were regularly examined microscopically for the presence of mycoplasmas and circled with a marker if positive.

Identification was carried out as follows:

(a) mycoplasma-containing sections of agar were cut from the plate.

(b) four squares from each sample were placed in a large petri dish and secured by waxing.

(c) one drop of antisera against A.laidlawii, M.bovirhinis, M.bovis and M.dispar were placed on the square and left for 30 minutes.

(d) washing was then carried out with phosphate buffered saline, twice, in ten minutes.

(e) one drop of fluorescein isothiocyanate (FITC) was placed on all squares.

(f) washing as in (d) was repeated.

(g) mycoplasmas were then identified using a fluorescent microscope.

- bacteria

A separate nasopharyngeal swab was used for bacteriology. The swabs were applied as described above. On occasions, bacteriology was carried out immediately; on others the swab was stored in Amies transport medium with charcoal (Exogen, Vi-Pak Transport Swab System) and stored at -70°C for later examination.

Culture and isolation procedures were as outlined below:

(a) the swabs were plated on to 5% sheep blood agar and incubated aerobically at 37°C

(b) plates were examined after 24 and 48 hours and the colony numbers together with their cultural and morphological characteristics were recorded

(c) colonies were then purified on either sheep blood agar or McConkey agar for 24 hours at 37°C and findings were again noted.

Bacteria were identified on a "best-bet" basis by the first-stage procedure based on their individual biochemical reactions (Appendix 1,1). Thereafter, further biochemical tests were carried out for final identification (Appendix 1,2-3). Pure colonies of non-enteric Gram -ve oxidase positive rods were identified by the API Zone System; enterobacteria were identified by the API 20E System.

Identification of Pasteurella organisms

Antisera raised in rabbits were used to serotype P.haemolytica A1 and A2. The IHA method described by

(204) and modified by (203) was used to serotype the P.haemolytica isolates. The unknown serotypes and the positive control P.haemolytica A1 and A2 were grown overnight at 37°C in trypticase Soya broth (TSB, Oxoid Ltd., Hants, England). The broth cultures were heated at 56°C for 30 minutes to kill any viable organisms. Gluteraldehyde-fixed ox (bovine) red blood cells which had been prepared as described below, were added to the killed bacteria to the concentration of 0.5% and incubated at 37°C for 30 minutes to sensitise the bacteria. Each sensitized culture was then washed three times in 0.3 per cent neutral formalin in phosphate buffered saline (FPBS) at PH 7.0 to remove excess antigen, then made up to the original volume 0.025ml of the washed sensitized bacteria (antigen) was added to an equal volume of P.haemolytica serotype A1 or A2 antisera in round-bottomed microtitre plates (Flow Laboratories, Ayrshire, Scotland) using volume adjustable pipettes (Pipetman, Gilson, France) and allowed to stand at room temperature for two hours. Agglutination occurred when an even mat of red blood cells was formed over the bottom of the well in the microtitre plate. A negative reaction was considered to have been produced when a small button of red blood cells formed in the centre of the well.

Gluteraldehyde Fixation of bovine red blood cells (203)

Five ml of bovine blood was collected from the jugular vein into a universal bottle containing 0.76g (3.8% weight/volume) of sodium citrate as an

anticoagulant. The blood cells were washed twice in phosphate buffered saline (PBS) and resuspended in PBS to a 20% volume/volume concentration. The RBC suspension was mixed with an equal volume of gluteraldehyde (BDH Chemical Ltd.), Poole, England) which had been diluted to a 0.2% concentration in PBS. The mixture was then incubated at 37°C for 15 minutes, washed five times in 0.5% normal saline and resuspended to a final concentration of 10% volume/volume in PBS before being stored at 4°C for use within three to four weeks. Before being used, the RBC's were washed three times in FPBS and made up to a 5% concentration in FPBS before added to the killed P.haemolytica broth cultures as described above.

Microbiological procedures (tissues)

- mycoplasmas

Tissues were collected by the methods described above and stored in 1.8 ml sterile phosphate-buffered saline (PBS, ph 7.4) in sterile universal bottles. When isolations etc. were to be carried out, the samples of tissue were thawed and then incubated at 37°C in 5% CO_2 for 30 minutes. Each sample was lightly chopped and a section measuring 0.5cm^3 was processed as described above for culture and identification.

- bacteria

Tissue samples were incubated at 37°C for 30-45 minutes. A loopful of suspension was inoculated on to McConkey agar, 5% sheep blood agar and, occasionally, chocolate blood agar plates. The latter two media were

inoculated in duplicate with one of each pair of plates being incubated anaerobically at 37°C and the other aerobically at 37°C. Plates were examined after 24 and 48 hours incubation. Procedures were then as described above.

Serological procedures

Serum antibody determinations were limited to the indirect haemagglutination (IHA) test and were only carried out on the calves that formed the basis of the study reported in Chapter 5. The IHA technique for this and for the identification of P.haemolytica A1 and P.haemolytica A2 was by the technique described by Shreeve and colleagues (204).

CHAPTER 3

INFECTIONS OF THE UPPER AND LOWER RESPIRATORY TRACTS IN EXPERIMENTALLY-INDUCED PNEUMONIAS IN WEANED, CONVENTIONAL CALVES: EXPERIMENTAL VIRUS (PARAINFLUENZA TYPE 3) PNEUMONIA

CHAPTER 3. INFECTIONS OF THE UPPER AND LOWER RESPIRATORY TRACTS IN EXPERIMENTALLY-INDUCED PNEUMONIAS IN WEANED, CONVENTIONAL CALVES: EXPERIMENTAL VIRUS (PARAINFLUENZA TYPE 3) PNEUMONIA

INTRODUCTION

The infectious pneumonias of young calves represent a major source of economic loss to the cattle industry, particularly where large numbers of calves are subjected to conditions of intensive management (13,173, 232). Despite the availability of a number of vaccines against some of the agents that are considered to be of aetiological significance and a widespread belief in the importance of improved housing standards and stockmanship, it would still seem that the first - and often the only - contact that cattle practitioners have with such problems is when they are requested to control severe or troublesome clinical incidents (196).

While variations exist in the way individual veterinarians approach such incidents, antibiotic therapy usually forms the basis of that approach, administered either on an individual or a group basis. In addition, severely affected animals are often given some form of anti-inflammatory therapy which until recently usually took the form of one or more injections of a corticosteroid compound (122,149). However, the wisdom of this latter approach has been questioned since in at least one acute viral disease of cattle - infectious bovine rhinotracheitis (IBR) - there is evidence that the use of corticosteroids is commonly associated with subsequent clinical deterioration and higher mortality rates than in

cattle that have received antibiotics alone (99,227).

A number of alternative anti-inflammatory preparations are now available (207) and the following trials were designed to assess the effects of administering one such compound, the anti-prostaglandin substance flunixin meglumine to conventional weaned calves suffering from experimentally-induced virus (PI3) pneumonia. The major aim of this study was to assess whether or not the administration of flunixin meglumine reduced the inflammatory response within the lungs and thereby conferred clinical benefits. However, the opportunity was also taken to carry out a detailed study into the mycoplasmal and bacterial infections of the upper and lower tracts of the 20 calves concerned and to investigate the possibility that by reducing the extent of pulmonary inflammation in the ten calves treated with the anti-inflammatory compound, the range and/or extent of these infections might in some way be modified.

EXPERIMENT 1

MATERIALS AND METHODS

Experimental animals and their management

Ten 10-12 week-old Friesian bull calves, reared together on an *ad libitum* cold acid milk system and weaned two weeks before admission to the veterinary school, were obtained from a local dealer (Sinclair). They had been selected from a much larger group of similar animals on the basis of excellent bodily condition, lack of clinical signs of disease and reciprocal haemagglutination

inhibition (HAI) titres to PI3 virus <8.

Following admission, the calves were randomly allocated into two groups, group 1 (test calves, 1-5) and group 2 (controls, 6-10). Each group was then housed in adjacent and identical, self-contained, conventional loose-boxes. The calves were offered hay, concentrates (190 "Rosette Rearing Pencils", BOCM-Silcock) and water ad libitum.

Clinical procedures

Clinical examinations were carried out once daily (10.00) during the pre-infection period (day -14 to -1), twice daily (10.00 and 16.30) on day 0 and from day 4 until the termination of the study and four times daily (07.30, 10.00, 16.30 and 21.00) on days 1,2 and 3 (i.e. the 'treatment days').

Rectal temperatures and respiratory rates were measured on each occasion along with other information relating to demeanour, presence and degree of hyperpnoea and frequency of coughing. The concentrate intakes of each group were recorded as weight (Kg) of concentrate consumed daily by each group of calves between days -7 and 9.

The weights of the calves were assessed using a weighband (Dalton Supplies) on days -14, -7, 0, 3, 9 and 12. Clinical terms used are as defined elsewhere (197); 'fever' was defined as a rectal temperature of 102^{o}F or above unless otherwise stated and 'tachypnoea' as a respiratory rate in excess of 50 per minute.

Treatment procedures

Flunixin meglumine (Finadyne; Kirby-Warrick Animal Health, Kirby-Warrick Pharmaceuticals Ltd., Mildenhall, Bury St. Edmunds, Suffolk IP28 7AX) was administered to the test group intravenously at the manufacturer's recommended rate (for cattle) of 2.2 mg/kg liveweight at 11.00 on days 1, 2 and 3. No other drugs were administered to either of the groups.

Sampling procedures

Nasopharyngeal swabs, which were taken from all calves on days -14, -7, 0, 4 and 9, were examined by conventional methods for viruses, mycoplasmas and bacteria. The latter two procedures were carried out immediately whereas swabs for virological examination were stored in virus transport medium at -70^{o}C for examination at a later date. Blood samples were collected for serological examination before admission, day -14, day 0 and immediately before slaughter.

Microbiological procedures

Nasopharyngeal swabs and respiratory tract tissues were examined for mycoplasmas and bacteria by methods previously described (4). Samples were examined for PI3 virus on bovine nasal turbinate cell cultures and foetal calf lung cell cultures. The methods used to culture and identify the isolates were as described by Bryson and others (34). Antibodies to PI3 virus were detected by the haemagglutination inhibition (HAI) test (94).

Pathological procedures

Calves were slaughtered as follows: day 4 (calves 2,9), day 9 (calves 1,8) and day 12 (calves 3,4,5,6,7,10). Decisions as to which calves were to be slaughtered, and when, were made before the beginning of the trial. Following slaughter, the lungs of all animals were examined grossly and samples of nasal conchus (NC), trachea (TR), right cranial lobe (RC), right middle lobe (RM), right caudal lobe (RC) and bronchial lymph node (BR) were collected for microbiological examination. Samples from adjacent sites were collected in 10% formol saline and processed by standard methods for routine histopathological examination.

All tissues were examined for the presence of PI3 virus antigen by a standard indirect immuno-fluorescent technique on trypsinised paraffin-embedded sections (213).

Infection procedures

The strain of PI3 virus used had originally been isolated from a severe, acute outbreak of pneumonia in six-month-old, home-bred calves on a local dairy farm (Selman, unpublished data). The virus had been considered to be of primary aetiological importance in this outbreak on the basis of clinical signs, pathological lesions, virus isolation from both dead and surviving calves, demonstration of virus antigen by immunofluorescent techniques and serological response.

The virus, at the fourth pass level, was grown in bovine nasal turbinate cell cultures. Infected cultures

were harvested at maximum cytopathic effect by freezing and thawing once, clarifying by centrifugation at 3000g for 30 minutes at $4^{o}C$ and divided into 20 ml aliquots. The titre of virus at this stage was 10^{5} TCID50/ml.

The inoculum was examined for the presence of contaminating bovine virus diarrhoea-mucosal disease virus by direct immunofluorescence on acetone-fixed coverslip preparations and was confirmed as negative.

Each of the ten experimental calves received 10ml by direct (percutaneous) intratracheal injection and 5ml by instillation into each nostril of the inoculum at 10.30 and 16.30 on days 0 and 1.

Statistical methods

Estimations were carried out by the methods described by Bishop (23). Deviations from the mean are expressed as standard deviations (mean + sd).

RESULTS

Clinical and pathological findings

The detailed findings regarding these aspects of the trial have been reported elsewhere and therefore will only be summarised below.

The infection procedure was judged to have been successful for the following reasons: infection was established with subsequent seroconversion; there was a prompt development of depression, fever and respiratory signs; appropriate pathological and histopathological lesions were found in all cases. In addition, PI3 viral

antigen was identified in association with these lesions in both calves slaughtered on day 4.

Coughing abruptly diminished to an almost imperceptible level within six hours of the test calves receiving their first dose of flunixin meglumine. Later, it appeared that the same animals were brighter than the untreated controls, although significant differences in group concentrate intake were not demonstrable. Therapeutic benefits were also obvious in that the test calves had fewer fevers (Table 1), lower mean respiratory rates (mean rates, days 2-7; test calves -46/min, controls - 69/min) and significantly ($p = <0.001$) fewer days marked by tachypnoea than the untreated control calves (Table 2). These effects were found to persist beyond the 3-day treatment phase of the trial.

Post mortem examinations revealed that while treatment with flunixin meglumine had no obvious effect on PI3 virus infection rates, the clinical benefits were almost certainly the result of a reduction in the extent of pulmonary consolidation. The mean consolidation scores for the test and control calves were 8.1% and 16.6%, respectively.

Mycoplasmal isolations

Mycoplasmal isolations made from nasopharyngeal swabs and upper and lower respiratory tract tissues are presented in Table 3. Information regarding the mycoplasmal isolations made from individual calves is presented in Appendix 22, 1-10.

TABLE 1. The effect of flunixin meglumine ("Finadyne") on the rectal temperature of pneumonic experimental calves*

Rectal temp. (°F)	Data relating to:	Designation (no.)	No.of fevers** detected (mean+s.d)		Significance
>102.0	Group 1 (Sinclair) calves	Test (5)	6.0	2.00	p = n.s.***
		Controls (5)	7.8	3.70	
	Group 2 (Borland) calves	Test (5)	9.2	1.92	p = n.s.
		Controls (5)	10.4	0.90	
>102.5	Group 1 (Sinclair) calves	Test (5)	2.6	1.34	p = n.s.
		Controls (5)	3.4	2.19	
	Group 2 (Borland) calves	Test (5)	6.0	2.35	p = n.s.
		Controls (5)	6.0	3.24	
>103.0	Group 1 (Sinclair) calves	Test (5)	0.4	0.55	p = <0.1
		Controls (5)	1.8	1.30	
	Group 2 (Borland) calves	Test (5)	2.6	2.07	p = n.s.
		Controls (5)	3.0	2.35	

* data for treatment period only (i.e. days 1-4)
** "fever" defined variously (see column 1)
*** n.s. = not statistically significant

TABLE 2. The effect of flunixin meglumine ("Finadyne") on the respiratory rates of pneumonic experimental calves*

Respiratory rate (per min)	Data relating to:	Designation (no.)	No.of tachypnoeas** detected (mean+s.d)		Significance
>50	Group 1 (Sinclair) calves	Test (5)	3.8	2.28	p = <0.001
		Controls (5)	10.6	0.55	
	Group 2 (Borland) calves	Test (5)	8.8	2.17	p = n.s.***
		Controls (5)	10.4	0.90	
>60	Group 1 (Sinclair) calves	Test (5)	1.8	1.3	p = <0.001
		Controls (5)	8.0	2.0	
	Group 2 (Borland) calves	Test (5)	7.0	1.87	p = n.s.
		Controls (5)	7.8	1.92	
>70	Group 1 (Sinclair) calves	Test (5)	0.2	0.45	p = <0.001
		Controls (5)	5.4	2.51	
	Group 2 (Borland) calves	Test (5)	4.0	2.83	p = n.s.
		Controls (5)	4.0	1.0	

* data for treatment period only (i.e. days 1-4)
** "tachypnoea" defined variously (see column 1)
*** n.s. = not statistically significant

TABLE 3. Mycoplasmal isolations from nasopharyngeal swabs and tissues of ten Group 1 (Sinclair) calves.

	Treated (calves 1-5)				Untreated (calves 6-10)			
	Nph* swabs		Tissue		Nph swabs		Tissue	
Agent	Total	(last**)	Upper	Lower	Total	(Last***)	Upper	Lower
A.laidlawii	3	(2)	3	3	4	(2)	1	3
M.bovirhinis	7	(1)	2	1	6	(0)	4	6
M.bovis	2	(0)	2	5	5	(1)	3	6
M.dispar	2	(1)	0	0	1	(0)	0	0
U.diversum	5	(1)	0	0	1	(0)	2	3
	19	(5)	7	9	17	(3)	10	18

* Nph = nasopharyngeal
** one calf (5) negative
*** three calves (6,9,10) negative

In all, a total of 80 mycoplasmal isolations were made during the duration of this study and five different species were identified, namely A.laidlawii (21%), M.bovirhinis (33%), M.bovis (29%), M.dispar (4%) and U.diversum (14%).

- nasopharyngeal swabs

All ten calves were positive for Mycoplasma sp. on at least one occasion.

The two species of Mycoplasma that are generally considered to be non-pathogenic for the bovine respiratory tract, namely A.laidlawii and M.bovirhinis, together represented 56% of nasopharyngeal swab isolations. Both species were isolated on a sporadic basis from most calves and isolations were reasonably evenly distributed over the "settling-in" and the experimental periods.

Mycoplasma bovis was isolated on seven occasions from five calves. Again, isolations were of a sporadic nature although most, five, came from the untreated control group. Mycoplasma dispar was isolated on only three occasions and U.diversum on six. Five of the latter isolations were made from three calves in the test (treated) group.

- upper respiratory tract tissues

Only one calf (5, test group) was negative for Mycoplasma sp. at the two sites (nasal conchus and trachea) tested.

Acholeplasma laidlawii and M.bovirhinis were once again commonly to be found, forming between them 35% of total upper tract tissue isolations.

Five isolations of M.bovis were made from five calves while the only other organism found in this region was U.diversum, on a total of two occasions from two different calves.

- lower respiratory tract tissue

Two calves (2,5) both of which were from the test (treated) group were uniformly negative for mycoplasmas at the four sites (three lung lobes, bronchial lymph node) tested. Each had, however, been positive on nasopharyngeal swab on earlier occasions for one or more of the following: A.laidlawii, M.bovirhinis, U.diversum.

Acholeplasma laidlawii and M.bovirhinis made up 48% of total mycoplasmal isolations from the lower tract. There were substantially more lower tract isolations of the latter organisms among the controls (six isolations) than from the treated group of calves (one isolation).

Mycoplasma bovis was isolated on 11 occasions, that is 41% of total mycoplasmal isolations from the lower tract tissues with five and six isolations from the control and test groups, respectively. However, it should be noted that the majority of these isolations, seven, came from only two calves (3,6).

Mycoplasma dispar was never isolated from the lower tract of any calf while U.diversum was isolated on three occasions from two control calves.

- isolation patterns

While repeated nasopharyngeal swabbing was certainly successful in demonstrating the presence of a variety of mycoplasmal infections, no evidence was found indicating a clearcut correlation between swab and tissue isolations. Indeed, in several instances, for example with M.bovis (both groups) and with M.bovirhinis and U.diversum (control group) the last nasopharyngeal swabs either largely or totally failed to detect the presence of upper and lower tract infections. Not surprisingly, perhaps, this disparity was even more marked when one considered lower tract isolates alone.

- the effect of treatment with flunixin meglumine

Little difference was found in the number and range of mycoplasmal infections between treated or untreated groups of calves as judged by nasopharyngeal swab and upper tract tissue isolations. However, there were twice as many lower tract isolations from the controls as from the calves that had received flunixin meglumine. This difference was almost entirely due to the greater number of isolations of M.bovirhinis and U.diversum. While M.bovis infections were relatively common, no difference was found to exist between treated and untreated groups in this respect.

Bacterial isolations

Information regarding the range of species of bacteria isolated from the ten Group 1 (Sinclair) calves and the number of different species isolated by

nasopharyngeal swab or from tissues is summarised in Table 4. Detailed information regarding isolations from various sites of individual calves is presented in Appendix 2, 1-10.

A total of 18 different bacterial species were isolated during this study, most of which were obtained by routine nasopharyngeal swabbing.

- nasopharyngeal swabs

Thirteen different bacterial species were isolated and identified from nasopharyngeal swab samplings carried out over the settling-in and experimental periods. In all, a total of 76 isolations were made, representing 65% of total bacterial isolations. A summary of the percentage of swabs positive for specific bacterial species is presented in Table 5 from which comparisons may be made with similar data for the second trial involving the Group 2 (Borland) calves.

The commonest nasopharyngeal isolate (represented almost equally between test and control groups of calves) was M.(M)bovis with a total of 24 isolations. Pasteurella haemolytica A1 was isolated on 9 separate occasions again almost equally from test and control calves.

The relatively large number of isolations made by routine nasopharyngeal swabbing is at least in part due to the many and varied isolations made during the "settling-in" period. It can be seen that far fewer isolations, in all 16, were made from the last nasopharyngeal swabs to be collected prior to slaughter.

TABLE 4. Bacterial isolations from nasopharyngeal swabs and upper and lower respiratory tract tissues of the Group 1 (Sinclair) calves.

	Treated (No's 1-5)				Untreated (No's 6-10)			
	Nph* swabs		Tissues		Nph* swabs		Tissues	
Agent	Total	(last)	Upper	Lower	Total	(last)	Upper	Lower
A.anitratus	4	(1)	5	1	0		0	1
A.faecalis	0		0	3	0		0	0
A.lignieresi	1	(1)	0	0	1	(0)	0	0
A.lwoffii	4	(1)	0	0	3	(0)	7	11
A.pyogenes	1	(0)	0	0	0		0	0
B.coagulans	0		0	0	1	(0)	0	0
B.licheniformis	0		0	0	0		0	0
C.bovis	3	(1)	0	1	0		0	0
F.meningosepticum	3	(0)	0	0	0		0	0
Micrococcus sp.	2	(1)	0	0	2	(1)	0	1
M.(B.)catarrhalis	4	(2)	2	1	7	(2)	3	0
M.(M.)bovis	11	(1)	1	0	13	(3)	1	0
M.(M.)nonliquefaciens	3	(1)	0	0	3	(1)	0	0
N.subflava	1	(0)	0	0	0	(0)	2	0
P.haemolytica A1	4	(0)	0	0	5	(0)	0	0
P.haemolytica A2	0		0	0	0		0	0
S.aureus	0		1	0	0		0	0
S.bovis	0	0	0	0	0		0	0
Total isolations	41	(9)	(9)	6	35	(7)	13	13

(* Nph = nasopharyngeal)

TABLE 5. Nasopharyngeal swab isolates from 20 experimental calves: percent of swabs positive for specific bacterial species.

	Percent of total swabs positive		
	Group 1(Sinclair) (10)	Group 2(Borland) (10)	All calves (20)
Agent			
A.anitratus	8	50	29
M.(M.)bovis	46	12	29
M.(B.)catarrhalis	21	25	23
A.lwoffii	14	19	16
P.haemolytica A1	17	8	13
C.bovis	6	14	10
Micrococcus sp.	8	10	9
M.(M.)nonliquefaciens	12	2	7
F.meningosepticum	6	6	6
P.haemolytica A2	0	10	5
A.lignieresi	4	0	2
B.coagulans	2	4	2
B.licheniformis	0	2	1
A.pyogenes	2	0	1
N.subflava	2	0	1
S.bovis	0	2	1
No.of agents represented	13	13	16

- upper respiratory tract tissue

A total of 22 isolations were made from material obtained from the two upper respiratory tract sites, namely nasal conchus and trachea. In all, six different bacterial species were represented namely, A.anitratus (5), A.lwoffii (7), M.(M.)bovis (2), M.(B.)catarrhalis (5), N.subflava (2) and S.aureus (1). Of these, A.anitratus was confined to the test calves whereas A.lwoffii was confined to the controls.

A summary of the percentage of tissues positive for specific bacterial species isolated from the upper tract of the Group 1 (Sinclair) calves is presented in Table 6.

- lower respiratory tract tissues

Nineteen isolations were made from the four lower respiratory tract sites (three lung lobes and bronchial lymph node). However, this number was unevenly spread between the test and control groups insofar as only six isolations, representing four bacterial species (A.anitratus (1), A.faecalis (3), C.bovis (1) and M.(B.)catarrhalis (1)), were made from the five test calves while 13 isolations, representing three bacterial species (A.anitratus (1), A.lwoffii (11) and Micrococcus sp. (1)), were made from the untreated controls. In this context, it should be remembered that A.lwoffii was also the commonest upper tract tissue isolate from this latter group.

A summary of the percentage of tissues positive for specific bacterial species isolated from the lower

TABLE 6. Upper tract* isolates from 20 experimental calves: percentage of swabs positive for specific bacterial species.

	Percent of total swabs positive		
	Group 1(Sinclair) (10)	Group 2(Borland) (10)	All calves (20)
Agent			
A.anitratus	25	30	28
A.lwoffii	35	5	20
M.(M.)bovis	10	25	18
M.(B.)catarrhalis	25	10	18
N.subflava	10	20	15
S.aureus	5	5	5
B.coagulans	0	5	3
F.meningosepticum	0	5	3
Micrococcus sp.	0	5	3
No.of agents represented	6	9	9

(*two standard sampling sites/calf: nasal conchus, trachea)

tract of the Group 1 (Sinclair) calves is presented in Table 7.

- isolation patterns

As has already been stated, the large number of isolations and the wide range of species represented on nasopharyngeal swab samples must in part, at least, reflect the number of times such samples were collected. The difference between total and "last" swabs in terms of the range of bacterial species identified must also indicate the transient nature of many such infections. In addition, the wide range of species identified on "last" nasopharyngeal swabs compared with upper tract tissues might well reflect the fact that many transient infections are situated in the area of the external nares rather than established within the upper airways.

The significance of the situation regarding A.lwoffii is unclear. It does however serve to indicate the limited value of nasopharyngeal swabbing in that the organism was found on four swabs taken from the test calves which were found to have no upper or lower tissue infections while only three swabs were positive in the control group which was later found to have seven and 11 infections in upper and lower tract sites, respectively.

Pasteurella haemolytica A1 was only found by nasopharyngeal swab and proved to be an early, transient infection present in both groups of calves.

- effect of treatment with flunixin meglumine.

Differences were noted between test and control

TABLE 7. Lower tract* isolates from 20 experimental calves: percentage of swabs positive for specific bacterial species

	Percent of total swabs positive		
	Group 1(Sinclair) (10)	Group 2(Borland) (10)	All calves (20)
Agent			
A.anitratus	5	35	20
A.lwoffii	28	3	15
A.faecalis	8	0	4
F.meningosepticum	0	8	4
Micrococcus sp.	3	5	4
M.(M.)bovis	0	8	4
M.(B.)catarrhalis	3	5	4
C.bovis	3	0	1
N.subflava	0	3	1
S.aureus	0	3	1
No.of agents represented	6	8	10

(* four standard sampling sites/calf: three right lung lobes, bronchial lymph node)

groups in terms of both upper and lower tract tissue isolations. Total upper tract isolations from test and control groups were nine and 13, respectively; total lower tract isolations from test and control groups were six and 13, respectively. It has already been noted that this effect was due to the presence of numerous upper and lower tract infections by the Acinetobacter spp.

EXPERIMENT 2

MATERIALS AND METHODS

All procedures used were identical to those employed in the previous study. Similarly the ten experimental calves were selected by the same methods and managed in the same way as the previous experimental animals, once admitted to the Veterinary School. However, it should be noted that the latter calves originated from a different source (Borland) and while they were clinically normal on admission and, moreover, were selected from a larger group of clinical normal and similarly-sized individuals, it was quite obvious that they were of a somewhat poorer quality than those used in the first experiment, thinner and less well grown.

RESULTS

Clinical and pathological findings

Once again, only a summary of this aspect of the study will be presented below.

The infection procedures were deemed to have been successful for the same reasons as outlined for the

previous study. However, during the "settling-in period" it became clear that there were slightly more fevers and tachypnoeas present in the calves than had been the case previously. Consequently, the effect of flunixin meglumine was less marked in respect to its ability to control fever and tachypnoea than was the case with the Group 1 (Sinclair) calves (see Tables 1 and 2).

Post mortem examination revealed that the Group 2 (Borland) calves, while having lesions attributable to, and characteristic of, acute viral (PI3) pneumonia, also had widespread and, in some cases, moderately extensive anterior lung lobe lesions of "cuffing" and other chronic low-grade pneumonias (Appendix 2, 11-20). Two were found to have "cuffing pneumonia" on post-mortem examination, three others had fairly severe lesions of chronic suppurative pneumonia and a further five had lesions of chronic non-suppurative pneumonia (severe bronchitis and bronchiolitis with fibrosis around the airways). In contrast, although lesions of cuffing pneumonia were present in a number of the Group 1 (Sinclair calves) these were minimal.

Mycoplasmal isolations

Mycoplasmal isolations from nasopharyngeal swabs and upper and lower respiratory tract tissues are presented in Table 8. Information regarding the mycoplasmal isolations made from individual calves is presented in Appendix 2, 11-20.

In all, a total of 128 mycoplasmal isolations were made and the same five species were detected in the

TABLE 8. Mycoplasmal isolations from nasopharyngeal swabs and tissues of ten Group 2 (Borland) calves.

	Treated (calves 16-20)				Untreated (calves 11-15)			
	Nph* swabs		Tissue		Nph* swabs		Tissue	
Agent	Total	(Last*)	Upper	Lower	Total	(Last)	Upper	Lower
A.laidlawii	6	(2)	1	9	1	(0)	5	15
M.bovirhinis	5	(0)	5	3	15	(4)	2	1
M.bovis	1	(1)	4	11	2	(0)	5	13
M.dispar	0	(0)	0	2	0	(0)	0	1
U.diversum	2	(0)	0	0	9	(3)	4	6
	14	(3)	10	25	27	(7)	16	36

* two calves (21,24) negative

earlier trial were again represented, albeit to a varying degree.

- nasopharyngeal swabs

One calf (21) was uniformly negative for mycoplasmas although it should be noted that in its particular case, swabbing was only carried out on three occasions.

Once again, the two species most frequently isolated (53% of total isolations, 66% of nasopharyngeal swab isolations) were A.laidlawii and M.bovirhinis. However, in this study three times as many isolations of the latter organism were made from the untreated control calves than from the test animals. This did seem to reflect (see below) a higher level of tissue infection in these animals.

Mycoplasma bovis was isolated on only three occasions while M.dispar was not isolated at all. Ureaplasma diversum on the other hand, was isolated on 11 separate occasions, nine of which were when untreated control calves were swabbed.

- upper respiratory tract tissue

All ten calves were positive on isolation in at least one upper tract site (Table 8).

Thirteen isolations (50%) from these same sites proved to be positive for either A.laidlawii or M.bovirhinis although their relative prevalence differed between groups. Mycoplasma bovis was isolated on nine occasions with little difference between groups.

Mycoplasma dispar was never isolated while U.diversum was found only four times, in each instance in untreated controls.

- lower respiratory tract tissue

All ten calves were positive on numerous occasions at the sites tested (Table 8).

Acholeplasma laidlawii was found on 24 occasions, thereby representing 39% of all positive isolations from this area. In contrast, M.bovirhinis was far less frequently found, with only four positive isolations.

Mycoplasma bovis was also found on 24 occasions and, as with A.laidlawii, there were no obvious differences between the isolation rates of treated and untreated Group 2 calves. Mycoplasma dispar was encountered only three times, U.diversum, six. However, in the latter instance, all six isolations were from untreated control calves.

- isolation patterns

The total number of mycoplasmal isolations made from the Group 2 (Borland) calves (128) was far greater than that (80) from the Group 1 (Sinclair) animals. This was largely due to the far greater number of lower tract isolations made from the Group 2 individuals, particularly with respect to A.laidlawii (24 as opposed to 6) and M.bovis (26 as opposed to 11).

Even given these greater numbers, however, no clearcut correlation was obvious between nasopharyngeal swab and tissue isolates.

The single calf (21) that was negative for Mycoplasma sp. when swabbed on three separate occasions nevertheless had six positive isolations made from its upper and lower tract tissues: A.laidlawii (2), M.bovirhinis (2), M.bovis (2).

The isolations made from last nasopharyngeal swabs and lower tract tissues were as follows for certain selected Mycoplasma sp.:A.laidlawii (untreated controls), 0-15; M.bovis (treated calves), 1-11; M.bovis (untreated controls), 0-13. Overall figures do, however, indicate greater numbers of nasopharyngeal swab isolates in the presence of greater numbers of lower tract tissue isolates when the two separate trials are compared.

Bacterial isolations

Information regarding the range of species of bacteria isolated from the ten Group 2 (Borland) calves and the number of different species isolated by nasopharyngeal swab or from tissues is summarised in Table 9. Detailed information regarding isolations from various sites of individual calves is presented in Appendix 2, 11-20.

A total of 18 different bacterial species were isolated during this part of the study most of which were obtained by routine nasopharyngeal swabbing.

- nasopharyngeal swabs

Thirteen bacterial species were isolated and identified from nasopharyngeal swabs during the period of the study. In all, a total of 84 isolations were made,

TABLE 9. Bacterial isolations from nasopharyngeal swabs and upper an lower respiratory tract tissues of the Group 2 (Borland) calves.

	Treated (No's 16-20)				Untreated (No's 11-15)			
	Nph* swabs		Tissues		Nph* swabs		Tissues	
Agent	Total	(last)	Upper	Lower	Total	(last)	Upper	Lower
A.anitratus	15	(5)	4	11	11	(1)	2	3
A.faecalis	0		0	0	0		0	0
A.lignieresi	0		0	0	0		0	0
A.lwoffii	5	(2)	1	0	5	(1)	0	1
A.pyogenes	0		0	0	0		0	0
B.coagulans	2	(0)	0	0	0		1	0
B.licheniformis	1	(0)	0	0	0		0	0
C.bovis	3		0	0	4		0	0
F.meningosepticum	3	(0)	1	3	0		0	0
Micrococcus sp.	0		0	1	5	(1)	1	1
M.(B.)catarrhalis	3	(1)	2	2	10	(0)	0	0
M.(M.)bovis	2	(1)	2	2	4	(3)	3	1
M.(M.)nonliquefaciens	0		0	0	1	(1)	0	0
N.subflava	0		0	0	0		4	1
P.haemolytica A1	4	(0)	0	0	0		0	0
P.haemolytica A2	1	(0)	0	0	4	(0)	0	0
S.aureus	0		1	1	0		0	0
S.bovis	1	(0)	0	0	0		0	0
Total isolations	40	(9)	11	20	44	7	11	7

(*Nph = nasopharyngeal)

representing 63% of total bacterial isolations made from the ten members of this group. A summary of the percentage of swabs positive for specific bacterial species isolated from nasopharyngeal swabs obtained from the Group 2 (Borland) calves is presented in Table 5.

The commonest nasopharyngeal isolate was A.anitratus with a total of 26 isolations that is 15 and 11 isolations from the test and control groups, respectively. The second most commonly isolated organism was M.(B.)catarrhalis with a total of 13 isolations; however in this case by far the greatest number of isolations (10) was made from the untreated control calves.

Pasteurella haemolytica A1 was isolated from test calves only on a total of four occasions. As in the earlier study these infections were found to be of a transient nature and fairly early during the settling-in period. However, unlike the earlier trial, P.haemolytica A2 was also found to be present with five isolations, four of which were found in the control group.

- upper respiratory tract tissue

Nine different bacterial species were identified in samples obtained from upper respiratory tract sites, the commonest being A.anitratus (4) and N.subflava (4). A total of 22 infections were identified from these same sites, 11 in each of the two groups.

A summary of the percentage of tissues positive for specific bacterial species isolated from the upper

respiratory tract tissues of the Group 2 (Borland) calves is presented in Table 6.

- lower respiratory tract tissue

A total of 27 infections were identified involving eight different bacterial species. The commonest species to be identified, A.anitratus was isolated on 14 (52%) occasions, 11 of which occurred in the test group of calves. A summary of the percentage of tissues positive for specific bacterial species isolated from the lower respiratory tract tissues of the Group 2 (Borland) calves is presented in Table 7.

- isolation patterns

As previously, the relatively large number of nasopharyngeal swab isolates reflected on the relative frequency with which such sampling was carried out.

The only obvious correlation between the various sampling activities was seen with the commonest isolate A.anitratus, in the test group of calves. Here, five "last" nasopharyngeal swabs, related well to four upper and 11 lower tract tissue isolations. On the other hand, N.subflava was not detected by nasopharyngeal swab although its presence was detected in four upper tract and one lower tract tissue samples.

- effect of treatment with flunixin meglumine

As in the earlier trial, the relatively small number of isolations from upper and lower tract tissues overall made evaluation difficult in this respect. However, the bacterium, A.anitratus, was found on many

more occasions in the upper and lower tract tissues of the treated calves (15 isolations) than in those of the untreated controls (five isolations).

DISCUSSION

The main aim of the above two experiments was to study the effects of the non-steroidal anti-inflammatory compound, flunixin meglumine, on experimentally-induced bovine viral (PI3) pneumonia. Consequently, the studies did not include provision for additional control calves to which PI3 virus had not been administered. Nevertheless, it was considered that the trials afforded a useful opportunity to assess, first, the range and extent of mycoplasmal and bacterial infections in conventional calves with clinical viral pneumonia and, second, whether or not a correlation could be found between nasopharyngeal swab isolations and those obtained at post mortem examination from upper and lower respiratory tract tissues.

The pulmonary mycoplasmal infections of calves of similar ages, types and backgrounds to those studied above were defined and discussed in the early and mid-1970's (4,5). Infections with the apparently non-pathogenic mycoplasmas, A.laidlawii and M.bovirhinis, were found to be a frequent occurrence in both pneumonic and non-pneumonic calves as was the case in the calves in the present studies. However, certain marked differences between the results of the two investigations - separated as they were by 12-15 years - are worthy of further

comment. Such differences basically revolve around the isolation of certain of the pathogenic mycoplasmas.

By far the most striking difference was that in the earlier investigations (4,5) M.bovis was never isolated whereas in the present studies that same species proved to be the most frequently isolated pathogenic mycoplasma. This finding can be explained in that while M.bovis has long been recognised as a cause of pneumonia and/or polyarthritis in North America (215), its presence was only reported in Britain in 1975 (215) and in the west of Scotland in 1979 (7). The present work together with other studies carried out recently in the same area (Allan, unpublished observations) reveal that M.bovis is now a widespread infection in young cattle in the region.

Another point regarding M.bovis isolations that is worthy of mention is that while little difference was noted within each of the two groups in terms of whether or not calves received the test compound flunixin meglumine, clearcut differences were found to exist when the two groups were compared with each other. Far more isolations of M.bovis were made from the Group 2 (Borland) animals than from the Group 1 (Sinclair) individuals. This, when viewed against the clinical appearance (albeit retrospectively), and the performance of the former animals, the post mortem findings of widespread low-grade pneumonias and the fact that most isolations were from lower tract tissues would suggest that M.bovis was aetiologically involved in at least certain of the pneumonias affecting the animals in this

group. Such a view might also be supported by the fact that certain of the pathological features of these pneumonias were similar to those described for both experimental and 'naturally occurring' M.bovis pneumonia (7,159,215).

Another major difference between the results of the earlier and the present studies relates to M.dispar isolations. Allan (4,5) isolated this species relatively frequently, particularly from the lungs of pneumonic calves and linked it, possibly in association with "Ureaplasma sp." (Sic) to a particular type of calf pneumonia, "cuffing pneumonia". Such an association, especially with respect to M.dispar, had also been highlighted by the work of other British workers (86). In the present studies, M.dispar was only isolated on relatively few occasions and while U.diversum was found far more frequently, the majority of such isolations were from nasopharyngeal swabs or upper tract tissue samples. Such findings perhaps also add weight to the view that the low-grade pneumonia affecting the Group 2 (Borland) calves was associated with, or perhaps even caused by, M.bovis.

The effect of treatment with flunixin meglumine, while clearly limiting the extent of pulmonary consolidation - most particularly in the Group 1 (Sinclair) calves - had little or no effect on specific mycoplasmal isolation rates. The single possible exception to this statement might relate to U.diversum isolations from the Group 2 (Borland) calves in which

many more isolations were made from untreated controls. Total mycoplasmal isolations were, however, quite striking with a far greater number of isolations from untreated Group 1 and 2 calves (108 in all) than from the treated animals (84). Such a difference proved to be true for upper and lower tract tissue isolations for both Group 1 and 2 calves and for nasopharyngeal swab isolations for the Group 2 (Borland) animals. The exception, that is the nasopharyngeal swab isolates from the Group 1 (Sinclair) calves, had numbers that were virtually identical. Given such small numbers of animals, the significance of these differences is questionable but if significance exists at all it is difficult to account for the differences except on the basis of decreased inflammation allowing decreased chances for mycoplasmas to establish themselves, to survive and to multiply.

As with mycoplasmal isolations, comparisons between the bacteriological findings in the present studies and of those carried out on relatively large numbers of similar calves from the same area some 12-15 years ago are possible. As in these earlier studies (4,5,148), which involved groups of pneumonic and non-pneumonic calves, a wide range of different bacteria were isolated from the various sites that were sampled. Again, as previously, many of the species that were identified were not recognised pulmonary pathogens while others, such as A.lignieresi, M.(M)bovis and M.(B)catarrhalis are pathogens in their own right despite being more usually found in association with disease states other than

pneumonia. Certain bacteria, for example H.somnus were notable by their absence, and others such as A.pyogenes (formerly C.pyogenes) - traditionally held to be the classic secondary invader in ruminants - was only isolated on a relatively small number of occasions. In fact, the only bacterium that is widely held to be a pulmonary pathogen - and debate still exists as to whether or not it has a primary capability for inducing pulmonary lesions or is merely a particularly aggressive secondary invader (80,234) - P.haemolytica A1, was isolated relatively infrequently and only then sporadically by nasopharyngeal swab during the earlier stages of the study. Other organisms, such as A.anitratus and A.lwoffii (see below) were notable isolates insofar as they were very frequently detected by nasopharyngeal swab and also by sampling tissues of the upper and lower respiratory tract.

Discussion regarding the isolation of Pasteurellae from the bovine respiratory tract is hampered by several factors such as failure by early authors to define sites sampled, whether or not calves were pneumonic or, if they were pneumonic, whether or not they had lesions generally accepted as typical of bovine pneumonic pasteurellosis (5,80). Similarly, it is only relatively recently that the various biotypes and serotypes of P.haemolytica have been regularly cited in field and experimental studies. A number of early studies reviewed by Allan (5) found "Pasteurella spp." in the lungs of pneumonic calves (age and type of calf and

nature of the "pneumonia" often unspecified) at rates ranging from 23.7 - 32.0% of affected lungs. Other workers (Collier and Rossow, 1964 (49)) failed to isolate any Pasteurella spp. from the lungs of 88 non-pneumonic two-year-old cattle. In the study carried out during the early and mid-1970's on calves similar to those used in the present studies, Allan (4,5) isolated Pasteurella spp. from 23.7% of pneumonic lungs; however, in the same study an approximately similar proportion of non-pneumonic calves (20.6%) were also found to harbour these same species in their lower airways. Further comparisons between these and the present findings are not possible, unfortunately, because at the time the earlier studies were conducted it was not possible to define the biotypes and serotypes of P.haemolytica isolates. However, P.multocida was not isolated in the present studies and, as already stated, the P.haemolytica A1 isolation patterns indicated only transient, early infections insofar as they were only detected by nasopharyngeal swab. In fact, while P.haemolytica A1 was isolated from both Group 1 and Group 2 calves, most (77%) of the total of 13 isolates were on the day of admission and only one calf was found to be positive for this particular organism on day 0. Pasteurella haemolytica A2, on the other hand, was limited to the Group 2 (Borland) animals, fewer isolations (5) were made and all were between days 0-7. Again, this organism was never found in tissue studies.

For many years it has been postulated (Yates) that "transit" or "shipping" fever is not primary

pasteurellosis but the result of a viral-bacterial interaction involving PI3 (sometimes, perhaps IBR) virus and Pasteurella spp. In recent years, P.haemolytica A1 has been spot-lighted as the biotype/serotype most commonly involved (Gibbs). Certainly P.haemolytica A2 does not seem to be commonly associated with field incidents of bovine pneumonic pasteurellosis (10) and, unlike P.haemolytica A1, appears to be incapable of inducing, per se, typical bovine pneumonic pasteurellosis in experimental conventional calves (Gibbs, unpublished observations). Many reasons, therefore, could be advanced as to why, despite having administered a highly virulent strain of PI3 virus to groups of calves known to have been infected with either P.haemolytica A1 or A2, typical "transit fever" signs and lesions were not induced. The first and most obvious of these is that neither the A1 or the A2 strains isolate were present in sufficient numbers by the time the virus was administered in that the A1 infection appeared to be receding and the A2 infection was only just emerging. In this context, it is also worth noting that workers using a PI3 or IBR-Pasteurella model (11) have emphasised the importance of timing in terms of the different infections; for best results, the virus has to be administered four days before the bacterium. The next possibility is that neither the A1 nor the A2 isolates were pathogenic. Pasteurella haemolytica A1 isolates with differing sensitivity patterns have been shown to have different biological capabilities when administered to experimental conventional calves (228).

Furthermore P.haemolytica A2 does not appear to be pathogenic for experimental calves despite its known effects in sheep (80). In other words, these two infections were merely transient and clinically insignificant phenomena that would normally have passed unnoticed.

A further interesting finding was that Acinetobacter spp. were isolated only from pneumonic lungs of 4.7% of calves examined by Allan (4,5) and never from non-pneumonic lungs. In contrast, A.anitratus and A.lwoffii were the organisms that were most commonly isolated of all and, in fact, were isolated from the lungs of 55% of the 20 calves in those studies. However marked differences were found to exist between groups. Acinetobacter lwoffii was only infrequently isolated from nasopharyngeal swabs taken from the untreated Group 1 (Sinclair) calves but was, nevertheless by far the commonest upper and lower tract tissue isolate in these same animals. In contrast, the same organism was only an infrequent isolation from the Group 2 (Borland) calves while A.anitratus was the commonest isolate. When the data from these two groups of calves was combined these two species were the commonest isolates from nasopharyngeal swabs (45% of all isolates) and lower tract tissues (35% of all isolates). Recently (22), it has been proposed that these two species should no longer be viewed as separate and that they should be viewed as a single species, A.calcoaceticus. Such a situation, of course, renders discussions relating to group differences

irrelevant. The widespread presence of this (or these) organisms is, however, of enormous interest, particularly in view of the number of isolations made from lower respiratory tract tissues in the present studies. While the situation in humans regarding lower tract respiratory bacterial infections may well be very different from that of ruminants (see below) it should be noted that Acinetobacter spp. are now being viewed as an increasingly important "nosocomial" (i.e. hospital-acquired) infections. Thus, their presence in the upper and lower airways of intensively-managed calves with viral pneumonia might well be of greater significance than hitherto believed.

Apart from the latter infection(s) being common in all three types of sample examined, nasopharyngeal swabbing was not a reliable guide to even upper tract tissue bacteria let alone those in the lower parts of the lung. Indeed, because several infections appeared to be limited to the upper airways, nasopharyngeal swabs could well, under certain circumstances, provide highly misleading information. The presence of such a wide range of very different organisms from nasopharyngeal swab material (18 different bacterial species in all) must to a degree reflect upon the large number of swabs that were taken compared with the "one-off" (albeit multiple-site) samples obtained following slaughter. Many of these species could well have been, given the life-style of intensively-managed calves, transient, accidental contaminations rather than true, active infections. Such

a view might in part be born out by the larger number of species isolated from swabs compared with upper tract tissues. In other words, many of these species were probably obtained from the external nares rather than from further inside the nasal cavities. If this were to be true, it casts even graver doubts upon the widespread habit of veterinarians to attempt to base therapeutic decisions upon nasal, rather than even nasopharyngeal swab, isolates. Similarly, the suggestion that certain infections of the lower tract, might be due to soil contamination (49) is also worthy of further examination.

The whole subject of viral-bacterial interactions and the inactivation and clearance of bacterial and other particulate matter from the airways of normal and pneumonic subjects has been reviewed recently (101). In humans and small laboratory animals it is widely-held that "the distal airways and alveoli are normally sterile" (101). Furthermore, while no one bacterial species is responsible for all post-influenzal bacterial pneumonias in humans (101), the prevalence of a specific superinfecting microbe during such epidemics may well depend upon the presence of that organism within a given community at the time of an influenzal epidemic (101). From the results of the present study, the situation in intensively-managed calves with an experimentally-induced virus pneumonia is quite different in that multiple and differing infections of the lower tract are clearly quite common, even when such calves share a common loose-box. Moreover, such infections have been shown to occur in

non-pneumonic cattle (4,5,49).

The presence of bacteria in the lower airways depends upon host, pathogen and environmental factors (101). A single examination of tissue obtained at slaughter cannot, as yet, indicate whether a particular isolate is in the process of being inactivated or cleared or, indeed, whether it is capable of, or undergoing, proliferation (101). Thus, the significance of the finding of numerous bacterial species in the lower airways of pneumonic cattle (that is pneumonic from other known cause(s)) must often be in doubt - that is, unless the organism concerned has been defined as a pulmonary pathogen by field and experimental studies.

The suggestion (49), that in normal cattle many lung isolates may be merely the result of contamination by soil organisms therefore needs further attention, particularly since the commonest isolate, A.calcoaceticus, is recognised as an organism that exists widely in soil and sewage (22). Contamination by such organisms could arise in several different ways. Inspiration of ruminal contents not infrequently occurs during, or immediately following, death or slaughter; that apart, inhalation of small numbers of food-borne or environmental organisms may occur almost on a regular, almost minute-to-minute, basis. However, in the case of ruminants, due note must be made of the fact that 40% of eructated air is inhaled by ruminants (193) and that this is perhaps a much more likely way of small numbers of otherwise harmless organisms entering their "distal

airways". It seems, therefore, inappropriate to closely compare humans or small laboratory animals with ruminants - not least because to do so may, perhaps, give pathological credibility to many bacterial species which do not really deserve it. In short, the pathogenic capability of frequent pulmonary isolates can only be established by experimental infection studies. Given the results of the present investigations, it would appear that A.calcoaceticus is a prime candidate for such studies.

CHAPTER 4

MYCOPLASMAL AND BACTERIAL INFECTIONS OF THE UPPER AND LOWER RESPIRATORY TRACT OF D.viviparus INFECTED CALVES AND UNPARASITISED CONTROLS

CHAPTER 4. MYCOPLASMAL AND BACTERIAL INFECTIONS OF THE UPPER AND LOWER RESPIRATORY TRACT OF D.viviparus INFECTED CALVES AND UNPARASITISED CONTROLS

INTRODUCTION

The literature regarding bovine parasitic bronchitis in terms of aetiology, epidemiology, clinical and pathological features and prevention has already been reviewed. This being so, the following brief introduction will confine itself to certain aspects relating to the treatment of that condition.

In the two clinical forms of the disease in which lungworms of various ages are present in the airways, that is the prepatent and patent phases, treatment with a wide variety of anthelmintics is usually effective, though not without certain risks (194). On the basis of the gross appearance of the lungs, particularly in view of the existence of much green pus in the airways of patent cases (102) it has been postulated that secondary bacterial infections are a common sequel to parasitic infections (89). However, this view has been challenged since detailed histopathological studies have indicated that the pus is basically composed of eosinophils rather than neutrophils and is basically a reaction to live and dead lungworms and their contents (102). Nevertheless it has been advocated that in severe (102) or febrile cases of parasitic bronchitis (220) antibiotics should be administered.

An examination of the literature would suggest that detailed studies on the microbiological infections

associated with D.viviparus- induced pneumonia and bronchitis have not been carried out. It was therefore decided to instigate such studies under controlled circumstances using artificially-administered lungworm infections.

MATERIALS AND METHODS

Experimental animals

Ten Friesian-cross steers, aged approximately six months were acquired from a local commercial source and admitted to the Veterinary School. These calves had been reared indoors on concrete, had not been vaccinated for parasitic bronchitis and had neither grazed nor been fed cut grass. Once admitted they were randomly allocated to two groups of five animals and housed in adjacent pens in the same large loose-box. Throughout the study feeding was with hay and water ad lib and approximately 2 kg of concentrate feed/day. The animals had no history of ill-health, were clinically normal when purchased and, apart from the pneumonia that arose in the five animals that received lungworm larvae, developed no significant clinical abnormalities during the trial.

Parasitological procedures

Lungworm larvae were kindly donated by the Department of Parasitology of the University of Glasgow Veterinary School. On day 0 of the study, larvae were administered orally, in normal saline, at the rate of 50 larvae/kg bodyweight. Details of the weights of these calves, larval doses etc. are presented in Table 10.

TABLE 10. Parasitological data for the five calves experimentally infected with D.viviparus larvae on day 0.

Group	Calf nos.	Bodyweight (kg)	No.larvae administered	No. larvae detected* (per gm. faeces) day 20	day 24	day 27	day 31	day 34	Slaughter day	No.of adult worms
Test	68	140	7000	-	-	-	-	-	10	-
	65	125	6250	-	-	-	-	-	20	1925
	64	140	7000	-	1700**	-	-	-	25	1245
	69	160	8000	-	10	20	-	-	30	720
	71	135	6750	-	100	200	500	156	35	370

(* Data cited for McMaster test results)
(** also 100 Capillaria eggs)

Parasitological procedure

Faecal larval counts were made twice weekly from all animals. Both McMaster and Baermann techniques were used but only McMaster results are cited below.

Adult worms were carried out on the lungs of all calves by counting the worms present in the left lung and doubling the figure obtained.

Microbiological procedures

Nasopharyngeal swabs were obtained from each animal twice weekly and examined for viruses, mycoplasmas or bacteria. Procedures were as described earlier.

Tissue samples were removed from the upper and lower respiratory tract and associated lymphatic tissues of all calves. Samples taken were as follows: nasal conchus, trachea, retropharyngeal lymph node, tonsil, right cranial, middle and caudal lung lobes, bronchial lymph node.

RESULTS

Since the main aim of this study was to investigate upper and lower respiratory tract infections in calves with experimentally-induced parasitic bronchitis and to compare findings with uninfected controls, only a brief account will be given of clinical, parasitological and pathological findings. Detailed findings are presented in Appendices 3,1-23.

Clinical findings

The five control calves remained clinically

normal throughout the duration of the study apart from the appearance of infrequent coughing between days 13-22 (Figure 1).

The five calves that received infective (L3) D.viviparus larvae on day 0 of the study were clinically normal during the 14-day "settling-in" period. The first evidence of parasite-induced respiratory disease was a sharp increase in respiratory rates and coughing on day 7, that is early in the prepatent phase of the disease (Figure 2). These signs persisted and became more severe over the following days and, in fact, remained an obvious feature of the calves that were allowed to survive until the later stages of the study. Fever, that is a rectal temperature in excess of 102.0°F was also noted in the majority of lungworm-infected calves from day 11 onwards (Figure 3). Appetite remained good until the patent stage of the disease (day 25 onwards) but thereafter the surviving calves were dull and ate little. Latterly, the remaining calves were extremely dull, with gross dyspnoea and widespread squeaks and crackles were detected on auscultation.

Parasitological findings

Findings are summarised in Table 10.

Faeces from the lungworm-free controls were uniformly negative throughout the study. However, in one animal (60) 100 capillaria eggs were found.

Dictyocaulus viviparus larvae were found in the faeces of all three experimentally-infected calves that

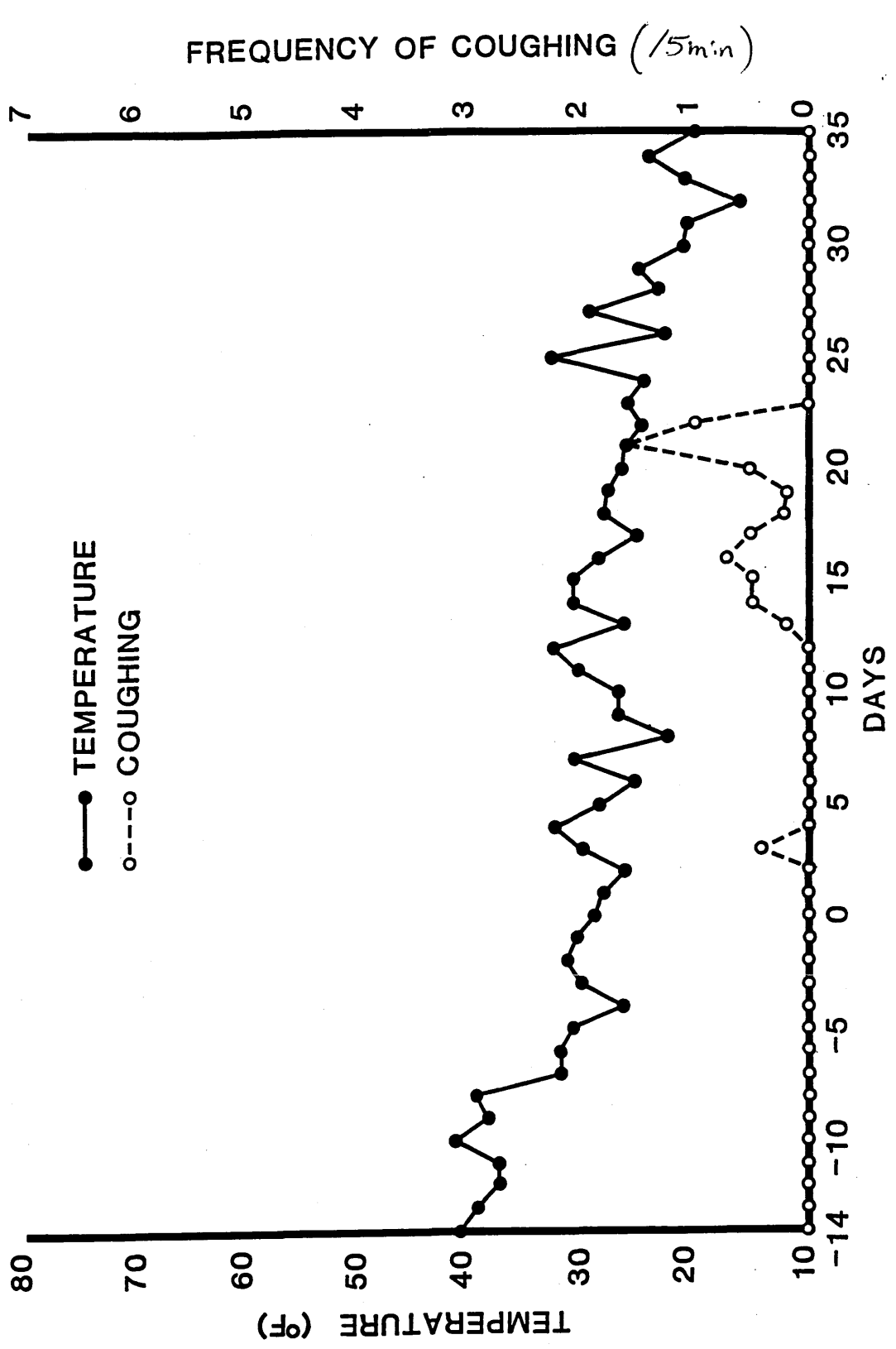

FIGURE 1. Mean daily rectal temperatures and coughing scores of five control calves.

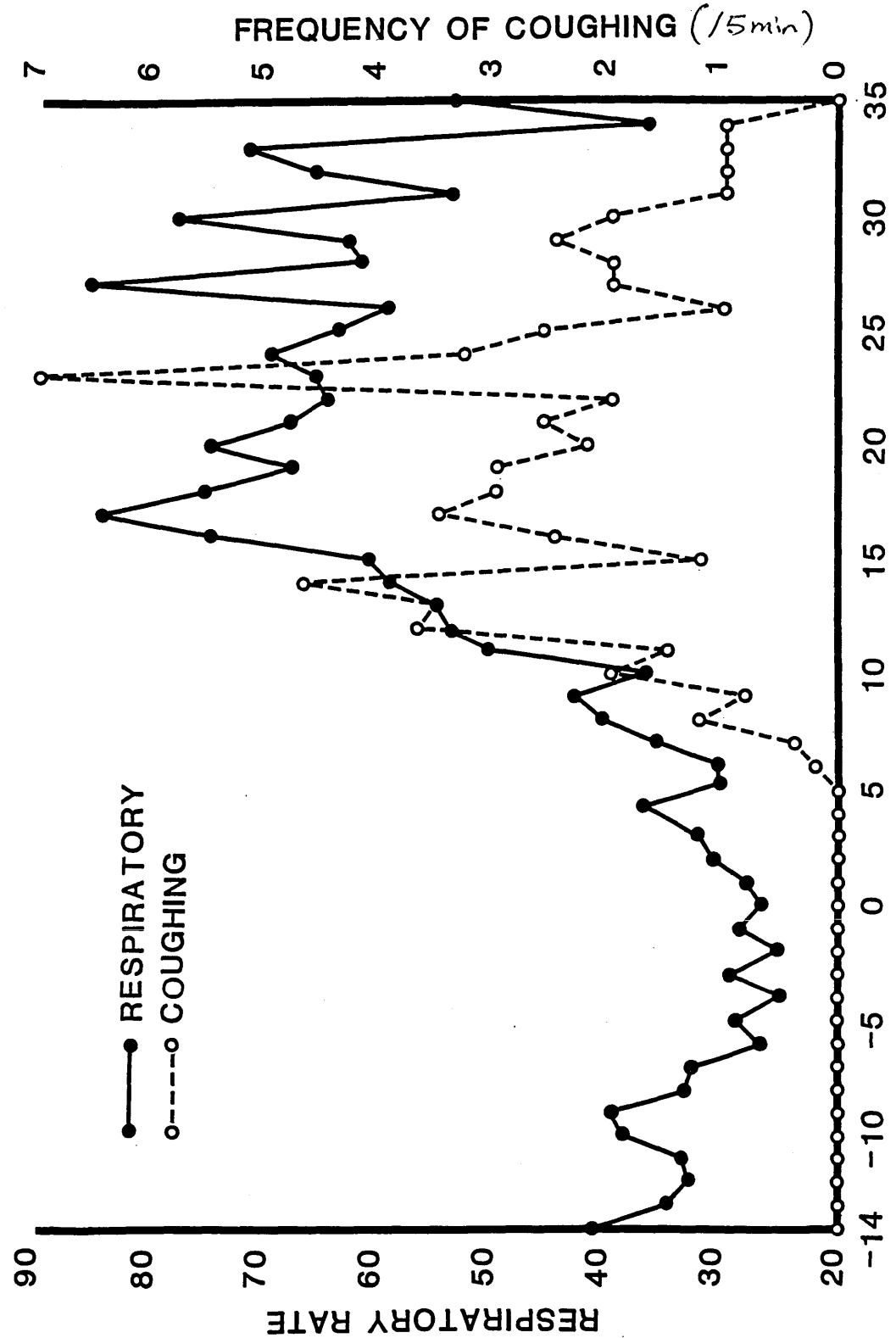

FIGURE 2. Mean daily respiratory rates and coughing scores of five calves experimentally infected with the cattle lungworm, D.viviparus on day 0

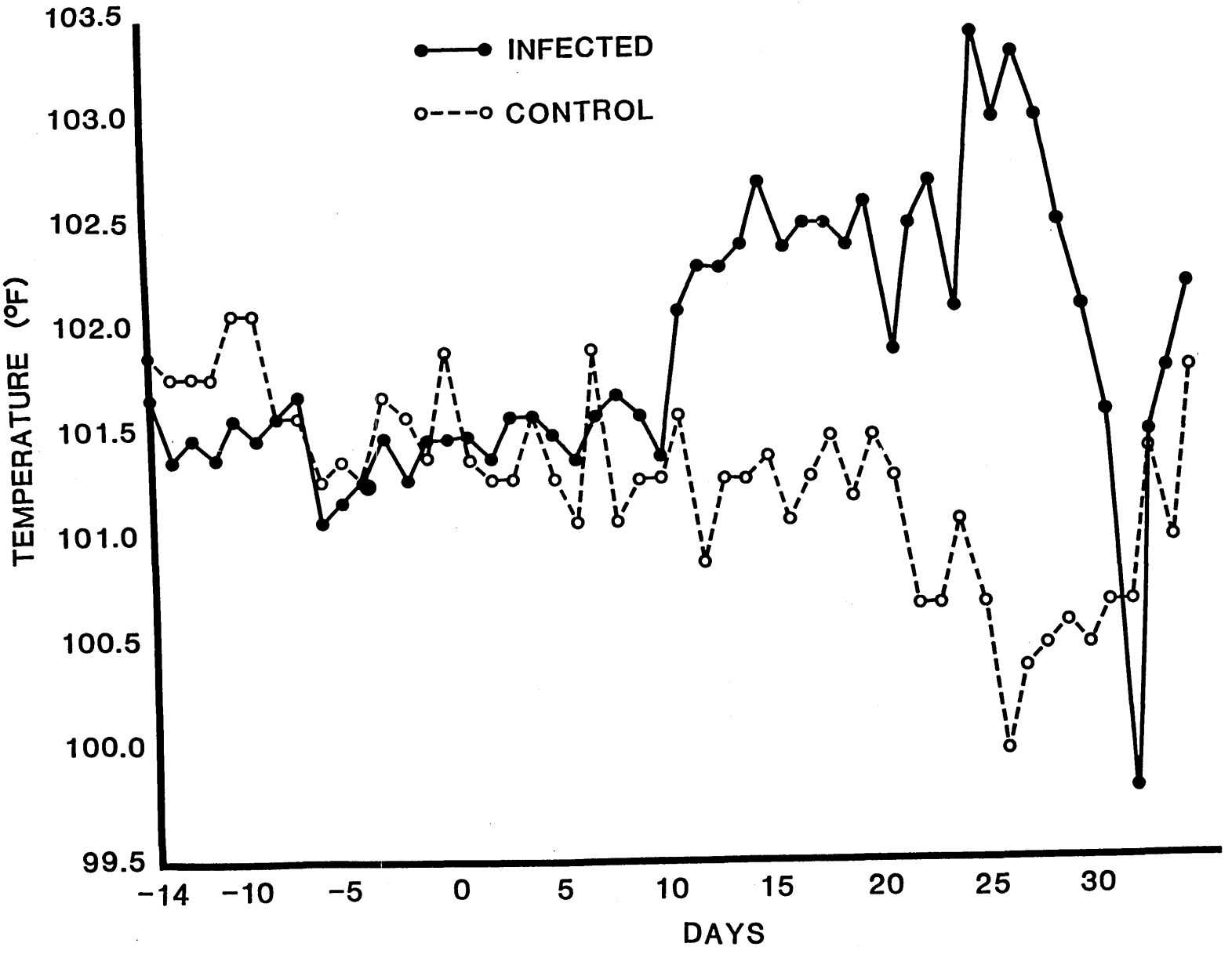

FIGURE 3. The mean daily rectal temperatures of five calves experimentally infected with the cattle lungworm, D.viviparus, on day 0 and five uninfected controls.

survived beyond day 24 although a degree of individual variation was evident (Table 10).

The number of adult lungworms found at post-mortem examination also varied considerably (Table 10), with a range of 370-1925. The greatest numbers were detected in the calf slaughtered on day 20 and thereafter, numbers progressively fell.

Pathological findings

Findings are tabulated in Appendix 3,11-13. Gross and histopathological findings were consistent with a diagnosis of severe parasitic bronchitis.

Mycoplasmal isolations

- nasopharyngeal swabs

A summary of the results obtained when nasopharyngeal swabs from the ten experimental calves in this study were examined for mycoplasmal infections is presented in Table 11. Detailed information regarding isolations from each of the animals is presented in Appendix 3,14-23.

A total of 115 nasopharyngeal swabs were collected and examined, that is 49 during the 14-day "settling-in period" and 66 during the variable periods that each of the calves survived between day 0 (when D.viviparus larvae were administered to the test animals) and slaughter. Only 39 of these swabs (33.9%) proved to be positive for Mycoplasma sp., 17 and 22 from control and lungworm-infected groups, respectively.

TABLE 11. Mycoplasmal infections detected by nasopharyngeal swabs collected from ten experimental calves, five with an experimentally-induced D.viviparus infection and five lungworm-free controls

	No. of mycoplasmal infections	
	Controls (5)	Lungworms infected (5)
No. of swabs examined	(57)	(58)
A.laidlawii	7	7
M.bovirhinis	6	10
M.bovis	4	4
M.dispar	0	1
U.diversum	0	0
Total no. of isolations	17	22

The greatest number of the above isolates, that is 30 (77%) were of the non-pathogenic species, A.laidlawii and M.bovirhinis which were represented in almost equal proportions (Table 11). Eight isolates (20.5%) were made of M.bovis, again in equal proportions from the two groups, while only a single swab yielded M.dispar. Ureaplasma diversum was never isolated during the period of the study.

Mycoplasmal infections that were detected during the various phases of lungworm infection were investigated and compared with those in the uninfected controls over the same periods (Table 12). When allowances were made for variations in the numbers of swabs collected, no obvious differences were found either between phases or between groups. The total lack of isolates obtained during the patent phase of D.viviparus infection in particular, should be viewed against the very limited number (3) of swabs collected at this stage of the study.

- upper respiratory tract and associated lymphatic tissues

Results of tissue examinations carried out on the four animals slaughtered on either days 10 or 20 of the study (i.e. during the prepatent phase of the lungworm infection) are summarised in Table 13. Individual data is presented in Appendix 3,14-23.

Only two mycoplasmal infections were detected from the total of 16 tissues examined and both were M.bovirhinis. One was from a test and the other a control animal.

TABLE 12. Mycoplasmal infections detected by nasopharyngeal swab during each phase of experimental D.viviparus infection and in lungworm-free controls

	Phases of D.viviparus infection (days)								
	Preinfection (-14 -0)		Penetration (0-7)		Prepatent (7-25)		Patent (25-35)		
Groups	Control	Test*	Control	Test	Control	Test	Control	Test	
Total no.of swabs taken	25	24	10	10	21	22	1	2	Total no.of isolations
A.laidlawii	4	3	0	1	3	3	0	0	14
M.bovirhinis	2	4	3	2	1	4	0	0	16
M.bovis	2	2	0	0	2	2	0	0	8
M.dispar	0	1	0	0	0	0	0	0	1
U.diversum	0	0	0	0	0	0	0	0	0
Total no. of isolations	8	10	3	3	6	9	0	0	39

(*test = lungworm-infected)

TABLE 13. Mycoplasmal infections in upper respiratory tract and associated lymphatic tissues in two calves (65,68) experimentally-infected with D.viviparus and two lungworm-free controls (55,58) slaughtered on either days 10 or 20 of the study (i.e. during the prepatent phase of the lungworm infection)

	No. of mycoplasmal infections			
	Controls (2)		Lungworm-infected (2)	
	tract	lymphatic tissue	tract	lymphatic tissue
No.of samples examined	(4)	(4)	(4)	(4)
A.laidlawii	0	0	0	0
M.Bovirhinis	0	1	1	0
M.bovis	0	0	0	0
M.dispar	0	0	0	0
U.diversum	0	0	0	0
Total no.of isolations	0	1	1	0

Results of tissue examinations carried out on the six animals slaughtered on either days 25, 30 or 35 of the study (i.e. during the patent phase of the lungworm infection) are summarised in Table 14. Individual data is presented in Appendix 3,14-23.

Only three infections were detected at this stage of the lungworm infection and all were of M.bovirhinis from tonsillar tissue. Two isolates were from lungworm-infected animals and the other from one of the control animals.

- lower respiratory tract and associated lymphatic tissues

Results of tissue examinations carried out on the four animals slaughtered on either days 10 or 20 of the study (i.e. during the prepatent phase of the lungworm infection) are summarised in Table 15. Individual data is presented in Appendix 3,14-23.

In all, 16 tissues were examined but only two mycoplasmal infections were detected both of which were M.bovirhinis in the lungs of lungworm-infected cattle.

Results of tissue examinations carried out on the six animals slaughtered on either days 25, 30 or 35 of the study (i.e. during the patent phase of the lungworm infection) are summarised in Table 16. Individual data is presented in Appendix 3,14-23.

A total of four infections were detected all of which were in the lungworm-infected group of animals. Three of these were M.bovirhinis, the fourth was A.laidlawii and all were found in lung tissue.

TABLE 14. Mycoplasmal infections in upper respiratory tract and associated lymphatic tissues in three calves (64,69,71) experimentally-infected with D.viviparus and three lungworm-free controls (56,60,61) slaughtered on either days 25, 30 or 35 (i.e. during the patent phase of the lungworm infection).

	No. of mycoplasmal infections			
	Controls (3)		Lungworm-infected (3)	
	tract	lymphatic tissue	tract	lymphatic tissue
No.of samples examined	(6)	(6)	(6)	(6)
A.laidlawii	0	0	0	0
M.bovirhinis	0	1	0	2
M.bovis	0	0	0	0
M.dispar	0	0	0	0
U.diversum	0	0	0	0
Total no.of isolations	0	1	0	2

TABLE 15. Mycoplasmal infections in lower respiratory tract and associated lymphatic tissues in two calves (65,68) experimentally-infected with D.viviparus and two lungworm-free controls (55,58) slaughtered on either days 10 or 20 (i.e. during the prepatent phase of the lungworm infection).

	No. of mycoplasmal infections			
	Controls (2)		Lungworm-infected (2)	
	tract	lymphatic tissue	tract	lymphatic tissue
No.of samples examined	(6)	(2)	(6)	(2)
A.laidlawii	0	0	0	0
M.bovirhinis	0	0	2	0
M.bovis	0	0	0	0
M.dispar	0	0	0	0
U.diversum	0	0	0	0
Total no.of isolations	0	0	2	0

TABLE 16. Mycoplasmal infections in lower respiratory tract and associated lymphatic tissues in three calves (64,69,71) experimentally-infected with D.viviparus and three lungworm-free controls (56,60,61) slaughtered on either days 25,30 or35 (i.e. during the patent phase of the lungworm infection).

	No. of mycoplasmal infections			
	Controls (3)		Lungworm-infected (3)	
	tract	lymphatic tissue	tract	lymphatic tissue
No.of samples examined	(9)	(3)	(9)	(3)
A.laidlawii	0	0	1	0
M.Bovirhinis	0	0	3	0
M.bovis	0	0	0	0
M.dispar	0	0	0	0
U.diversum	0	0	0	0
Total no.of isolations	0	0	4	0

- isolation patterns

A comparison of the overall isolations made from upper and lower respiratory tract and associated lymphatic tissue is presented in Table 17 in relation to the commonest isolate, M.bovirhinis. It can be seen that while only two isolations were made from the five lungworm-free control animals, a total of eight were made from the five lungworm-infected control animals. To this must be added the single isolation of A.laidlawii which was also made from the lung tissue of the latter group.

Bacteriological findings

- nasopharyngeal swabs

A summary of the results obtained from bacteriological examinations carried out on a total of 115 nasopharyngeal swabs collected throughout the study on the ten experimental calves is presented in Table 18. Individual data is presented in Appendix 3,14-23.

It can be seen from Table 18 that a total of 33 different species were identified over the experimental period, 23 from the lungworm-free controls and 24 from the lungworm-infected test calves. The total number of isolations numbered 157, 87 from the controls and 70 from the test calves.

The bacterial isolations that were made during each phase of the study are summarised in Table 19. In all, isolations made during the preinfection, penetration, prepatent and patent phase of the study in the lungworm-infected test calves and during equivalent

TABLE 17. Total M.bovirhinis isolations from tissues of five calves experimentally-infected with D.viviparus and the lungworm-free controls.

	No. of infections	
	Controls (5)	Lungworms infected (5)
Upper tract/ lymphatic tissue	2	3
Lower tract/ lymphatic tissue	0	5
Total no. of isolations	2	8

TABLE 18. Bacterial infections detected by nasopharyngeal swabs collected from ten experimental calves, five with an experimentally induced D.viviparus infection and five lungworm-free controls

	No.(%) of bacterial infections		
	Controls (5)	Lungworm-infected (5)	Total
No.of swabs examined	57	58	115
S.aureus	16 (18.4)	10 (14.3)	26 (16.6)
Bacillus sp.	12 (13.8)	8 (11.4)	20 (12.7)
N.subflava	7 (8.0)	9 (12.9)	16 (10.2)
E.coli	9 (10.3)	6 (8.6)	15 (9.6)
Micrococcus sp.	6 (6.9)	6 (8.6)	12 (7.6)
Corynebacterium sp.	6 (6.9)	2 (2.9)	8 (5.1)
Moraxella sp.	3 (3.5)	5 (7.1)	8 (5.1)
Flavobacterium sp.	4 (4.6)	2 (2.9)	6 (3.8)
A.viridans	4 (4.6)	2 (2.9)	6 (3.8)
Proteus sp.	3 (3.5)	2 (2.9)	5 (3.2)
A.calcoaceticus	3 (3.5)	1 (1.4)	4 (2.6)
A.lignieresii	1 (1.1)	2 (2.9)	3 (1.9)
Aerococcus sp.	1 (1.1)	1 (1.4)	2 (1.3)
A.faecalis	0	2 (2.9)	2 (1.3)
A.pyogenes	1 (1.1)	1 (1.4)	2 (1.3)
P.haemolytica	0	2 (2.9)	2 (1.3)
S.epidermides	0	2 (2.9)	2 (1.3)
S.pyogenes	2 (2.3)	0	2 (1.3)
Streptococcus sp.	2 (2.3)	0	2 (1.3)

TABLE 19. Bacterial isolations detected by nasopharyngeal swab during each phase of experimental D.viviparus infection and in lungworm-free controls

	Phases of D.viviparus infection (days)								
	Preinfection (-14 -0)		Penetration (0-7)		Prepatent (7-25)		Patent (25-35)		
Groups	Control	Test*	Control	Test	Control	Test	Control	Test	
Total no.of swabs taken	25	24	10	10	21	22	1	2	Total no.of isolations
S.aureus	10	5	2	2	4	2	0	1	26
Bacillus sp.	7	7	2	1	3	0	0	0	20
N.subflava	3	5	1	1	3	3	0	0	16
E.coli	7	1	2	2	0	3	0	0	15
Micrococcus sp.	1	4	3	2	2	0	0	0	12
Corynebacterium sp.	5	1	1	1	0	0	0	0	8
Moraxella sp.	1	5	1	0	1	0	0	0	8
Flavobacterium sp.	2	1	1	0	1	1	0	0	8
A.viridans	2	1	2	1	0	0	0	0	6
Proteus sp.	0	1	2	0	1	1	0	0	6
A.calcoaceticus	0	0	2	1	1	0	0	0	5
Others	5	2	2	7	5	6	2	2	31
Total no. of isolations	43	33	21	18	21	16	2	3	157

(*test = D.viviparus infected)

times in the uninfected controls were 76, 39, 37 and 5, respectively. Further, little difference could be seen between the total number of isolations when test and control calves were compared by phases; indeed, in all save the patent phase, most isolations were made from control calves. The small number of swabs taken during the patent phase made any comparisons meaningless.

An examination of individual bacterial species indicated that the vast majority were probably incidental infections, probably of no pathogenic significance. The only recognised pulmonary pathogens for the bovine respiratory tract that were isolated - Pasteurella sp. - were, on the other hand, only made from swabs collected from two test calves during the prepatent phase of the study.

- upper respiratory tract and associated lymphatic tissues

A summary of the number and identities of bacteria isolated from upper respiratory tract and associated lymphatic tissues of two test and two control calves slaughtered in the prepatent phase of the study are summarised in Table 20. Individual data is presented in Appendix 3,14-23.

Reference to Table 20 reveals that a total of 23 isolations of nine different species were made from the four calves and that no obvious differences existed between either the number or the identity of isolations from the test and control calves.

Similar examinations carried out on tissues of three control and three test calves slaughtered during

TABLE 20. Bacterial infections in upper respiratory tract and associated lymphatic tissues in two calves (65,68) experimentally-infected with D.viviparus and two lungworm-free controls (55,58) slaughtered on either days 10 or 20 of the study (i.e. during the prepatent phase of the lungworm infection)

	No. of bacterial infections				
	Controls (2)		Lungworm-infected (2)		
	tract	lymphatic tissue	tract	lymphatic tissue	
No.of samples examined	(4)	(4)	(4)	(4)	Total no. of isolations
E.coli	0	4	0	4	8
S.aureus	1	2	2	0	5
A.salmonicida	0	0	0	2	2
A.viridans	1	1	0	0	2
Micrococcus sp.	2	0	0	0	2
A.calcoaceticus	0	0	1	0	1
Bacillus sp.	0	0	1	0	1
Pseudomonas sp.	0	0	1	0	1
S.bovis	1	0	0	0	1
Total no.of isolations	5	7	5	6	23
Total no.of species represented/site	4	3	4	2	9

the patent phase of the lungworm infection are presented in Table 21. Individual information relating to these calves is presented in Appendix 3,14-23. From Table 21, it can be seen that 16 different bacterial species were isolated on 32 separate occasions. At this stage, however, differences between test and control calves were apparent in that almost twice as many isolations were made from the test calves than from the controls involving 15 as opposed to nine different species.

- lower respiratory tract and associated lymphatic tissues

Information obtained from bacteriological studies of lower respiratory tract and associated lymphatic tissue from two test and two control calves slaughtered during the prepatent phase of the disease is summarised in Table 22. Individual data is presented in Appendix 3,14-23.

It can be seen that a total of 23 isolations were made involving ten different bacterial species. As with the upper tissue samples, no clear differences were found between the test and control calves.

Similar studies carried out on lower tract tissues of calves slaughtered during the patent phase of the disease (Table 23). As with studies on upper tract tissues from calves killed at this stage (see above) obvious differences were found between test and control calves. Again, approximately twice as many isolations and twice as many bacterial species were obtained from the lungworm-infected calves compared with the lungworm-free controls.

TABLE 21. Bacterial infections in upper respiratory tract and associated lymphatic tissues in three calves (64,69,71) experimentally-infected with D.viviparus and three lungworm-free controls (56,60,61) slaughtered on either days 25,30 or 35 of the study (i.e. during the prepatent phase of the lungworm infection)

	No. of bacterial infections				
	Controls (3)		Lungworm-infected (3)		
	tract	lymphatic tissue	tract	lymphatic tissue	
No.of samples examined	(6)	(6)	(6)	(6)	Total no. of isolations
E.coli	0	3	0	4	7
N. subflava	0	0	2	1	3
S.equinus	1	1	1		3
A-D group	0	0	1	1	2
A.liquefaciens	1	0	0	1	2
Bacillus sp.	0	0	2		2
Flavobacterim sp.	1	1	0		2
Micrococcus sp.	0	0	0	2	2
S.aureus	1	0	1	0	2
A.calcoaceticus	0	0	1	0	1
A.lignieresii	1	0	0	0	1
Aerococcus sp.	0	0	0	1	1
B.circulans	0	0	1	0	1
Neisseria sp.	0	0	1	0	1
P.multocida	0	0	1	0	1
S.zooepidermicus	0	1	0	0	1
Total no.of isolations	5	6	11	10	32
Total no.of species represented/site	5	4	9	6	15

TABLE 22. Bacterial infections in lower respiratory tract and associated lymphatic tissues in two calves (65,68) experimentally-infected with D.viviparus and two lungworm-free controls (55,58) slaughtered-on either days 10 or 20 of the study (i.e. during the prepatent phase of the lungworm infection)

	No. of bacterial infections				
	Controls (2)		Lungworm-infected (2)		
	tract	lymphatic tissue	tract	lymphatic tissue	
No.of samples examined	(6)	(2)	(6)	(2)	Total no. of isolations
S.aureus	3	0	2	1	6
Micrococcus sp.	1	0	2	0	3
Moraxella sp.	1	0	2	0	3
S.pyogenes	3	0	0	0	3
A.lignieressi	0	0	1	1	2
Corynebacterium sp.	1	0	0	1	2
A.faecalis	0	0	1	0	2
A.viridans	1	0	0	0	1
Bacillus sp.	0	1	0	0	1
S.bovis	0	0	0	1	1
Total no.of isolations	10	1	8	4	23
Total no.of species represented/site	6	1	5	4	10

TABLE 23. Bacterial infections in lower respiratory tract and associated lymphatic tissues in three calves (64,69,71) experimentally-infected with D.viviparus and three lungworm-free controls (56,60,61) slaughtered on either days 25,30 of 30 of the study (i.e. during the patent phase of the lungworm infection)

	No. of bacterial infections				
	Controls (3)		Lungworm-infected (3)		
	tract	lymphatic tissue	tract	lymphatic tissue	
No.of samples examined	(9)	(3)	(9)	(3)	Total no. of isolations
S.aureus	0	0	2	2	4
N.subflava	0	0	2	1	3
Moraxella sp.	0	0	2	0	2
S.equinus	2	0	0	0	2
S.zooepidermicus	1	1	0	0	2
A.pyogenes	0	0	1	0	1
Flavobacterium sp.	0	0	1	0	1
Micrococcus sp.	0	1	0	0	1
P.haemolytica	0	0	1	0	1
Proteus sp.	1	0	0	0	1
Total no.of isolations	4	2	9	3	18
Total no.of species represented/site	3	2	6	2	10

- isolations patterns

No obvious differences were found between the numbers of isolations or species isolated from the upper and lower respiratory tract and associated lymphatic tissues of the test and control calves slaughtered in the prepatent phase of the study. In contrast, greater numbers of isolations and species were found in the upper tissues of the test and control calves slaughtered during the patent phase of the lungworm infections. Moreover, in the latter calves the number of isolations and species isolated from both upper and lower tissues of the lungworm-infected calves greatly exceeded those of the lungworm-free controls (Table 24).

DISCUSSION

The chosen dose-rate of infective (L3) larvae of the cattle lungworm, D.viviparus (50/kg.bodyweight), proved to be highly effective in producing severe pneumonia. Clinical signs became apparent approximately one week after the larvae were administered to the five test calves, that is soon after the onset of the prepatent phase of the disease. Thereafter the clinical picture gradually worsened in those calves that were allowed to survive into the patent phase. The clinical signs were identical to those seen in field cases of parasitic bronchitis as were the pathological findings. Larvae were found in the faeces of all calves that survived up to and beyond the twenty-fifth day of the study and large numbers of adult worms were present in

TABLE 24. Bacterial infections detected by nasopharyngeal swabs collected from ten experimental calves, five with an experimentally induced D.viviparus infection and five lungworm-free controls

	No.(%) of bacterial infections		
	Controls (5)	Lungworm-infected (5)	Total
No.of swabs examined	57	58	115
S.aureus	16 (18.4)	10 (14.3)	26 (16.6)
Bacillus sp.	12 (13.8)	8 (11.4)	20 (12.7)
N.Subflava	7 (8.0)	9 (12.9)	16 (10.2)
E.coli	9 (10.3)	6 (8.6)	15 (9.6)
Micrococcus sp.	6 (6.9)	6 (8.6)	12 (7.6)
Corynebacterium sp.	6 (6.9)	2 (2.9)	8 (5.1)
Moraxella sp.	3 (3.5)	5 (7.1)	8 (5.1)
Flavobacterium sp.	4 (4.6)	2 (2.9)	6 (3.8)
A.viridans	4 (4.6)	2 (2.9)	6 (3.8)
Proteus sp.	3 (3.5)	2 (2.9)	5 (3.2)
A.calcoaceticus	3 (3.5)	1 (1.4)	4 (2.6)
A.lignieresii	1 (1.1)	2 (2.9)	3 (1.9)
Aerococcus sp.	1 (1.1)	1 (1.4)	2 (1.3)
A.faecalis	0	2 (2.9)	2 (1.3)
A.pyogenes	1 (1.1)	1 (1.4)	2 (1.3)
P.haemolytica	0	2 (2.9)	2 (1.3)
S.epidermidis	0	2 (2.9)	2 (1.3)
S.pyogenes	2 (2.3)	0	2 (1.3)
Streptococcus sp.	2 (2.3)	0	2 (1.3)

TABLE 24. contd.

	No.(%) of bacterial infections		
	Controls (5)	Lungworm-infected (5)	Total
No.of swabs examined	57	58	115
A-D Group	0	1 (1.4)	1 (0.6)
A.liquefaciens	1 (1.1)	0	1 (0.6)
Aeromonas sp.	0	1 (1.4)	1 (0.6)
B.circulans	0	1 (1.4)	1 (0.6)
M.roseus	0	1 (1.4)	1 (0.6)
M.(B.)catarrhalis	1 (1.1)	0	1 (0.6)
Neisseria sp.	1 (1.1)	0	1 (0.6)
P.multocida	0	1 (1.4)	1 (0.6)
Pseudomonas sp.	0	1 (1.4)	1 (0.6)
S.bovis	1 (1.1)	0	1 (0.6)
S.equinus	1 (1.1)	0	1 (0.6)
S.faecalis	1 (1.1)	0	1 (0.6)
S.pneumoniae	0	1 (1.4)	1 (0.6)
S.zooepidermicus	1 (1.1)	0	
Total	87 (99.6)	70 (100.1)	157 (99.7)

the airways of all cases that survived beyond day 20. Thus, the model chosen appeared to offer a good opportunity to investigate whether or not secondary infections occur in parasitic bronchitis and, if so, are likely to be of any significance.

An examination of nasopharyngeal swabs revealed the presence of four different mycoplasmal species although the two species that were in by far the greatest majority, A.laidlawii and M.bovirhinis are generally reckoned to be non-pathogenic. No obvious differences were found between the number or type of isolations made from test or control calves. However, examination of tissues for mycoplasmal species indicated a somewhat different situation, particularly in respect of the calves slaughtered during the patent phase of the disease. In all, nine mycoplasmal isolations were made from the tissues of lungworm-infected calves as against only two from the tissues of controls. Six of these infections were found in the tissues of patent cases four of which were in the lungs or associated lymphatic tissues.

Nasopharyngeal swabs yielded large numbers of bacteria of very many different species but even when comparisons between test and control calves were made within the various phases of the study, no obvious differences were found to exist between them.

An examination of upper and lower tract tissues obtained from calves slaughtered on either days 10 or 20 of the study (i.e. during the prepatent phase of the

lungworm infection), while again yielding many bacterial species, also revealed no obvious difference between test and control calves. The situation regarding samples obtained from calves slaughtered later in the study, as with mycoplasmal isolations, was found to be quite different, however. Isolations from upper tract and associated lymphatic tissues of the lungworm-infected calves were almost twice as numerous and involved a far wider range of species than those from the lungworm-free controls. An almost identical situation was found when lower tract tissues were examined although total numbers of isolation and species were somewhat lower.

The majority of bacterial species that were isolated, as in the previous study, were not recognised pulmonary pathogens. Most, and this would appear to be particularly true in the case of nasopharyngeal swab isolations, would appear to have been the result of contamination. Pasteurella sp. were isolated on two occasions and, despite the occurrence of a great deal of pus in the lungs of the airways of the patent cases of parasitic bronchitis, A.pyogenes (previously C.pyogenes) was isolated only once. As has been previously reported (102) the copious yellow-green pus in these animals was due to the plugging of airways with lungworms, larvae and eosinophilic debris and a resultant foreign-body pneumonia. In view of the fact that no histopathological evidence was found indicating the presence of an acute exudative component to the pneumonia, the finding of more numerous mycoplasmal and bacteriological infections in

the patent cases cannot simply be viewed as "secondary infection", despite the fact that these calves had been febrile for several days prior to death.

The questions posed when bacteria, for example, are found in lower airways was addressed in the previous chapter. Such a finding may indicate active infection or (as would seem to be the case in this instance where no exudative reaction was described) a lack, or decreased rate, of pulmonary clearance. Given the nature of the lesions that arise as a result of lungworm infection in cattle, interference with clearance mechanisms would seem very likely. If the suggestion that the lungs of normal ruminants are regularly infected with bacteria that are inspired with eructated gases is correct (see above) then a gradually increasing lower tract loading of bacteria can be visualised in parasitic bronchitis where the airways lesions develop progressively from the onset of the prepatent phase of the disease. It seems likely that, given a sufficient number of organisms or infections, then an exudative reaction will almost inevitably arise. Alternatively, should sufficient numbers of a pathogenic organism such as P.haemolytica A1 accumulate in the lower airways then the pathological reactions specific to that organism will arise in addition to those attributable to the presence of lungworms. Such a situation, that is an acute fibrinous pneumonia and pleurisy, arising in the late patent phase of "naturally occurring" parasitic bronchitis has been described (Gibbs, personal communication).

While secondary bacterial pneumonia has been stated as "not being as common as is generally supposed" (102) it is nevertheless usual to recommend the administration of antibiotics to the most severe and/or febrile cases of parasitic bronchitis (220). On the basis of the findings of this study, such advice would appear to be well-founded.

CHAPTER 5

CLINICAL, PATHOLOGICAL, MICROBIOLOGICAL AND SEROLOGICAL STUDIES ON YOUNG CALVES DURING A "NATURAL" OUTBREAK OF PNEUMONIC PASTEURELLOSIS

CHAPTER 5. CLINICAL, PATHOLOGICAL, MICROBIOLOGICAL AND SEROLOGICAL STUDIES ON YOUNG CALVES DURING A "NATURAL" OUTBREAK OF PNEUMONIC PASTEURELLOSIS

INTRODUCTION

Mycoplasmal and bacterial infections that may arise in pneumonic and non-pneumonic calves have been studied by a number of workers (4,5,49,148). In one such study (5), it was demonstrated that the range of bacterial infections to be found in the lungs of pneumonic and non-pneumonic calves were not significantly different, although their numbers were frequently greater, where disease was found to exist. Additional pathological studies carried out on the same animals revealed that the commonest type of pneumonia that was encountered - "cuffing" pneumonia - was commonly associated with infection by M.dispar and, less frequently, by Ureaplasma sp. "Cuffing" pneumonia has long been recognised as a common, possibly almost unavoidable, respiratory disease of young calves (4), particularly when they are reared artificially in groups such as happens on most dairy farms and rearing units in Britain.

In an attempt to monitor the upper and lower respiratory tract infections that may arise during the first few months of a calf's life, an investigation was designed whereby dairy and dairy-cross calves, obtained from local markets, were to be examined by nasopharyngeal swab twice weekly with pairs of calves being slaughtered at monthly intervals for detailed pathological and micro-

biological study. However, within a few days of these calves being admitted to the Veterinary School, one calf developed acute pneumonia and promptly died. A similar problem was then identified in others of the same group by which time the first case had been positively identified as one of pneumonic pasteurellosis.

This situation was considered to offer an unequalled opportunity to study an outbreak of pneumonic pasteurellosis by the methods that had been proposed and the original experimental design was modified to allow for this. At the same time, a decision was made to withhold all forms of therapy in order that the natural history of an uncomplicated outbreak of pneumonic pasteurellosis might be studied, albeit in a class of livestock that would appear not to be as commonly affected with this disease as are unweaned and weaned single-suckled calves (80).

MATERIALS AND METHODS

Experimental animals

Ten dairy and dairy-cross calves, aged 4-7 days, were obtained through a local dealer from nearby markets and brought to the University of Glasgow Veterinary School along with a larger number of similar animals destined for other, quite separate, investigations. The ten calves involved in this study were placed in a completely separate loose-box to the other calves in the study where they were housed in adjacent pens holding three, three and four calves.

Whole milk from the bulk tank of a local dairy farm was fed at the approximate rate of 5% bodyweight twice daily until weaning at six weeks of age. Hay and water were available ad lib from admission and a commercial concentrate ration (190 calf rearing pencils, BOCM-Silcock) was offered ad lib from two weeks post-admission.

Clinical examination

All calves were examined once daily. Information regarding demeanour, appetite, presence or absence of diarrhoea, severity of diarrhoea, presence or absence of respiratory signs, their nature and their severity were recorded along with any other clinical abnormalities was recorded. Clinical terms used were as defined earlier (197).

Sampling procedures

All calves were sampled by nasopharyngeal swab twice weekly from admission until the termination of the study. Two swabs were taken on each occasion from each animal for mycoplasmal and bacterial isolations, respectively.

Pathological examination

Post mortem examinations were carried out according to the routine described elsewhere (4). Detailed pathological studies were carried out only on the first five calves that died, that is during the acute episode of pneumonia. Microbiological studies were limited in these calves to an examination for the

presence and extent of P.haemolytica infection. In the remaining five calves, post mortem examination was limited to a gross evaluation of respiratory lesions and microbiological studies were not carried out.

RESULTS

Clinical findings

- general information

Details regarding the individual calves involved in this study on day 0, when they were admitted to the University of Glasgow Veterinary School, are presented in Table 25.

All ten calves were considered to be bright on admission and all drank well from their pails. Calf weights on admission ranged from 29-43 kg (mean 36.1 kg). Weekly weight changes over the first nine weeks of the study are presented in Table 26. It can be seen that all calves that died lost weight prior to so doing. In contrast, there was a relatively steady weight gain in three of the others (14,18,24) and an overall, albeit erratic, weight gain in the other two (17,23).

Five calves (15,19,20,22,25) were mild or moderately diarrhoeic on admission (Table 25). This persisted over the following three days in three (15,19,25). By day 4, all five calves that subsequently died from pneumonia (see below) were, or had been, diarrhoeic; in contrast, only two of those that survived beyond day 28 (17,24) had been diarrhoeic (Table 27).

TABLE 25. Details regarding ten calves and clinical findings on admission, day 0

Calf no.	Breed	Weight (kg)	Zinc Sulphate Turbidity Values	Respiratory rates (per min.)	Rectal temperature (°F)	Diarrhoea*	Cough**	Other respiratory signs
14	Friesian	31	16	23	101.2	-	0	None
15	"	35	45	20	102.8	++	0	"
17	"	37	37	20	101.0	-	3	"
18	"	42	25	26	102.8	-	0	"
19	Hereford X	35	59	21	102.2	+	0	"
20	"	39	45	20	103.2	++	0	"
22	"	30	26	22	102.0	+	0	"
23	Friesian	40	16	20	104.0	-	0	"
24	Hereford X	29	23	20	101.4	-	0	"
25	Holstein	43	16	24	102.1	++	0	"

* (+ = mild, ++ = moderate diarrhoea)

** (/ 5 min. observation)

TABLE 26. Weekly weight changes (kg) from admission for nine weeks of ten calves

Calf no.	Weeks 1	2	3	4	5	6	7	8	9
15	35(d)								
25	43	38(d)							
19	35	38	34(d)						
22	30	30	30	28(d)					
20	39	41	37	37(d)					
14	31	35	39	42	45(s)				
18	42	46	47	49	50	50(s)			
24	29	30	31	31	30	31	30(s)		
23	40	40	37	42	42	42	41	41(d)	
17	37	40	39	40	41	40	42	40	40(d)

(d = died; s = slaughtered)

TABLE 27. Diarrhoea scores in ten calves over 20 days from admission

Calf no.	Survival time(days)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	20
15	3	++	+++	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	9	++	++	++	+	+	0	0	0	0	0	-	-	-	-	-	-	-
19	17	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0	0	-
22	25	+	0	0	0	++	++	+	0	0	0	0	0	0	++	0	0	0
20	27	++	0	0	0	++	++	++	+	0	0	0	0	0	++	+	0	0
14	35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	36	0	0	0	0	0	0	0	0	0	0	+	0	+++	+++	+++	++	+
24	45	0	+	+	+	++	+	+	0	0	0	0	0	0	0	0	0	0
23	52	0	0	0	0	0	0	+	+	++	++	0	0	+++	+	0	0	0
17	68	0	0	+	+	0	0	0	0	0	0	++	+	0	0	0	0	0

(+,++,+++ = mild, moderate, severe diarrhoea, respectively; 0 = non-diarrhoeic)

The zinc sulphate turbidity values of the ten calves on admission (Table 25) ranged from 16-59 units (mean 30.8 units), that is levels not generally associated with severe diarrhoea or diarrhoeic deaths in calves of this type maintained under these conditions. In the event, only one calf that died had exhibited severe (+++) diarrhoea and diarrhoea of such severity was only exhibited by one other calf (18) towards the end of the second week of the study (Table 27).

- the respiratory event

Detailed information regarding the degree and frequency of coughing among the ten individual calves is presented in Appendix 4, 1-10.

One calf (17) was heard to be coughing on admission (Table 25). Coughing gradually increased over the first few days following admission and by day 9 all surviving calves were coughing to a greater or lesser extent. Frequent, paroxysmal coughing was noted in three calves (17,23,24) although in the other individuals this feature was mostly a sporadic event.

Individual respiratory rates are presented in Appendix 4, 1-10. On day 0 the mean respiratory rate was 22/min (range 20-24/min). This had almost doubled (i.e. to 43/min) by day 6 although there was, at this stage considerable individual variation (19-100/min). On day 10 the mean value was 38/min (range 20-62); later, on days 20 and 30, the mean respiratory rates were 62/min. and 49/min., respectively. Dyspnoea was noticed for a

limited period only in two calves that died (19,20) and one other (23). On auscultation, harsh sounds were audible over days 4-20 in all calves examined and the more severely affected animals were noted as often having low-pitched squeaks and crackles over the cranio-ventral parts of the thorax. Gentle percussion indicated thoracic pain in three calves (17,22,23).

The only other obvious abnormalities that arose in the calves were discharges from the nostrils and the eyes. Almost all calves developed a seromucoid nasal discharge which gradually became more purulent as the respiratory problem progressed. Occasionally, dried nasal exudate was to be noticed around the nostrils and muzzle. All calves developed a serous bilateral ocular discharge which also progressed to mucopurulence over a period of a few days. This appeared to be the result of conjunctivitis; corneal lesions were not apparent. (This latter feature regularly occurs at this stage in market calves reared in the Veterinary School (159), is of unknown aetiology although apparently infectious and will not be discussed further).

Fever (i.e. a rectal temperature in excess of 102.5^{o}F) was noted on admission in four calves on admission (15,18,20,23) (Table 25). All calves subsequently developed fevers, save for the animal (15) that died on day 2 of the study. However, one calf (14) which was never diarrhoeic (Table 27), which showed the greatest weight-gain (Table 26) and which was only very mildly pneumonic during the acute episode, only showed

very occasional fevers until the later stages of the observational period (days 26-35).

The highest fever attained was 106.8°F (calf 23, day 6). On day 10, when the acute pneumonic episode reached crisis point, the mean rectal temperature of the eight surviving was 104.2°F (range 102.2-106.1°F). In general, maximum rectal temperatures correlated well with maximum respiratory rates.

Course and events

The incidence, morbidity and mortality rates of the acute pneumonic episode are presented in Table 28, from which it can be seen that the episode, on the grounds of clinical observations, lasted from days 2-26 with a peak morbidity rate (100%) on day 11. The five calves that died, did so on days 3 (15), 9(25), 15(19), 25(22) and 26(20).

The five calves that survived beyond day 26 were all, save one (14), affected to a greater or lesser degree by chronic pneumonia.

Pathological findings

Details regarding the gross and/or histopathological findings in the five calves (15,19,20,22,25) that died between days 3 and 26 of the study are presented in Table 29. It can be seen that all five were found to have lesions consistent with a diagnosis of bovine pneumonic pasteurellosis although, in addition, one (22) also had lesions of chronic suppurative pneumonia.

TABLE 28. Incidence, morbidity and mortality rates of acute pneumonia episode that arose in ten young calves between admission (day 0) and day 26.

Day of study	No.of pneumonic calves*	Morbidity rate(%)	Deaths	Mortality rate(%)**
2	3/10	30	0	0
3	4/9	44	+	10
5	4/9	44	0	10
6	8/9	89	0	10
9	7/8	88	+	20
11	8/8	100	0	20
15	6/8	75	+	30
25	5/7	71	+	40
26	4/6	67	+	50

(* acute pneumonia as judged by clinical signs)
(** 50% of calves died by day 26 but four of the remaining calves died or were slaughtered over the following weeks because of chronic respiratory disease)

TABLE 29. Pathological findings in five fatal cases of bovine pneumonic pasteurellosis.

Calf no.	Day of death	Gross findings	Histopathological findings
15	3	A similar reaction was seen in both sets of lungs with severe anterior lobe consolidation also involving the anterior part of the caudal lobes. There was a marked fibrinous pleurisy over the affected areas in both animals. The tissue was firm, dark red-purple, slightly oedematous and, on section, had the characteristic mottled appearance of acute fibrinous pneumonia.	N.D.
25	9	Very severe, classical fibrinous pneumonia with mild to moderately severe pleurisy over the right lung. There were marked adhesions between the lobes and the pericardium.	N.D.
19	15	Very severe, classical fibrinous pneumonia with extensive pleurisy over the left lung and pericardium. On section, the tissue was firm, necrotic, haemorrhagic with thrombi. There was the usual mottled appearance and a little oedema oozed from the tissue.	There was severe acute exudative fibrinous pneumonia. There was much congestion and the interlobular septa were greatly dilated and contained much fibrin as did the lymphatics which also contained clots of fibrin. There was a little bronchiolar epithelial necrosis.
22	25	All lobes totally consolidated apart from the right caudal lobe. Many adhesions, pleurisy and large abscesses were present and there was a chronic suppurative pneumonia with an overlying fibrinous pneumonia.	N.D.
20	26	Lesions present in all lobes. The lungs were solid, mottled, with many small abscesses, particularly in the anterior lobes. Foci of necrosis were seen throughout with many dark haemorrhagic areas. There was a slight fibrinous reaction.	Severe acute exudative pneumonia with some fibrinous involvement.

Bacteriological examinations were carried out on upper and lower tract tissues in four calves (19,20,22,25) and P.haemolytica A1 was isolated from most sites sampled from all calves.

Two chronically pneumonic calves (18,24) were slaughtered on humane grounds on days 36 and 45 of the study; two others (17,23) died 68 and 52 days after having been admitted to the Veterinary School. These four calves were not subjected to a detailed post mortem study but gross pathological findings were consistent with a diagnosis of chronic (irreversible) suppurative pneumonia. Microbiological examinations were not carried out on these four animals.

Microbiological findings

- nasopharyngeal swabs

In all, 157 isolations of 24 different bacterial species were made from nasopharyngeal swabs collected from the ten calves involved in this study.

Two species, Bacillus sp. and S.aureus, comprised 29% of total isolations. Pasteurella haemolytica A1 formed 11% of total isolates and was isolated on one or more occasions, to a maximum of four, from all calves save No.15 (i.e., the first to die on day 3). The timing of P.haemolytica A1 isolations is summarised in Table 31 from which it can be seen that most isolations were made between days 6-13 with a peak on day 10 when (63%) of nasopharyngeal swabs were found to be positive.

Mycoplasmal isolations are also summarised in

TABLE 31. Isolation patterns of P.haemolytica A1 from nasopharyngeal swabs from calves with naturally-acquired pneumonic pasteurellosis

Day of sampling	No.of calves surviving	No.(%) of positive* isolations	Individual calf no's
6	9	3 (30)	18,19,25
10	8	5 (63)	17,18,20,22,24
13	8	2 (25)	22,23
17	7	1 (14)	17
20	7	1 (14)	18
26	6	3 (50)	18,23,24
29	5	2 (40)	14,23

(* positive for P.haemolytica A1)

Table 30. It can be seen that only one species, M.dispar, was found and at that on only one occasion.

- tissues

Standard sampling sites from four (19,20,22,25) of the five calves that died during the acute pneumonic episode were examined for the presence of Pasteurella sp. The presence of P.haemolytica A1 was confirmed in the tissues of the upper and lower respiratory tract of all four animals.

- in vitro sensitivity patterns

In vitro sensitivity tests were carried out on the 17 P.haemolytica A1 isolates obtained from nasopharyngeal swabs taken from the calves in this study. The results are summarised in Table 32.

An identical resistance profile was obtained in 15 of these isolates obtained from a total of 8 different calves (14,17,18,19,20,23,25). The same pattern was found in similar studies carried out on tissue isolates from four of the five calves that died (19,20,22,25). An isolate of P.haemolytica A1 with a clearly different sensitivity pattern was obtained on two occasions from a single calf (24) on swabs collected on days 10 and 26 (Table 32).

Serology

On admission, two calves were found to have reciprocal serum antibody (IHA) titres to P.haemolytica A1. One of these (24) survived the first month of life but developed chronic respiratory disease and was

TABLE 32. The results of in vitro sensitivity tests carried out on 17 isolates of P.haemolytica A1 during the course of an outbreak of bovine pneumonic pasteurellosis

Calf no.	18	19	25	17	18	20	22	24	22	23	20	18	18	23	24	14	23
Day post-admission	6	6	6	10	10	10	10	10	13	13	17	20	26	26	26	29	29
Ampicillin	S	S	S	S	S	S	S	R	S	S	S	S	S	S	R	S	S
Chloramphenical	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Lincomycin	R*	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Oxytetracycline	S	S	S	S	S	S	S	R	S	S	S	S	S	S	R	S	S
Penicillin	S	S	S	S	S	S	S	R	S	S	S	S	S	S	R	S	S
Streptomycin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Co-trimoxazole	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

(*S = sensitive; R = resistant)

slaughtered on humane grounds on day 45 of the study; the other (25) died on day 9. Three other calves developed reciprocal titres of 4 or 8 over the first 22 days of the study; one of these (20) on day 25, one (23) died of the effects of chronic respiratory disease on day 52 and the third (14) survived in good health.

DISCUSSION

Many different studies have been carried out since the mid-1960's on very young calves acquired from local markets and admitted to the premises in which the above investigations were conducted. The bulk of such studies (100,135,192) have been aimed at investigating various aspects of neonatal calf diarrhoea with particular reference to the importance of maternally-derived (colostral) globulins in deciding the fate of young calves during their first month of life. The results of such studies have revealed that calves, agamma or markedly hypogammaglobulinaemic on admission, frequently die either of colisepticaemia or the effects of severe neonatal diarrhoea; in contrast, calves with high concentrations of serum globulins maintained under identical conditions, may or may not develop diarrhoea but rarely die (135). Other studies, carried out on the same type of calves, maintained under the same conditions, have revealed that if calves become pneumonic and die within the first month of admission, the problem is usually limited to individuals that have recently been very severely diarrhoeic and the specific lesion is

almost always of an acute exudative nature (159). More widespread pneumonia usually arises after one month and is usually non-fatal; this time the lesions are of the "cuffing" variety (4,5,159).

Given this common pattern of events, the original aim of this study was to examine nasopharyngeal isolates twice weekly over a period of two months, with sequential (weekly) slaughterings of pairs of calves in order to investigate the nature and severity of any pulmonary lesions and tissue microbiology. However, the unexpected and very unusual development of an acute, widespread pneumonia soon after admission was considered to be sufficiently interesting to justify a change of plans aimed at studying the natural history of this event in the absence of any form of therapy.

As already stated, an acute, febrile respiratory syndrome developed within a few days of admission. Such was the dramatic nature of the incident that by day 3 one calf had died of pneumonia and four others were pneumonic; by day 11 all nine of the surviving animals were affected to a greater or lesser extent. In all, five calves died between days 3 and 26 and four of the five that survived their first month of life died or were slaughtered on humane grounds because of chronic irreversible lung damage. The pathological features that were found in the fatal cases were of an extensive exudative and fibrinous pneumonia superimposed, in one instance, upon severe chronic suppurative pneumonia. Tissue bacteriology revealed the presence in the upper

and lower respiratory tract of P.haemolytica A1. Thus, the problem exhibited all of the classical features of bovine pneumonic pasteurellosis (80).

Field studies carried out within the last decade in Scotland (80) have demonstrated that bovine pneumonic pasteurellosis (transit fever) is most commonly found as a problem of newly-weaned, recently assembled single-suckled (ranch-type) beef calves. The organism generally associated with such outbreaks has been P.haemolytica A1 (10) and there is increasing field and experimental evidence indicating that this biotype at least is capable of being a primary pathogen for the bovine respiratory tract (80,81). A limited number of outbreaks of what has appeared to be primary pneumonic pasteurellosis have been encountered and investigated by workers from the Glasgow Veterinary School in groups of very young, artificially reared and intensively managed dairy-cross calves (Gibbs, unpublished observations) although the opportunity has never been available to study such problems in as much detail as in the present study.

The origin of the infection that caused the present problem is uncertain and the fact that one calf was coughing and four calves were febrile on admission, including the first to die, cannot be overlooked. Thus, it is possible that some calves were infected with the causal agent on admission; against this, however, is the fact that no calves were found to be infected by nasopharyngeal swabbing until six days post-admission. In fact, an alternative explanation exists and that is that

the infection was acquired by horizontal transmission from experimental calves in nearby loose-boxes. Such a possibility is supported by the fact that horizontal transmission of P.haemolytica A1 has been described (80,81) and that studies involving P.haemolytica A1 of an identical in vitro sensitivity profile to the causal agent were being carried out at the same time as this present study, albeit in quite separate accommodation sited some 30 metres away. The isolation of another strain of P.haemolytica A1, again as judged by sensitivity profile, merely reflects the complex nature of the problem at hand and underlines the potential dangers of relying totally upon information derived from an insufficient number of nasopharyngeal swabs. Similarly, the fact that the five calves that died were all mildly or moderately diarrhoeic when admitted is of unknown significance. However, it should be noted that diarrhoea was not thought to have been the cause of death in any of these cases, nor would this have been expected given the levels of colostral-derived serum globulins present in the calves on admission (135).

The fact that the original experimental design included taking nasopharyngeal swabs from each calf twice weekly allowed for the relatively early detection of P.haemolytica A1, that is six days following admission. However, by this stage one calf had already died and had been examined pathologically and eight of the nine surviving calves were exhibiting signs of an acute, febrile pneumonia. Thus, examination by nasopharyngeal

swab was not really necessary for the diagnosis of pneumonic pasteurellosis to be made. It did, though, act as (retrospective) diagnostic support and gave the opportunity for in vitro sensitivity tests to be carried out. In this particular outbreak, 15 of the 17 nasopharyngeal swab isolates exhibited the same sensitivity profiles as did the tissue isolates from fatal cases. However, this is not always the case in classic outbreaks of the disease (10) and therefore, information obtained from upper tract swabbing may sometimes be misleading. As in the earlier studies, a large number of bacterial species were obtained by nasopharyngeal swab, mostly it would appear, as the result of contamination rather than active infection although this view cannot be completely substantiated on the evidence available. In contrast, only one mycoplasmal isolation was made, a finding that might reflect on the far younger age of the calves compared with the earlier studies.

The administration of antibiotics, usually Oxytetracycline hydrochloride, by the parenteral route to all members of a group in which cases of classic pneumonic pasteurellosis are occurring, forms the basis of the current approach to treatment, at least in Scotland (80,201). Whether this would have been as successful as is usually the case (201), given the very young age of the calves in the present study, is open to some doubt. However, the very high mortality (50%) together with the fact that four of the five surviving

calves later succumbed to the effects of chronic, irreversible, suppurative pneumonia would surely indicate that, under different circumstances, prompt and intensive antibiotic therapy must always be considered as the major course of action.

CHAPTER 6

BACTERIAL AND MYCOPLASMAL INFECTIONS OF THE RESPIRATORY TRACT OF CHRONICALLY PNEUMONIC CATTLE

CHAPTER 6. BACTERIAL AND MYCOPLASMAL INFECTIONS OF THE RESPIRATORY TRACT IN CHRONICALLY PNEUMONIC CATTLE.

INTRODUCTION

While certain cattle pneumonias, for example contagious bovine pleuro-pneumonia (CBPP), tuberculosis and fog fever (acute bovine pulmonary emphysema, ABPE) have been recognised since the early 19th Century (193,226) it is only within the last 20-25 years that it has become appreciated just what a wide range of diseases exist under the umbrella term "pneumonia" (30,31,195,198).

For obvious reasons the greatest emphasis in terms of research priorities has been placed upon those respiratory diseases that may arise as major epizootics (e.g. CBPP), others that may pose public health dangers (e.g. tuberculosis) and those that give rise to high economic losses (e.g. the acute pneumonias of young calves, parasitic bronchitis, pneumonic pasteurellosis and fog fever). However, much is now known about the causes, prevalence and impact of a large number of other respiratory diseases that have been defined only relatively recently (30,31,32,193). It is, for example, now widely recognised that one such problem, chronic suppurative pneumonia, while conveniently viewed as a disease of individual cattle (195,197) may arise in such numbers following acute epizootics of viral, parasitic or bacterial pneumonia that it should best be viewed as a condition that may at least sometimes assume the proportions of a high morbidity event.

Unfortunately, such cases do not respond to antibiotic therapy despite the presence of lesions indicating that bacterial infections must play an important role in the initiation and persistence of the disease (195). As a consequence, many of these, and other superficially similar, cases are eventually referred to the Department of Veterinary Medicine of the University of Glasgow Veterinary School as teaching material. Perhaps, surprisingly, little effort has been made to carry out detailed studies into the mycoplasmal and bacterial infections that may exist in these cases. The opportunity was therefore taken to investigate the flora of the upper and lower respiratory tract in a small series of chronically pneumonic cattle that were admitted during the course of the earlier reported studies.

MATERIALS AND METHODS

Clinical material

The 14 cattle involved in this study formed part of the 400 or so routine bovine admissions that are admitted to the Department of Veterinary Medicine each year for student teaching purposes. These cattle are purchased from farmers in Scotland and Northern England usually after their veterinarians have identified them as poor prognosis cases needing prompt disposal.

On admission, the cattle were examined by at least one clinician and sampled for later biochemical, haematological and faecal analysis. Such procedures were carried out by conventional methods and repeated weekly

until the cattle were either slaughtered (13) or died (1). Post mortem examinations were carried out on all cases although in only six did this examination include histopathological investigations.

Diffuse fibrosing alveolitis (DFA)

Two pathologically-confirmed cases of DFA were examined in detail. Individual clinical, pathological and microbiological findings are recorded in Appendix 5,1-2.

- nasopharyngeal swabs

A total of 17 nasopharyngeal swabs were collected from these cases during their period of hospitalisation. Findings are summarised in Table 33. It can be seen that a wide range of bacteria were found but by far the commonest isolate was A.calcoaceticus at 31% of total isolates.

All swabs were examined for mycoplasmas but only one isolation was made, that being M.bovirhinis, from case no. 96737.

- upper tract and associated lymphatic tissues

Individual isolations from case nos. 96141 and 96737 are recorded in Appendix 2,1-2. A summary of the combined findings is presented in Table 34.

Nine different bacterial species were isolated on a total of 23 occasions. The commonest species isolated, Micrococcus sp. (52%) was found at almost all sites sampled in both cases. The only other species to be found in any number, Proteus sp. was confined to the tissues of

TABLE 33. Bacteria isolated from nasopharyngeal swabs collected from two pathologically-confirmed cases of DFA.

	Case nos.		
	96141	96737	
No.of Nph swabs collected	(10)	(7)	Total (%) isolations
A.calcoaceticus	9	3	12 (31)
B.coagulans	2	2	4 (10)
Flavobacterium	2	1	3 (8)
A.lignieresii	-	2	2 (5)
A.viridans	2	-	2 (5)
C.bovis	1	1	2 (5)
Micrococcus sp.	2	-	2 (5)
M.(B.)catarrhalis	2	-	2 (5)
M.(M.)liquefaciens	-	2	2 (5)
P.haemolytica A1	-	2	2 (5)
B.lentus	1	-	1 (3)
Moraxella sp.	-	1	1 (3)
N.subflava	-	1	1 (3)
P.haemolytica A2	-	1	1 (3)
S.aureus	1	-	1 (3)
S.bovis	1	-	1 (3)
Total	23	16	39

(* nasopharyngeal)

TABLE 34. Bacteria isolated from the upper respiratory tract and associated lymphatic tissues of two pathologically-confirmed cases of DFA.

	Sampling sites						
	NC	NP	L	TR	TON	RTN	Total(%)
Micrococcus sp.	2	2	2	4	1	1	12 (52)
Proteus sp.	2	-	1	-	1	-	4 (17)
Bacillus sp.	-	1	-	-	-	-	1 (4)
Corynebacterium sp.	-	1	-	-	-	-	1 (4)
M.(M.)nonliquefaciens	-	1	-	-	-	-	1 (4)
Moraxella sp.	1	-	-	-	-	-	1 (4)
N.subflava	-	-	-	-	1	-	1 (4)
S.aureus	-	-	-	-	-	1	1 (4)
Staphylococcus sp.	-	-	-	-	-	1	1 (4)
Total	5	5	3	4	3	3	23

only one animal (96141). All sites were examined for mycoplasmas but none were found.

- lower tract and associated lymphatic tissues

Individual isolations from case nos. 96141 and 96737 are recorded in Appendix 5,1-2. A summary of the combined findings is presented in Table 35.

It can be seen that only two bacterial species were isolated on ten separate occasions; of these, the three isolations of M.(M.)nonliquefaciens came from one case (96141) and the seven isolations of Micrococcus sp. from the other (96737). No mycoplasmas were isolated.

Chronic suppurative/non-suppurative pneumonia

Four pathologically-confirmed cases of chronic suppurative/non-suppurative pneumonia were examined in detail. Individual clinical, pathological and microbiological findings are recorded in Appendix 5,3-6. The ages of the four animals concerned ranged from six weeks to four years. An examination of histories together with a consideration of the nature, extent and siting of the various pathological features strongly suggested that the youngest calf had become a respiratory cripple following an earlier episode of acute pneumonia, the two yearlings had previously suffered from parasitic bronchitis and the adult animal had developed an inhalation pneumonia subsequent to having been affected with milk fever.

TABLE 35. Bacteria isolated from the lower respiratory tract and associated lymphatic tissues of two pathologically-confirmed cases of DFA.

	Sampling sites				
	RC	RM	RD	BR	Total(%)
Micrococcus sp.	2	2	2	1	7 (70)
M.(M.)nonliquefaciens	2	1	-	-	3 (30)
Total	4	3	2	1	10

- nasopharyngeal swabs

The four cases underwent nasopharyngeal swabbing on a total of 30 occasions (Table 36). A total of 59 isolates were obtained involving 22 different bacterial species (Table 36), the commonest of which was A.calcoaceticus at 14% of total isolates. P.haemolytica A1 was isolated on three occasions from one case (97439) and P.multocida on one occasion only from another (96887).

Mycoplasma bovirhinis was isolated from one case (97439) on one occasion only.

- upper tract and associated lymphatic tissues

Individual isolations from these four animals are recorded in Appendix 5,3-6. A summary of the combined findings is presented in Table 37.

The number of isolations made from the four animals ranged from six to 15 and between four and eight bacterial species were encountered.

In all, a total of 38 different isolations were made involving 14 bacterial species. As with nasopharyngeal swab isolations, the commonest species encountered, at 18% of total isolations, was A.calcoaceticus. Pasteurella haemolytica A1 was isolated from three sites (twice from nasal conchus samples and once from nasopharynx) obtained from the same animal that was positive for this organism on nasopharyngeal swab. Pasteurella haemolytica A2 was also isolated from one site (nasopharynx) of the same animal.

TABLE 36. Bacteria isolated from nasopharyngeal swabs collected from four pathologically-confirmed cases of chronic suppurative/ non-suppurative pneumonia.

	Case nos.				
	96203	96887	97439	97669	
Age	4 years	1 year	10 months	6 weeks	
Previous respiratory problem	Inhalation pneumonia	Parasitic bronchitis	Parasitic bronchitis	Acute pneumonia	
No.Nph. swabs collected	(6)	(9)	(9)	(6)	Total(%)
A.calcoaceticus	5	-	1	2	8 (14)
Micrococcus sp.	2	1	1	2	6 (10)
Bacillus sp.	-	-	2	3	5 (9)
M.(B.)catarrhalis	1	-	1	3	5 (9)
N.subflava	1	-	2	2	5 (9)
A.viridans	2	2	-	-	4 (8)
N.pharyngisatus	-	3	-	-	3 (5)
P.haemolytica Al	-	-	3	-	3 (5)
S.aureus	-	-	2	1	3 (5)
Aerococcus sp.	-	-	-	2	2 (3)
C.bovis	-	2	-	-	2 (3)
Flavobacterium sp.	1	-	-	1	2 (3)
M.(M.)nonliquefaciens	-	2	-	-	2 (3)
A.lignieresii	-	-	1	-	1 (2)
B.circulans	-	1	-	-	1 (2)
C.murium	-	1	-	-	1 (2)
E.coli	1	-	-	-	1 (2)
Moraxella sp.	1	-	-	-	1 (2)
P.multocida	-	1	-	-	1 (2)
Proteus sp.	-	-	1	-	1 (2)
S.bovis	-	1	-	-	1 2)
Streptococcus sp.	-	-	-	1	1 (2)
Total	14	14	14	17	59

TABLE 37. Bacteria isolated from upper respiratory tract and associated lymphatic tissue of four pathologically-confirmed cases of chronic suppurative/non-suppurative pneumonia.

	Sampling sites						
	NC	NP	L	TR	Ton	RTN	Total(%)
A.calcoaceticus	-	1	-	3	2	1	7 (18)
Micrococcus sp.	1	1	2	-	-	1	5 (13)
A.lignieresii	1	-	-	1	1	1	4 (10)
Proteus sp.	-	1	1	2	-	-	4 (10)
A.viridans	-	1	-	-	1	1	3 (8)
N.subflava	-	-	1	-	1	1	3 (8)
P.haemolytica A1	2	1	-	-	-	-	3 (8)
Flavobacterium sp.	-	-	-	2	-	-	2 (5)
S. aureus	2	-	-	-	-	-	2 (5)
C.murium	1	-	-	-	-	-	1 (3)
K.oxytoca	-	-	-	-	-	1	1 (3)
M.(B.)catarrhalis	-	1	-	-	-	-	1 (3)
P.haemolytica A2	-	1	-	-	-	-	1 (3)
S.lactis	-	-	1	-	-	-	1 (3)
Total	7	7	5	8	5	6	38

- lower respiratory tract and associated lymphatic tissue

Individual isolations from these cases are recorded in Appendix 5,3-6. A summary of the combined findings is presented in Table 38.

Between seven to nine isolations were made from the lower tract tissues, involving two to six different bacterial species.

In all, a total of 33 different isolations were made involving twelve different species of bacteria. The commonest isolate was A.calcoaceticus (33% of total isolates) although this organism was not found in one of the cases (96203). No Pasteurellae were detected in the tissues of any of the cases.

Miscellaneous pneumonias

While the previous studies were being carried out, the opportunity was taken to collect nasopharyngeal swab samples from a number of cattle of various ages all of which had been referred as cases of chronic pneumonia. Post-mortem examinations were carried out on all of these cases but was limited to a gross examination of the animals concerned. Tissue microbiology was not carried out.

Details regarding the eight individual cases are recorded in Appendix 5,7. Nasopharyngeal swab findings are summarised in Table 39.

In all, a total of 71 nasopharyngeal swabs were examined for bacteria. One hundred and forty-five separate isolations were made involving twenty-seven

TABLE 38. Bacteria isolated from lower respiratory tract and associated lymphatic tissue of four pathologically-confirmed cases of chronic suppurative/non-suppurative pneumonia.

	Sampling sites				
	RC	RM	RD	BR	Total(%)
A.calcoaceticus	3	3	3	2	11 (33)
Proteus sp.	2	2	1	-	5 (15)
A.lignieresii	2	1	-	1	4 (12)
C.xerosis	-	1	1	1	3 (9)
E.coli	-	-	1	1	2 (6)
Micrococcus sp.	-	-	1	1	2 (6)
A.viridans	-	-	-	1	1 (3)
B.lentis	-	-	1	-	1 (3)
C.bovis	-	1	-	-	1 (3)
C.hofmannii	-	1	-	-	1 (3)
Lactobacillus sp.	-	-	1	-	1 (3)
M.(B.)catarrhalis	-	-	1	-	1 (3)
Total	7	9	10	7	33

TABLE 39. Bacteria isolated from nasopharyngeal swabs collected from eight pathologically-confirmed cases of chronic pneumonia

No.of Nph* swabs collected	CASE NUMBERS								Total(%)
	101904	102045	102215	103105	103393	104016	104254	104427	
S.aureus	-	3	2	10	2	3	-	1	21 (15)
A.lignieresii	3	-	-	5	1	2	1	1	13 (9)
A.pyogenes	2	-	-	5	2	-	-	2	11 (8)
Bacillus sp.	-	2	4	-	2	-	2	1	11 (8)
Streptococcus sp.	1	3	-	2	2	-	1	2	11 (8)
M.(B.)catarrhalis	3	1	-	-	1	3	-	1	9 (6)
A.viridans	-	3	-	-	1	2	-	2	8 (6)
Branhamella sp.	-	-	1	3	2	2	-	-	8 (6)
M.rosens	-	3	-	-	-	3	-	-	6 (4)
Staphylococcus sp.	2	-	-	-	2	-	2	-	6 (4)
A.calcoaceticus	1	-	-	2	-	-	-	1	4 (3)
Flavobacterium sp.	-	1	-	-	-	2	-	1	4 (3)
Moraxella sp.	-	-	1	-	-	2	-	1	4 (3)
S.bovis	1	-	-	-	-	2	1	-	4 (3)
B.coagulans	-	1	-	-	-	2	-	-	3 (2)
Micrococcus sp.	-	-	-	-	-	-	1	2	3 (2)
P.multocida	-	-	1	1	1	-	-	-	3 (2)
S.faecalis	-	1	-	-	-	2	-	-	3 (2)
Corynebacterium sp.	-	-	-	-	-	1	1	-	2 (1)
Enterobacteria sp.	-	-	-	2	-	-	-	-	2 (1)
M.(M.)lacunata	-	1	-	-	1	-	-	-	2 (1)

TABLE 39. contd.

No.of Nph* swabs collected	CASE NUMBERS 101904	102045	102215	103105	103393	104016	104254	104427	Total(%)
P.haemolytica**	-	-	-	2	-	-	-	-	2 (1)
Actinobacillus sp.	-	1	-	-	-	-	-	-	1 (1)
Aerococcus sp.	-	-	-	-	-	-	1	-	1 (1)
A.faecalis	-	-	-	1	-	-	-	-	1 (1)
N.subflava	-	-	-	-	-	-	1	-	1 (1)
S.pneumoniae	-	-	-	-	1	-	-	-	1 (1)
Total no. of isolates	13	20	9	33	18	26	11	15	145
Total no. of species	7	11	5	10	11	12	9	11	27

(* Nph. = nasopharyngeal. ** P.haemolytica; untyped but not A1 or A2)

bacterial species. The number of isolations made per case ranged from nine to 33; the number of bacterial species identified in each individual case ranged from five to 12.

The commonest bacterial isolate was S.aureus, although this figure was distorted by the very large number of isolations of that organism from one particular animal (103105). This animal also provided far higher isolation rates than the others of two other organisms namely A.lignieresii and A.pyogenes.

DISCUSSION

Although the two respiratory diseases that were investigated in detail, that is diffuse fibrosing alveolitis and chronic suppurative pneumonia, have similar presenting signs of chronic respiratory disease and weight-loss it is nevertheless possible to differentiate between them and to make a reasonably confident diagnosis on the basis of clinical signs alone (197,198). Pathologically they are quite different (31) with diffuse pulmonary fibrosis and alveolar epithelial hyperplasia and metaplasia in diffuse fibrosing alveolitis and localised, usually cranial (dependant) lobe lesions in chronic suppurative pneumonia.

It would appear that detailed studies on the respiratory tract flora of cases of diffuse fibrosing alveolitis have not been carried out; however, the nature of the pathology and histopathology of the condition is such that "secondary infection" would not seem to be a

major component of the problem. In contrast, there is ample evidence (e.g. areas of advancing acute exudative pneumonia, architectural damage, presence of pus, fever etc.) that in chronic suppurative pneumonia additional infections, particularly bacterial infections, are of major importance. While the latter type of case is notorious for not responding to antibiotic therapy the reason that is often advanced to account for this is that clearance mechanisms are diminished or abated and the presence of lesions in the cranial (dependent) lobes results in persistent infections. On the other hand, detailed microbiological studies would not appear to have been conducted on such cases either.

Nasopharyngeal swabs from cases of diffuse fibrosing alveolitis yielded a total of 39 positive isolations with a range of ten different bacterial species per case. In all, sixteen different species were identified. In both cases, the most frequently identified species was A.calcoaceticus. Overall, almost a third of all isolates were of this latter species.

Similar studies on four cases of chronic suppurative / non suppurative pneumonia revealed very similar results, insofar as while more isolations were made (59 as compared with 39) the fact that more than twice as many nasopharyngeal swabs were collected from the latter types of cases than from the two cases of diffuse fibrosing alveolitis. Similarly, the larger number (22) of species identified could be explained on the basis of more swabs increasing the chance of more

species being detected. As with diffuse fibrosing alveolitis, the commonest isolate was A.calcoaceticus.

Studies on tissues removed from the upper respiratory tract again revealed more isolations and a wider range of bacterial species from the cases of chronic suppurative/non-suppurative pneumonia. However, while the most frequent isolate was again A.calcoaceticus in these latter cases, the commonest species in the upper tract tissues of the two cases of diffuse fibrosing alveolitis was Micrococcus sp.

It was not until interest was focussed on tissue isolations from lower respiratory tract tissues that clear differences between the two types of case became apparent. It was found that twice as many cases of chronic suppurative/non-suppurative pneumonia yielded more than three times as many isolates than the cases of diffuse fibrosing alveolitis. Moreover, while 12 different species were isolated from the chronic suppurative/non-suppurative pneumonia cases, the total for the others was two. Once again, the commonest isolate in the chronic suppurative/ non-suppurative cases was A.calcoaceticus, that for the cases of diffuse fibrosing alveolitis was Micrococcus sp., due almost entirely to isolations from one single individual.

A number of additional interesting findings emerged from the study. First, was the total lack of A.pyogenes (formerly C.pyogenes) from the swabs and tissues of the pathologically-confirmed cases of chronic suppurative/non-suppurative pneumonia. The second feature

of note was that while this last-named organism, S.aureus and A.lignieresii were the three most commonly isolated bacteria from nasopharyngeal swabs taken from the eight additional cases of chronic pneumonia, this situation was almost entirely due to the effects of isolation rates from one individual. The third point of interest was that while P.haemolytica A1 was isolated on a total of six occasions from swabs and upper tract tissues of one case of chronic suppurative pneumonia (97439), this organism was not isolated from lower tract tissues nor were any lesions that are generally attributable to bovine pneumonic pasteurellosis found in this particular animal. Finally, simultaneous (tissue) infections of P.haemolytica A1 and A2 were detected on a small number of occasions, a situation that has been noted as being a relatively rare event (10).

In short, the results of the above study have clearly shown that it is possible to detect a greater number of infections involving a wider range of bacterial species, from the lower tract tissues of cases of chronic suppurative/non-suppurative pneumonia than from cases of diffuse fibrosing alveolitis. As in several of the earlier studies, the organism most frequently isolated was A.calcoaceticus. Clearance mechanisms are almost certainly diminished in cases of chronic suppurative pneumonia due, first, to the nature of the airways damage and the consequent presence of debris and, second, to the vertical nature of the major airways in the dependent (most commonly affected) lobes. Hence, greater numbers of

bacteria could be explained on the basis of diminished clearance; however, the regular occurrence of the above organism in a number of different pulmonary disorders, at least suggests that it may well be exerting deleterious effects. Such a possibility seems all the more likely in view of its known pathogenic capability in humans (22). Such a situation can only be properly investigated under strictly controlled experimental circumstances.

GENERAL DISCUSSION

For many years the major interest of most research workers who have been involved in studies on the bovine respiratory system has been in the field of the various forms of pneumonia. Their prevalence and economic impact under almost all forms of cattle husbandry are such that to do otherwise would have been almost totally without justification. However, as already stated, the effort and expenditure that has been invested has only been rewarded by the development of truly effective vaccines or other prevention techniques in a relatively small number of very specific instances.

Detailed investigational and experimental work has established that the pneumonic bovine lung frequently harbours a wide range of micro-organisms, particularly mycoplasmas and bacteria. Unfortunately, their very presence appears to have often clouded the fact that little or no evidence exists to support the view that, if present (at least in the lower airways) such organisms must surely be adversely affecting the host animal. In short, these organisms are frequently referred to as "secondary" or "opportunistic" invaders and their (often presumed) presence is widely taken to be sufficient justification for antimicrobial therapy - even in those pneumonias where the primary cause is likely, or certain, to be non-microbial.

A number of earlier workers have, however, also demonstrated the presence of a similar range of mycoplasmas and bacteria in the lungs of normal (non-

pneumonic) cattle. However, it would seem reasonable to suppose that, given a normal array of pulmonary defence mechanisms, these agents are usually only transient residents perhaps having been inadvertently inhaled along with eructated ruminal gases. The species involved would certainly support this contention insofar as they are very frequently soil or pasture-born bacteria. Thus, the view that the distal airways are normally sterile, while possibly true for man, the mouse and perhaps other monogastrics, is most certainly not true for ruminants - or, at any rate, cattle.

The experimental studies that were carried out in the present programme of work, investigated the question of mycoplasmal and bacterial infections of calves with experimentally-induced pneumonias in two different ways.

In the study in which calves were infected with PI3 virus, the extent (but not the nature) of the subsequent pulmonary inflammatory response was reduced when the antiprostaglandin compound, flunixin meglumine, was administered. In the first of two groups of calves studied in this way (the "Sinclair" calves) this reduction in pulmonary consolidation was associated with a reduction of total mycoplasmal and bacterial isolations but not the range of species isolated from the lower tract and associated lymphatic tissue. The situation in the second group studied (the "Borland" calves) was very similar with regard to mycoplasmas but the reverse of the former situation with regard to bacteria. However, the response to flunixin meglumine had been noted as being

far less obvious in the latter calves possibly because of the presence of fairly extensive (albeit sub-clinical) chronic pneumonia, very probably the result of M.bovis infection.

In the second experimental study, two groups of calves were compared one of which was experimentally-infected with lungworms, the other being left as an uninfected control group. These latter (non-pneumonic) calves were found to harbour a large number of different bacterial species in their lower airways and associated tissues. In the calves slaughtered during the patent phase of the disease, the total number of bacterial isolations made from the various sampling sites in the lower tract and associated tissues and the total number of bacterial species isolated were both twice the numbers obtained from similar sites in the uninfected controls. This situation was similar for mycoplasmas but less impressive due to the smaller numbers involved.

These studies served to illustrate the fact that additional microbiological burdens within the lower airways may be found when pneumonic cattle are compared with non-pneumonic cattle and when different levels of pneumonic consolidation are compared. If there appears to be a difference between the first of these two findings and the results of earlier studies cited above, then perhaps this is a function of the fact that this is the first time that such detailed work has been carried out on cattle with parasitic bronchitis. Of course, it is not possible to state with certainty why these organisms were

present in greater numbers in the test cattle; it is possible that defective clearance mechanisms were merely reducing the effects, for example, of the mucociliary "escalator" effect. However, their presence would appear to justify the use of antibiotics in cases of viral or parasitic pneumonia particularly in the light of the fact that there had been a pronounced febrile response in the patent cases of parasitic bronchitis.

In neither of the above studies was there any clearcut correlation between upper and lower tract isolates. In many individual instances the isolations made from either nasopharyngeal swabs or upper tract tissues never reflected the infections present in the lower airways. Thus, the chance-occurrence of an acute outbreak of pneumonic pasteurellosis as reported in Chapter 5 gave a good opportunity to study this question in calves suffering from a severe and, apparently, uncomplicated bacterial pneumonia.

The incident in question was in many ways quite remarkable not least insofar as it very quickly came to affect the whole group of ten calves resulting in the prompt death of five and the death or necessary slaughter on humane grounds of four of the remainder later in the study. Certainly, the causal agent, P.haemolytica A1, was repeatedly isolated by nasopharyngeal swab during and after the acute incident. On the other hand, given the availability of dead calves (one died within three days of admission), the characteristic appearance of the pulmonary lesions and the opportunities for low tract

tissue bacteriology it is suggested that a diagnosis based merely upon the results of nasopharyngeal swab isolations might well have proved misleading and, anyway, would have taken as long as a proper investigation of the dead calves. Most important of all, if such an approach to diagnosis had been carried out in certain of the other groups of calves studied, a diagnosis of bovine pneumonic pasteurellosis might have been made in the absence of any low tract Pasteurella infections whatsoever.

The results of bacteriological studies carried out on the cases of chronic pneumonia confirmed the wide range of bacteria that may exist at all levels of the respiratory tract in chronic suppurative and/or non-suppurative pneumonia and the relative dearth of bacteria in the cases of diffuse fibrosing alveolitis. On the basis of the histopathological appearances of these pneumonias, this is hardly surprising.

Finally, of the wide range of bacteria that have been isolated from the 54 individuals of various ages involved in these studies, one organism, A.calcoaceticus, stands out as being a common resident of the upper and lower respiratory tract of both pneumonic and non-pneumonic cattle. Given that this organism is now an acknowledged cause of nosocomial infection in the human, it is suggested that further investigations should be carried out in calves - in order to confirm or refute its possible role as, at least, a particularly aggressive "secondary" invader.

APPENDIX 1,1-3

(Procedures cited in Chapter 2)

Appendix 1,1.

First-stage step for bacteria identification.

Microbiology

Date

Sample I.D.

Character of Colony

Gram's Stain

Purity

Motility

Aerobic

Anaerobic

Catalase

Oxidase

Glucose

Oxidate/fermentation

Bacteria

Appendix 1,2.

Classification of Bacteria into Gram (+)ve and Gram (-)ve.

Gram (+)ve bacteria	Gram (-)ve bacteria
Micrococcus	Bacteroides
Staphylococcus	Veillonella
Streptococcus	Neisseria
Pediococcus	Branhamella
Gemella	Acinetobacter
Anaerobic cocci	Moraxella
Kurthia	Brucella
Corynebacterium	Bordetella
Listeria	Chromobacterium lividum
Erysipelothrix	Alcaligenes
Lactobacillus	Flavobacterium
Arachinia	Pseudomonas
Propionibacterium	Actinobacillus
Actinomyces	Pasteurella
Bifidobacterium	Necromonas
Clostridium	Cardiobacterium
Bacillus	Chromobacterium violaceum
Nocardia	Benecka
Mycobacterium	Vibrio
	Plesiomonas
	Aeromonas
	Haemophilus
	Campylobacter
	Streptobacillus
	Mycoplasmas

APPENDIX 1,3. API 20E. Identification of non-enteric gram-negative rods.

INTERPRETATION TABLE

TESTS	SUBSTRATES		RESULTS NEGATIVE	RESULTS POSITIVE
NO_3	potassium nitrate	reduction of nitrates to nitrites	NIT 1 + NIT 2/5min: colourless	NIT 1 + NIT 2/5min: pink-red
		reduction of nitrates to nitrogen	Zn/5min: pink	Zn/5min: colourless
TRP	tryptophan	indole production	TRP/3-5min: yellow	TRP/3-5min: red
GLU	glucose	acidification	blue or green	yellow
ADH	arginine	arginine dihydrolase	yellow	orange/pink/red
URE	urea	urease	yellow	orange/pink/red
ESC	aesculin	hydrolysis (8-glucosidase)	yellow	grey/brown/black
GEL	gelatin	hydrolysis (protease)	no pigment diffusion	diffusion of black pigment
PNPG	p-nitrophenyl-BD-galactopyranoside	B-galactosidase	colourless	yellow
GLU	Glucose	assimilation	transparent	opaque
ARA	arabinose	assimilation	transparent	opaque
MNE	mannose	assimilation	transparent	opaque
MAN	mannitol	assimilation	transparent	opaque
NAG	N-acetyl-glucosamine	assimilation	transparent	opaque
MAL	maltose	assimilation	transparent	opaque
GNT	gluconate	assimilation	transparent	opaque
CAP	caprate	assimilation	transparent	opaque
ADI	adipate	assimilation	transparent	opaque
MLT	malate	assimilation	transparent	opaque
CIT	citrate	assimilation	transparent	opaque
PAC	phenyl-acetate	assimilation	transparent	opaque
OX	tetramethyl-p-phenylene diamine	cytochrome oxidase	colourless	violet

APPENDIX 2,1-20

(Individual mycoplasmal and bacterial isolations; pathological features; investigation reported in Chapter 3)

APPENDIX 2,1. Calf No.1 (Sinclair)

MICROBIOLOGICAL FINDINGS (PRE-MORTEM):

Day	Bacteriology	Mycoplasmology
-14	P.haemolytica A1 A.lignieresi	negative
-7	P.haemolytica A1 M.(M.)bovis	Ureaplasma sp. M.bovis
0	A.lwoffii M.(M.)nonliquefaciens	M.bovirhinis
+4	M.(M.)bovis N.subflava	M.bovirhinis
+7	M.(M.)bovis M.(B.)catarrhalis	M.bovirhinis M.dispar

MICROBIOLOGICAL FINDINGS (POST-MORTEM): DAY 9

Tissue	Bacteriology	Mycoplasmology
NC	M.(B.)catarrhalis A.anitratus	A.laidlawii
Tr	M.(B.)catarrhalis	M.bovirhinis
RC	A.faecalis	negative
RM	M.(B.)catarrhalis A.faecalis	M.bovirhinis
RD	A.faecalis	negative
BrLN	negative	negative

Pathological findings: There were a few scattered lobules of collapse in the anterior lobes which were soft, deep red-purple and, on section, the airways contained much thick mucopus. Microscopically the lesions represented a fairly mild cuffing pneumonia with several foci of bronchiolitis obliterans.

All six tissues were negative by immunofluorescence for PI_3 virus.

APPENDIX 2,2. Calf No.2 (Sinclair)

MICROBIOLOGICAL FINDINGS (PRE-MORTEM):

Day	Bacteriology	Mycoplasmology
-14	Micrococcus sp.	negative
-7	M.(M.)bovis F.meningosepticum	Ureaplasma sp. M.bovirhinis
0	A.lwoffii M.(M.)nonliquefaciens	Ureaplasma sp.

MICROBIOLOGICAL FINDINGS (POST-MORTEM): DAY 4

Tissue	Bacteriology	Mycoplasmology
NC	M.(M.)bovis S.aureus	negative
Tr	negative	M.bovis
RC	negative	negative
RM	negative	negative
RD	negative	negative
BrLN	negative	negative

Pathological findings: There were only a few scattered lobules of reaction in the anterior lobes. There was a moderately severe tracheitis. Microscopically, the lungs were virtually non-pneumonic, with a mild bronchitis, mild bronchiolar reaction consisting of increased numbers of peribronchiolar lymphocytes forming a layer. There were a few areas with alveolar epithelial hyperplasia with some oedema and intra-alveolar haemorrhage.

There was a marked neutrophilic infiltrate into the bronchial lymph node.

All tissues were negative by immunofluorescence for PI_3 virus except the right cranial lobe in which areas of fluorescence were seen within the alveolar tissue.

APPENDIX 2,3. Calf No.3 (Sinclair)

MICROBIOLOGICAL FINDINGS (PRE-MORTEM):

Day	Bacteriology	Mycoplasmology
-14	P.haemolytica A1	negative
-7	F.meningosepticum M.(M.)bovis	negative
0	A.anitratus A.lwoffii	negative
+4	M.(M.)bovis, A.anitratus	M.bovis
+7	A.pyogenes	M.bovirhinis
+9	C.bovis	A.laidlawii

MICROBIOLOGICAL FINDINGS (POST-MORTEM): DAY 12

Tissue	Bacteriology	Mycoplasmology
NC	A.anitratus	negative
Tr	A.anitratus	A.laidlawii, M.bovirhinis
RC	A.anitratus	M.bovis
RM	negative	A.laidlawii, M.bovis
RD	negative	M.bovis
BrLN	negative	M.bovis

Pathological findings: There was moderately severe consolidation in the right cranial lobe which was soft, grey, slightly oedematouss with thick creamy mucopus in the airways. Microscopically the areas of consolidation represented cuffing pneumonia with plugging of the airways with necrotic inflammatory exudate.

All six tissues were negative by immunofluorescence for PI_3 virus.

APPENDIX 2,4. Calf No.4 (Sinclair)

MICROBIOLOGICAL FINDINGS (PRE-MORTEM):

Day	Bacteriology	Mycoplasmology
-14	P.haemolytica A1	negative
-7	F.meningosepticum M.(M.)bovis	Ureaplasma sp. M.bovirhinis
0	M.(M.)bovis, M.(M.)nonliquefaciens	negative
+4	C.bovis A.anitratus	negative
+7	C.bovis, A.lwoffii	Ureaplasma sp., M.dispar
+9	Micrococcus sp.	A.laidlawii

MICROBIOLOGICAL FINDINGS (POST-MORTEM): DAY 12

Tissue	Bacteriology	Mycoplasmology
NC	negative	M.bovis
Tr	negative	A.laidlawii
RC	negative	M.bovis
RM	negative	A.laidlawii
RD	negative	negative
BrLN	negative	A.laidlawii

Pathological findings: There were a few scattered lobules of consolidation in the anterior lobes which were soft, grey-fawn, collapsed. There were thick clumps of yellow-green mucopus in the trachea and major airways. Microscopically the pulmonary lesions represented a mild bronchitis with a few peribronchiolar lymphoid aggregates and plugging of the bronchioles with necrotic inflammatory exudate.

All six tissues were negative by immunofluorescence for PI_3 virus.

APPENDIX 2,5. Calf No.5 (Sinclair)

MICROBIOLOGICAL FINDINGS (PRE-MORTEM):

Day	Bacteriology	Mycoplasmology
-14	M.(B.)catarrhalis	negative
-7	M.(M.)bovis	negative
0	M.(M.)bovis	M.bovirhinis
+4	M.(M.)bovis	A.laidlawii
+7	M.(B.)catarrhalis	negative
+9	M.(B.)catarrhalis A.anitratus	negative

MICROBIOLOGICAL FINDINGS (POST-MORTEM): DAY 12

Tissue	Bacteriology	Mycoplasmology
NC	A.anitratus	negative
Tr	A.anitratus	negative
RC	C.bovis	negative
RM	negative	negative
RD	negative	negative
BrLN	negative	negative

Pathological findings: There were only occasional foci of collapse which presented as mild cuffing pneumonia with necrotic inflammatory exudate in the airways.

All six tissues were negative by immunofluorescence for PI_3 virus.

APPENDIX 2,6. Calf No.6 (Sinclair)

MICROBIOLOGICAL FINDINGS (PRE-MORTEM):

Day	Bacteriology	Mycoplasmology
-14	M.(B.)catarrhalis	negative
-7	M.(M.)bovis	M.bovis
0	M.(M.)bovis	M.bovirhinis
+4	M.(M.)bovis	A.laidlawii
+7	M.(M.)bovis M.(B.)catarrhalis A.lignieresi	Ureaplasma sp. M.bovirhinis
+9	M.(M.)bovis	negative

MICROBIOLOGICAL FINDINGS (POST-MORTEM): DAY 12

Tissue	Bacteriology	Mycoplasmology
NC	A.lwoffii N.subflava	negative
Tr	N.subflava	M.bovis
RC	negative	M.bovirhinis
RM	negative	M.bovis
RD	negative	M.bovis
BrLN	negative	M.bovis

Pathological findings: There were many lobules of collapse scattered throughout all lobes; they were soft and deep red-purple in appearance. There was thick, creamy pus in the trachea and major airways. The lesions consisted of a mild cuffing reaction with many airways being plugged with necrotic inflammatory exudate.

All six tissues were negative by immunofluorescence for PI_3 virus.

APPENDIX 2,7. Calf No.7 (Sinclair)

MICROBIOLOGICAL FINDINGS (PRE-MORTEM):

Day	Bacteriology	Mycoplasmology
-14	M.(B.)catarrhalis	negative
-7	M.(B.)catarrhalis, M.(M.)nonliquefaciens	M.bovirhinis
0	Micrococcus sp.	M.bovirhinis
+4	M.(M.)bovis	M.bovis
+7	M.(M.)bovis, M.(B.)catarrhalis	M.bovis
+9	M.(M.)nonliquefaciens, M.(B.)catarrhalis	A.laidlawii

MICROBIOLOGICAL FINDINGS (POST-MORTEM): DAY 12

Tissue	Bacteriology	Mycoplasmology
NC	A.lwoffii, M.(B.)catarrhalis	M.bovirhinis
Tr	A.lwoffii	Ureaplasma sp., M.bovirhinis
RC	A.lwoffii	Ureaplasma sp., M.bovirhinis
RM	negative	M.bovirhinis
RD	negative	M.bovirhinis
BrLN	A.lwoffii	M.bovis

Pathological findings: The anterior portion of the right cranial lobe was consolidated, fibrous and pale grey-fawn and consisted of severe cuffing pneumonia with much necrotic inflammatory exudate in the airways. There was thick mucopus in the trachea and major airways; much of this would come from the right cranial lobe although there was a mild tracheitis and bronchitis in the other lobes.

All six tissues were negative by immunofluorescence for PI_3 virus.

APPENDIX 2,8. Calf No.8 (Sinclair)

MICROBIOLOGICAL FINDINGS (PRE-MORTEM):

Day	Bacteriology	Mycoplasmology
-14	P.haemolytica A1	negative
-7	P.haemolytica A1, M.(M.)bovis	M.bovirhinis
0	M.(M.)nonliquefaciens A.lwoffii	M.bovis
+4	M.(M.)bovis, A.anitratus	negative
+7	M.(M.)bovis	M.bovis, A.laidlawii

MICROBIOLOGICAL FINDINGS (POST-MORTEM): DAY 9

Tissue	Bacteriology	Mycoplasmology
NC	M.(M.)bovis	M.bovirhinis
Tr	negative	M.bovis, M.bovirhinis
RC	Micrococcus sp., A.anitratus	negative
RM	A.lwoffii	negative
RD	A.lwoffii	A.laidlawii
BrLN	Micrococcus sp.	M.bovis, M.bovirhinis

Pathological findings: There were a few lesions in the anterior lung lobes, most of which appeared as collapsed tissue except the right middle lobe which was firm, pale fawn-pink with fibrous adhesions to the cranial lobe. There was a marked tracheitis with thick yellow-green mucopus along its length and extending into the major bronchi. Microscopically, the lesions in the right middle lobe were of severe cuffing pneumonia with marked bronchiolitis obliterans and manh foci of alveolar epithelial hyperplasia. The other lesions consisted of a mild bronchitis and bronchiolitis.

All six tissues were negative by immunofluorescence for PI_3 virus.

APPENDIX 2,9. Calf No.9 (Sinclair)

MICROBIOLOGICAL FINDINGS (PRE-MORTEM):

Day	Bacteriology	Mycoplasmology
-14	P.haemolytica A1	negative
-7	A.lwoffii M.(B.)catarrhalis	M.dispar M.bovirhinis
0	Micrococcus sp.	negative

MICROBIOLOGICAL FINDINGS (POST-MORTEM): DAY 4

Tissue	Bacteriology	Mycoplasmology
NC	A.lwoffii M.(B.)catarrhalis	negative
Tr	A.lwoffii	M.bovis
RC	A.lwoffii	M.bovirhinis
RM	A.lwoffii	negative
RD	negative	M.bovis
BrLN	A.lwoffii	negative

Pathological findings: There were extensive areas of consolidation in the anterior lobes and anterior portion of the caudal lobes. The lesions in the anterior part of the cranial lobe were chronic and fibrous; they were pale pink-grey and characterised microscopically as severe cuffing pneumonia with bronchiolitis obliterans and a amarked neutrophilic exudation. The other lesions were soft, deep, red-purple and slightly collapsed. Microscopically there was bronchitis, bronchiolitis and alveolitis with thickening of the septa and the presence of small syncytia. The appearance of the bronchiolar epithelium was mostly of cytoplasmic vacuolation, regions of hyperplasia and occasional focal necrosis. There was an increase in peribronchiolar lymphocytes. Much of the inflammatory exudate in the airways was degenerating into an amorphous eosinophilic mass. Intracytoplasmic inclusion bodies were recognised in bronchial, bronchiolar and some alveolar cells.

The tissue from the right cranial lobe was found to be positive by immunofluorescence for PI_3, particularly in the bronchiolar epithelium.

APPENDIX 2,10. Calf No.10 (Sinclair)

MICROBIOLOGICAL FINDINGS (PRE-MORTEM):

Day	Bacteriology	Mycoplasmology
-14	P.haemolytica A1	negative
-7	M.(M.)bovis	negative
0	P.haemolytica A1	negative
+4	B.coagulans A.anitratus	negative
+7	A.lwoffii M.(M.)bovis	A.laidlawii
+9	M.(M.)bovis M.(B.)catarrhalis	negative

MICROBIOLOGICAL FINDINGS (POST-MORTEM): DAY 12

Tissue	Bacteriology	Mycoplasmology
NC	A.lwoffii M.(B.)catarrhalis	negative
Tr	A.lwoffii	Ureaplasma sp., A.laidlawii
RC	A.lwoffii	Ureaplasma sp., A.laidlawii
RM	A.lwoffii	Ureaplasma sp., A.laidlawii
RD	A.lwoffii	negative
BrLN	A.lwoffii	negative

Pathological findings: There was extensive consolidation in the anterior lobes of the lungs which were pale grey-fawn, firm with thick, sticky mucopus in the major airways and also in the trachea. There was a mild to moderately severe tracheitis. There were marked lesions of cuffing pneumonia in all lobes, particularly in the right cranial lobe together with bronchiolitis obliterans and a marked inflammatory exudate (macrophages and giant cells) in the alveoli. There was an obvious inflammatory exudate into the major airways.

All six tissues were negative by immunofluorescence for PI_3 virus.

APPENDIX 2,11. Calf No.11 (Borland)

MICROBIOLOGICAL FINDINGS (PRE-MORTEM):

Day	Bacteriology	Mycoplasmology
-14	A.anitratus M.(B.)catarrhalis	negative
-7	A.anitratus, M.(B.)catarrhalis	Ureaplasma sp. M.bovirhinis
0	Micrococcus sp. C.bovis	M.bovirhinis
+4	Micrococcus sp. C.bovis	M.bovirhinis
+7	M.(B.)catarrhalis, C.bovis	M.bovirhinis
+9	M.(M.)bovis M.(M.)nonliquefaciens	Ureaplasma sp. M.bovirhinis

MICROBIOLOGICAL FINDINGS (POST-MORTEM): DAY 11

Tissue	Bacteriology	Mycoplasmology
NC	M.(M.)bovis,M.(B.)pharyngis	M.bovirhinis
Tr	A.anitratus	M.bovis, M.bovirhinis, Ureaplasma sp.
RC	N.subflava	A.laidlawii
RM	negative	A.laidlawii
RD	negative	M.dispar, M.bovis
BrLN	negative	A.laidlawii, M.bovis

Pathological findings: There was moderately severe consolidation in the anterior lobes of the lungs which were deep red-purple, firm with thick yellow mucopus in the major airways. Microscopically the pulmonary consolidation was similar in all lobes with a moderately severe bronchitis, bronchiolitis and an obvious proliferative alveolitis with some alveolar collapse. There was a little bronchiolar epithelial necrosis and a few foci of fairly extensive intralobular haemorrhage.

All six tissues were negative by immunofluorescence for PI_3 virus.

APPENDIX 2,12. Calf No.12 (Borland)

MICROBIOLOGICAL FINDINGS (PRE-MORTEM):

Day	Bacteriology	Mycoplasmology
-14	A.anitratus A.lwoffii	M.bovirhinis
-7	M.(B.)catarrhalis	Ureaplasma sp.
0	Micrococcus sp., A.anitratus	Ureaplasma sp., M.bovirhinis

MICROBIOLOGICAL FINDINGS (POST-MORTEM): DAY 4

Tissue	Bacteriology	Mycoplasmology
NC	B.coagulans	A.laidlawii
Tr	negative	negative
RC	negative	A.laidlawii, M.bovis
RM	A.lwoffii	negative
RD	negative	negative
BrLN	negative	A.laidlawii M.bovirhinis

Pathological findings: There were severe lesions of chronic suppurative pneumonia in the right cranial lobe. There was marked consolidation in the middle lobe and small areas in the other anterior lobes. These lesions were soft, grey-purple with some thick mucopus in the major airways. These lesions were characterised by bronchitis, bronchiolitis and a proliferative alveolitis with some oedema. There was obvious bronchiolar epithelial hyperplasia with a few foci of necrosis. There was an increase in the number of lymphocytes surrounding the bronchioles often producing several layers. The bronchiolar and, in some areas, the alveolar exudate had an eosinophilic amorphous appearance due to degeneration of many of the cells. Eosinophilic intracytoplasmic inclusion bodies were seen in the bronchiolar and alveolar epithelia.

PI_3 viral antigen was recognised by immunofluorescence in the right cranial, right middle and to a small degree, in the right caudal lobes.

APPENDIX 2,13. Calf No.13 (Borland)

MICROBIOLOGICAL FINDINGS (PRE-MORTEM):

Day	Bacteriology	Mycoplasmology
-14	A.lwoffii M.(B.)catarrhalis	negative
-7	A.anitratus	negative
0	A.anitratus	M.bovirhinis
+4	A.anitratus	M.bovirhinis
+7	A.lwoffii	M.bovirhinis

MICROBIOLOGICAL FINDINGS (POST-MORTEM): DAY 9

Tissue	Bacteriology	Mycoplasmology
NC	negative	M.bovirhinis
Trq	Micrococcus sp.	M.bovis
RC	negative	A.laidlawii, M.bovis
RM	negative	A.laidlawii, M.bovis
RD	negative	M.bovis
BrLN	Micrococcus sp.	negative

Pathological findings: There was total consolidation of the right anterior lung lobes, accessory and anterior portions of the caudal lobes. The lesions were solid, deep red-purple to grey-fawn which had a uniform appearance on section with the major airways filled with a white frothy exudate. Microscopically the lesions were similar in all lobes being principally of a proliferative alveolitis with a small number of plasma cells and lymphocytes in the alveolar walls; these latter cells were present as a diffuse layer around many bronchioles, some of which showed epithelial necrosis while others had bronchiolitis obliterans. There was also a mild rhinitis and tracheitis.

All six tissues were negative by immunofluorescence for PI_3 virus.

APPENDIX 2,14. Calf No.14 (Borland)

MICROBIOLOGICAL FINDINGS (PRE-MORTEM):

Day	Bacteriology	Mycoplasmology
-14	A.anitratus M.(B.)catarrhalis	M.bovirhinis
-7	Micrococcus sp., A.anitratus	Ureaplasma sp.
0	P.haemolytica A2	Ureaplasma M.bovis
+4	P.haemolytica A2	Ureaplasma sp.
+7	M.(B.)catarrhalis P.haemolytica A2 C.bovis	Ureaplasma sp., A.laidlawii
+9	M.(B.)catarrhalis, M.(M.)bovis	Ureaplasma sp.

MICROBIOLOGICAL FINDINGS (POST-MORTEM): DAY 11

Tissue	Bacteriology	Mycoplasmology
NC	M.(M.)bovis, N.subflava	Ureaplasma sp., M.bovirhinis, A.laidlawii
Tr	N.subflava	Ureaplasma sp. A.laidlawii,M.bovis
RC	M.(B.)catarrhalis	Ureaplasma sp. A.laidlawii,M.bovis
RM	M.(B.)catarrhalis	Ureaplasma sp. A.laidlawii,M.bovis
RD	M.(B.)catarrhalis	Ureaplasma sp. A.laidlawii,M.bovis
BrLN	negative	A.laidlawii,M.bovis

Pathological findings: There were very severe pneumonic lesions in the anterior lung lobes and anterior parts of the caudal lobes. Apart from the right cranial lobe the lesions had a similar appearance being fawn-purple, collapsed, slightly mottled in appearance with a little pus in the airways. Microscopically there was a moderately severe bronchitis, bronchiolitis with a pronounced proliferative alveolitis. A few bronchioles showed epithelial necrosis and the majority were surrounded by a diffuse layer of lymphocytes and plasma cells. The exudate in many airways was necrotic. The lesions in the right cranial lobe were of a chronic nature and microscopically represented cuffing pneumonia although some lobules had an obvious proliferative alveolitis.
All six tissues were negative by immunofluorescence for PI_3 virus.

APPENDIX 2,15. Calf No.15 (Borland)

MICROBIOLOGICAL FINDINGS (PRE-MORTEM):

Day	Bacteriology	Mycoplasmology
-14	M.(B.)catarrhalis	negative
-7	M.(B.)catarrhalis, A.lwoffii	M.bovirhinis
0	Micrococcus sp. A.anitratus	M.bovirhinis
+4	M.(M.)bovis P.haemolytica A2	M.bovirhinis
+7	M.(M.)catarrhalis, A.lwoffii	M.bovis
+9	M.(M.)bovis A.anitratus	M.bovirhinis

MICROBIOLOGICAL FINDINGS (POST-MORTEM): DAY 11

Tissue	Bacteriology	Mycoplasmology
NC	N.subflava, M.(M.)bovis	M.bovis
Tr	A.anitratus	Ureaplasma sp., M.bovis
RC	A.anitratus	Ureaplasma sp., M.bovis,A.laidlawii
RM	A.anitratus	Ureaplasma sp., A.laidlawii
RD	A.anitratus	Ureaplasma sp., M.bovis,A.laidlawii
BrLN	M.(M.)bovis	M.bovis,A.laidlawii

Pathological findings: There was severe pulmonary consolidation in the anterior lobes of the right lung, caudal lobe and accessory lobe. The lesions were deep red-purple, soft, slightly oedematous and uniform in appearance. There was a mild tracheitis with much thick white mucopus along its length and in the major airways. Microscopically there was a moderately severe bronchitis, bronchiolitis and some degree of proliferative alveolitis and collapse. Many bronchioles were surrounded by a diffuse layer of plasma cells and lymphocytes. There was a mild to moderately severe neutrophilic exudate particularly into the small airways.

All six tissues were negative by immunofluorescence for PI_3 virus.

APPENDIX 2.16. Calf No.16 (Borland)

MICROBIOLOGICAL FINDINGS (PRE-MORTEM):

Day	Bacteriology	Mycoplasmology
-14	B.coagulans	negative
-7	A.lwoffii	negative
0	A.lwoffii,A.anitratus M.(M.)bovis	negative

MICROBIOLOGICAL FINDINGS (POST-MORTEM): DAY 4

Tissue	Bacteriology	Mycoplasmology
NC	A.lwoffii A.anitratus	negative
Tr	A.anitratus	A.laidlawii,M.bovis
RC	A.anitratus	M.bovirhinis
RM	A.anitratus	A.laidlawii,M.bovis M.bovirhinis
RD	A.anitratus	negative
BrLN	negative	negative

Pathological findings: There were severe lesions of chronic suppurative pneumonia in the right middle, caudal and accessory lobes. There were fairly extensive areas of consolidation in the other anterior lobes which were pale grey-purple, soft, slightly oedematous with obvious mucopus in the airways. Microscopically, they consisted of a proliferative alveolitis and bronchiolitis with areas of epithelial necrosis, some epithelial hyperplasia and plugging of the airways with necrotic inflammatory exudate. There was an increase in lymphocytes in the peribronchiolar spaces which appeared slightly oedematous. Eosinophilic intracytoplasmic inclusion bodies were seen in the bronchiolar epithelium.

There was positive fluorescence for PI_3 virus in the trachea, right cranial and right middle lobes, particularly in the bronchiolar epithelium.

APPENDIX 2,17. Calf No.17 (Borland)

MICROBIOLOGICAL FINDINGS (PRE-MORTEM):

Day	Bacteriology	Mycoplasmology
-14	P.haemolytica A1	negative
-7	S.bovis F.meningosepticum	negative
0	A.anitratus P.haemolytica A2	M.bovirhinis
+4	A.anitratus B.licheniformis	M.bovirhinis
+7	A.anitratus M.(B.)catarrhalis	A.laidlawii
+9	A.anitratus, M.(B.)catarrhalis	M.bovis

MICROBIOLOGICAL FINDINGS (POST-MORTEM): DAY 11

Tissue	Bacteriology	Mycoplasmology
NC	M.(B.)catarrhalis	negative
Tr	M.(B.)catarrhalis	M.bovis
RC	M.(B.)catarrhalis	A.laidlawii,M.bovis
RM	M.(B.)catarrhalis	A.laidlawii,M.bovis
RD	S.aureus	A.laidlawii
BrLN	A.anitratus	A.laidlawii,M.bovis

Pathological findings: There were moderately severe lesions in the right cranial lobe and accessory lobe; they were pale, firm, slightly fibrous with prominent airways which were filled with thick pus. Microscopically the lesions were similar and consisted of a moderately severe bronchitis, bronchiolitis and marked alveolar epithelial hyperplasia. In addition, there were areas with bronchiolitis obliterans, bronchiolar epithelial necrosis and peribronchiolar lymphoid aggregates.

All six tissues were negative by immunofluorescence for PI_3 virus.

APPENDIX 2,18. Calf No.18 (Borland)

MICROBIOLOGICAL FINDINGS (PRE-MORTEM):

Day	Bacteriology	Mycoplasmology
-14	P.haemolytica A1	negative
-7	A.anitratus A.lwoffii	negative
0	A.anitratus	M.bovirhinis
+4	B.coagulans C.bovis	negative
+7	A.anitratus, A.lwoffii	M.bovirhinis
+9	A.anitratus	A.laidlawii

MICROBIOLOGICAL FINDINGS (POST-MORTEM): DAY 11

Tissue	Bacteriology	Mycoplasmology
NC	A.anitratus	M.bovirhinis
Tr	A.anitratus	M.bovirhinis, M.bovis
RC	A.anitratus	A.laidlawii, M.bovis
RM	A.anitratus	A.laidlawii
RD	A.anitratus	A.laidlawii
BrLN	A.anitratus	A.laidlawii, M.bovis

Pathological findings: There was almost total consolidation of the right cranial lobe which was pale grey-fawn, firm with prominent airways which were filled with white mucopus; microscopically these lesions consisted of bronchitis, bronchiolitis, bronchiolitis obliterans with some epithelial necrosis. There were small areas of collapsed red-purple, soft lesions in the other anterior lobes which represented cuffing pneumonia. There was a moderately severe tracheitis.

All six tissues were negative by immunofluorescence for PI_3 virus.

APPENDIX 2,19. Calf No.19 (Borland)

MICROBIOLOGICAL FINDINGS (PRE-MORTEM):

Day	Bacteriology	Mycoplasmology
-14	Phaemolytica A1 M.(B.)catarrhalis	M.bovirhinis
-7	F.meningosepticum	M.bovirhinis
0	A.anitratus	negative
+4	F.meningosepticum C.bovis	M.bovirhinis
+7	A.anitratus	negative

MICROBIOLOGICAL FINDINGS (POST-MORTEM): DAY 9

Tissue	Bacteriology	Mycoplasmology
NC	negative	M.bovirhinis
Tr	F.meningosepticum	M.bovirhinis
RC	F.meningosepticum A.anitratus	M.dispar
RM	F.meningosepticum	M.bovis
RD	F.meningosepticum A.anitratus	M.dispar
BrLN	Micrococcus sp.	negative

Pathological findings: There was extensive consolidation of the anterior lobes and anterior portion of the caudal lobes. The lesions in the right cranial lobe were chronic suppurative pneumonia. The remaining lesions were firm, pale grey-fawn with the airways filled with frothy white fluid. Microscopically these lesions represented a proliferative alveolitis with areas of extensive oedema, some congestion and a marked infiltrate of neutrophils and macrophages. There was also a moderately severe bronchitis and many areas with bronchiolitis obliterans.

All six tissues were negative by immunofluorescence for PI_3 virus.

APPENDIX 2,20. Calf No.20 (Borland)

MICROBIOLOGICAL FINDINGS (PRE-MORTEM):

Day	Bacteriology	Mycoplasmology
-14	P.haemolytica A1	negative
-7	A.anitratus	Ureaplasma sp.
0	A.anitratus	Ureaplasma sp.
+4	M.(M.)bovis	M.bovirhinis
+7	C.bovis	negative
+9	A.anitratus A.lwoffii	A.laidlawii

MICROBIOLOGICAL FINDINGS (POST-MORTEM):

Tissue	Bacteriology	Mycoplasmology
NC	M.(M.)bovis S.aureus	M.bovirhinis
Tr	M.(M.)bovis	M.bovis
RC	M.(M.)bovis	M.bovis M.bovirhinis
RM	M.(M.)bovis	M.bovis
RD	negative	M.bovis
BrLN	negative	M.bovis

Pathological findings: There were mild anterior lobe lesions which were soft, oedematous, deep red-purple with fairly extensive intralobular haemorrhage in the right middle lobe. There was thick, white mucopus in the trachea and major airways of the consolidated tissue. Microscopically there was a moderately severe rhinitis, bronchitis, bronchiolitis with a few peribronchiolar lymphoid aggregates. There were areas of a proliferative alveolitis. There was a moderately severe infiltration of neutrophils in all lobes causing plugging of airways.

All six tissues were negative by immunofluorescence for PI_3 virus.

APPENDIX 3,1-23

(Individual clinical and pathological findings, mycoplasmal and bacterial isolations; investigations reported in Chapter 4)

Appendix 3.1. Clinical data, control calf no.55

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
-14	45	102.0	0
-13	40	101.9	0
-12	35	101.6	0
-11	36	102.0	0
-10	48	101.8	0
-9	42	101.6	0
-8	48	101.0	0
-7	30	101.0	0
-6	44	101.4	0
-5	35	101.2	0
-4	30	101.4	0
-3	36	101.5	0
-2	42	103.0	0
-1	36	101.8	0
0	35	102.0	0
+1	34	101.2	0
+2	29	101.0	0
+3	31	101.5	0
+4	35	102.0	0
+5	29	101.4	0
+6	31	101.5	0
+7	33	102.0	0
+8	18	101.0	0
+9	35	101.3	0
+10	33	101.5	0

Appendix 3.2. Clinical data, control calf no.58

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
-14	39	101.8	0
-13	38	101.9	0
-12	34	102.1	0
-11	34	101.4	0
-10	36	102.0	0
-9	30	101.8	0
-8	36	101.8	0
-7	30	103.0	0
-6	28	101.2	0
-5	36	101.5	0
-4	28	101.5	0
-3	36	101.3	0
-2	30	101.6	0
-1	30	102.4	0
0	30	101.8	0
+1	28	102.0	0
+2	27	101.5	0
+3	29	101.1	0
+4	35	101.6	0
+5	25	100.9	0
+6	24	100.5	0
+7	28	102.0	0
+8	27	101.4	0
+9	27	101.4	0
+10	25	101.4	0

Appendix 3.2. contd.

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
+11	33	101.6	0
+12	31	100.1	0
+13	29	101.4	0
+14	28	101.6	0
+15	30	101.4	0
+16	28	101.2	0
+17	26	101.1	0
+18	29	101.4	0
+19	29	101.3	0
+20	26	101.7	0

Appendix 3.3. Clinical data, control calf no.60

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
-14	40	101.8	0
-13	39	101.8	0
-12	44	101.8	0
-11	48	101.8	0
-10	48	101.8	0
-9	42	102.0	0
-8	42	101.6	0
-7	36	101.0	0
-6	30	101.4	0
-5	29	101.30	0
-4	26	101.2	0
-3	24	101.6	0
-2	24	101.0	0
-1	26	100.8	0
0	24	102.2	0
+1	27	100.8	0
+2	25	101.3	0
+3	35	101.4	2.0
+4	26	101.0	0
+5	31	101.4	0
+6	24	101.0	0
+7	32	101.7	0
+8	22	101.0	0
+9	22	101.4	0
+10	24	101.0	0

Appendix 3.3. contd.

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
+11	34	101.8	0
+12	34	100.7	0
+13	24	101.4	1.0
+14	39	101.6	2.0
+15	34	101.6	2.0
+16	31	101.4	0
+17	25	101.3	2.0
+18	33	101.4	0
+19	28	101.2	0
+20	25	101.1	1.0
+21	25	101.4	2.0
+22	23	99.9	1.0
+23	22	99.8	0
+24	26	101.0	0
+25	48	101.1	0

Appendix 3.4. Clinical data, control calf no.56

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
-14	40	102.4	0
-13	41	102.0	0
-12	40	102.1	0
-11	35	102.0	0
-10	40	102.8	0
-9	42	103.8	0
-8	36	102.7	0
-7	31	101.6	0
-6	30	101.8	0
-5	25	101.6	0
-4	24	1014.4	0
-3	25	101.8	0
-2	24	101.8	0
-1	31	101.0	0
0	24	102.0	0
+1	27	101.8	0
+2	25	101.6	0
+3	25	101.6	0
+3	25	101.6	0
+4	30	102.2	0
+5	28	101.8	0
+6	23	10.7	0
+7	28	102.0	0
+8	19	101.3	0
+9	20	101.5	0
+10	22	101.8	0

Appendix 3.4. contd.

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
+11	29	101.7	0
+12	31	101.8	0
+13	20	101.4	0
+14	26	101.5	0
+15	31	101.8	0
+16	25	101.3	0
+17	25	101.4	0
+18	19	101.5	0
+19	24	101.6	0
+20	26	101.7	0
+21	22	101.8	0
+22	25	101.7	1.0
+23	29	101.6	0
+24	21	101.4	0
+25	27	100.8	0
+26	23	100.4	0
+27	29	101.1	0
+28	22	100.1	0
+29	21	100.0	0
+30	20	101.1	0

Appendix 3.5. Clinical data, control calf no.61

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
-14	42	101.8	0
-13	40	101.7	0
-12	35	101.6	0
-11	36	101.8	0
-10	36	102.0	0
-9	36	101.6	0
-8	36	101.1	0
-7	33	101.4	0
-6	28	101.0	0
-5	30	101.4	0
-4	24	101.0	0
-3	30	102.2	0
-2	36	100.8	0
-1	30	101.2	0
0	32	101.4	0
+1	25	101.2	0
+2	26	101.3	0
+3	30	101.0	0
+4	36	101.2	0
+5	29	101.0	0
+6	25	100.8	0
+7	32	101.7	0
+8	25	100.7	0
+9	28	100.8	0
+10	28	101.0	0

Appendix 3.5. contd.

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
+11	26	101.5	0
+12	34	101.2	0
+13	34	101.2	0
+14	31	100.8	0
+15	29	100.8	0
+16	30	100.8	2.0
+17	25	10..6	0
+18	31	101.8	0
+19	30	101.0	0
+20	24	100.8	0
+21	31	100.9	3.0
+22	26	100.7	1.0
+23	27	100.8	0
+24	27	101.0	0
+25	25	100.4	0
+26	22	99.9	0
+27	29	99.9	0
+28	25	101.1	0
+29	29	101.3	0
+30	22	100.1	0
+31	21	100.8	0
+32	16	100.8	0
+33	21	101.4	0
+34	24	101.0	0
+35	20	10..8	0

Appendix 3.6 Clinical data, test calf no.68

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
-14	40	101.8	0
-13	28	101.5	0
-12	33	102.1	0
-11	26	101.6	0
-10	48	101.8	0
-9	42	101.2	0
-8	37	102.4	0
-7	31	101.8	0
-6	30	101.6	0
-5	35	101.4	0
-4	26	101.30	
-3	31	101.2	0
-2	24	101.8	0
-1	29	101.8	0
0	24	101.4	0
+1	32	102.0	0
+2	31	101.8	0
+3	32	101.8	0
+4	36	102.0	0
+5	34	101.6	0
+6	36	101.8	0
+7	45	103.3	2.0
+8	60	101.9	2.0
+9	60	101.6	1.0
+10	45	102.0	2.0

Appendix 3.7. Clinical data, test calf no.65

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
-14	38	102.0	0
-13	31	101.0	0
-12	34	101.6	0
-11	28	101.4	0
-10	30	101.0	0
-9	36	101.6	0
-8	30	101.8	0
-7	30	101.6	0
-6	24	101.2	0
-5	28	101.2	0
-4	24	101.5	0
-3	30	101.6	0
-2	24	101.2	0
-1	30	101.8	0
0	24	101.5	0
+1	26	101.6	0
+2	30	101.6	0
+3	31	101.8	0
+4	34	101.4	0
+5	26	101.2	0
+6	29	101.4	0
+7	30	101.0	0
+8	29	102.1	0
+9	31	101.6	0
+10	29	101.2	0

Appendix 3.7. contd.

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
+11	42	102.3	1.0
+12	40	102.0	2.0
+13	48	102.3	2.0
+14	61	102.6	2.0
+15	54	102.6	2.0
+16	72	103.0	3.0
+17	96	103.2	0
+18	54	102.8	2.0
+19	84	103.0	3.0
+20	72	103.5	5.0

Appendix 3.8. Clinical data, test calf no.64

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
-14	38	102.0	0
-13	30	101.1	0
-12	30	101.8	0
-11	42	101.6	0
-10	36	101.8	0
-9	42	102.0	0
-8	36	101.4	0
-7	36	101.0	0
-6	25	100.8	0
-5	29	101.4	0
-4	25	101.5	0
-3	29	101.7	0
-2	24	101.3	0
-1	29	101.4	0
0	30	101.6	0
+1	29	101.6	0
+2	32	101.0	0
+3	36	101.8	0
+4	35	101.8	0
+5	24	101.4	0
+6	28	101.4	1.0
+7	28	101.4	0
+8	27	101.7	0
+9	35	101.6	1.0
+10	33	101.2	1.0

Appendix 3.8. contd.

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
+11	41	101.6	2.0
+12	61	102.3	4.0
+13	54	101.5	3.0
+14	58	101.4	2.0
+15	60	103.0	0
+16	90	101.2	1.0
+17	84	102.6	3.0
+18	84	102.6	3.0
+19	72	102.4	1.0
+20	72	102.4	2.0
+21	84	102.2	1.0
+22	65	102.7	1.0
+23	66	102.7	6.0
+24	72	101.9	1.0
+25	60	103.0	3.0

Appendix 3.9. Clinical data, test calf no.69

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
-14	50	101.8	0
-13	48	102.0	0
-12	36	100.8	0
-11	36	101.1	0
-10	36	102.0	0
-9	36	101.6	0
-8	33	101.5	0
-7	35	1014.4	0
-6	30	101.2	0
-5	24	101.2	0
-4	25	101.7	0
-3	30	101.6	0
-2	30	101.4	0
-1	24	1015.5	0
0	30	101.5	0
+1	25	101.0	0
+2	29	101.0	0
+3	31	101.6	0
+4	37	101.4	0
+5	35	101.8	0
+6	35	101.4	0
+7	39	101.4	0
+8	59	101.8	4.0
+9	54	101.5	1.0
+10	48	101.4	2.0

Appendix 3.9. Contd.

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
+11	60	103.0	1.0
+12	71	103.6	5.0
+13	72	104.0	7.0
+14	66	104.1	10.0
+15	78	103.3	3.0
+16	84	103.6	4.0
+17	93	103.0	11.0
+18	93	103.0	4.0
+19	60	102.5	6.0
+20	84	102.5	1.0
+21	61	102.2	4.0
+22	70	103.0	3.0
+23	72	103.2	11.0
+24	72	102.4	8.0
+25	66	103.6	3.0
+26	59	102.9	1.0
+27	93	103.4	3.0
+28	65	103.1	3.0
+29	65	103.0	3.0
+30	84	102.4	1.0

Appendix 3.10. Clinical data, test calf no.71

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
-14	40	101.2	0
-13	38	101.3	0
-12	31	101.4	0
-11	36	101.2	0
-10	42	101.4	0
-9	42	101.0	0
-8	31	101.0	0
-7	32	101.4	0
-6	25	100.8	0
-5	28	100.8	0
-4	24	100.8	0
-3	26	100.8	0
-2	24	101.0	0
-1	30	101.0	0
0	24	101.6	0
+1	26	101.3	0
+2	29	101.6	0
+3	30	101.2	0
+4	45	101.4	0
+5	31	101.6	0
+6	24	101.0	0
+7	38	101.3	0
+8	29	101.5	0
+9	35	101.6	0
+10	29	101.5	2.0

Appendix 3.10. Contd.

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
+11	60	101.6	2.0
+12	44	101.3	4.0
+13	47	101.4	2.0
+14	54	101.8	2.0
+15	534	102.0	0
+16	54	101.8	2.0
+17	66	101.2	0
+18	72	101.8	3.0
+19	54	020	2.0
+20	72	102.1	1.0
+21	60	101.4	3.0
+22	60	102.0	2.0
+23	60	102.4	4.0
+24	77	102.0	1.0
+25	66	103.6	2.0
+26	60	103.2	1.0
+27	78	103.2	1.0
+28	59	102.9	1.0
+29	61	102.0	2.0
+30	72	101.9	2.0
+31	54	101.6	1.0
+32	66	99.8	1.0
+33	72	101.5	1.0
+34	37	101.8	1.0
+35	54	102.2	0

Appendix 3.11. Pathological findings of two test (65,68) and two control (55,58) calves slaughtered either on days 10 or 20.

Slaughtered	DAY 10		DAY 20	
Pathological findings	CONTROL (55)	INFECTED (68)	CONTROL (58)	INFECTED (65)
Macroscopic	Few lobules of collapse in the right cranial and middle lobes.	A little collapse in the right cranial and left cranial lobes. The mediastinal lymph node slightly enlarged. A little excess mucus in the trachea.	The lungs - normal. Few foci of collapse in the anterior lobes.	Extensive interstitial emphysema in all lobes. A little green pus in the trachea and the associated lymph node enlarged. Many lobules of fawn-purple consolidation, particularly in the caudal lobes. The airways plugged with greenish exudate and large numbers of lungworm larvae.
Histological	Mild lesions of cuffing pneumonia in the anterior lobes. A slight goblet cell hyperplasia in the nasal conchae and a mild eosinophilic infiltrate into the tonsil.	Small numbers of neutrophils and eosinophils infiltrated the epithelia of the nasal passages and trachea. Mild cuffing pneumonia in the anterior lobes. A marked widespread eosinophilia with many accumulating around airways and blood vessels. A moderately severe infiltrate of eosinophils into the bronchial lymph node and the spleen.	The lungs - normal. Mild bronchitis in the anterior lobes and occasional peribronchiolar lymphoid aggregates.	A mild tracheitis and tonsillitis with a mild eosinophilic infiltration into the lymph nodes. The pulmonary lesions (characteristic) of early patent husk with much interstitial emphysema, bronchitis and plugging of airways with lungworm larvae and eosinophilic debris.

Appendix 3.12. Pathological findings of two test (64,69) and two control (56,60) calves slaughtered on either days 25 or 30.

	DAY 25		DAY 30	
Pathological findings	CONTROL (60)	INFECTED (64)	CONTROL (56)	INFECTED (69)
Macroscopic	The lungs - normal.	Lesions of severe patent husk in all lobes. The tissue - pale, emphysematous with many lobules of consolidation, particularly in the caudal lobes. Much frothy exudate in the airways with flecks of thick yellow-green pus and large numbers of lungworm larvae. The lymph node enlarged.	The lungs - normal. Foci of collapse in the right cranial lobe.	Extensive lesions of patent husk with much interstitial emphysema, oedema, consolidation and many lungworms in the airways together with much yellow-green pus.
Histological	A few peribronchiolar lymphoid aggregates in the anterior lobes.	A mild rhinitis and tracheitis with many subepithelial lymphoid aggregates. A mild tonsillitis with a few crypts filled with necrotic debris. The pulmonary lesions - of patent husk with a few peribronchiolar lymphoid aggregates in the anterior lobes.	A mild tracheitis. Few foci of pulmonary collapse - due to mild cuffing pneumonia.	The pulmonary lesions - characteristic of patent husk with a marked foreign body pneumonia. A marked eosinophilic reaction in the bronchial lymph node.

Appendix 3.13. Pathological findings of one test (71) and one control (61) calf slaughtered on day 35.

Slaughtered	DAY 35	
Pathological findings	CONTROL (61)	INFECTED (71)
Macroscopic	The lung - normal. Foci of collapse in the right cranial lobe.	Severe anterior lobe lesions of chronic non-suppurative pneumonia with much thick mucopus in the airways. The caudal lobe lesions - of patent husk with many large lungworm larvae in the airways mixed with frothy exudate - dark brownish appearance. The lymph node enlarged.
Histological	A mild rhinitis and very mild tracheitis. Occasional peribronchiolar lymphoid aggregates in the anterior lobes together with small clusters of eosinophils. A mild neutrophilic infiltrate into the bronchial and retropharyngeal lymph node. A mild to moderately severe tonsillitis with much necrotic debris and inflammatory cells in the crypts.	A very mild rhinitis, tracheitis and laryngitis due to infiltrating neutrophils and eosinophils. A similar infiltrate in the bronchial and retropharyngeal lymph nodes. A moderate severe tonsillitis. Severe lesions of patent husk in all lobes with severe bronchitis, bronchiolitis (with marked bronchiolitis obliterans), alveolar epithelial hyperplasia, foreign body pneumonia and a severe diffuse eosinophilia. Areas of chronic non-suppurative pneumonia in the anterior lobes.

Appendix 3,14.

Calf no.55; Control; slaughtered day 10.

(i) Nasopharyngeal swab isolates

Day	Agent(s)
-14	Bacillus sp., S.aureus
-10	A.viridans, E.coli
-8	Bacillus sp., S.aureus
-3	E.coli, Moraxella sp., N.subflava
-1	Corynebacterium sp., S.bovis
3	A.calcoaceticus, E.coli
6	Micrococcus sp., S.aureus
10	A.viridans

(ii) Tissues

Site	Agent(s)
NC	Airidans, Micrococcus sp., S.bovis
TON	E.coli, S.aureus
RTLN	A.viridans, E.coli, S.aureus
TR	Micrococcus sp., Moraxella sp.
RC	A.viridans, Micrococcus sp., S.aureus
RM	Corynebacterium sp., Moraxella sp.,.aureus
RD	Moraxella sp., S.aureus
BRLN	A.viridans

Appendix 3,15.

Calf no.58; Control; slaughtered day 20.

(i) Nasopharyngeal swab isolates

Day	Agent(s)
-14	N.subflava, S.aureus
-10	E.coli, S.aureus
-8	Bacillus sp., S.pyogenes
-3	E.coli, S.aureus
-1	Corynebacterium sp., S.aureus
3	Bacillus sp., Moraxella sp.
6	E.coli, Flavobacterium sp.
10	Proteus sp., S.aureus
13	Bacillus sp., Flavobacterium sp.
17	A.pyogenes, S.aureus

(ii) Tissues

Site	Agent(s)
NC	S.aureus
TON	Bacillus sp., E.coli
RTLN	E.coli
TR	S.aureus, S.pyogenes
RC	S.pyogenes
RM	A.pyogenes
RD	S.aureus, S.pyogenes
BRLN	Bacillus sp., S.aureus

Appendix 3,16.

Calf no.60; Control; slaughtered day 25.

(i) Nasopharyngeal swab isolates

Day	Agent(s)
-14	Bacillus sp.
-10	S.aureus
-8	Corynebacterium sp.
-3	E.coli
-1	Bacillus sp., S.aureus
3	Corynebacterium sp.
6	Micrococcus sp.cterium sp.
10	A.viridans
13	S.pyogenes
17	N.subflava
20	Micrococcus sp., Streptococcus sp.
24	N.subflava

(ii) Tissues

Site	Agent(s)
NC	A.lignieresii
TON	Bacillus sp., Streptococcus sp.
RTLN	E.coli, S.aureus
TR	E.coli, Moraxella sp.
RC	A.viridans
RM	E.coli, S.aureus
RD	Micrococcus sp.
BRLN	Bacillus sp., E.coli

Appendix 3,17.

Calf no.56; Control; slaughtered day 30.

(i) Nasopharyngeal swab isolates

Day	Agent(s)
-14	Flavobacterium sp., S.aureus
-10	Bacillus sp., E.coli
-8	S.aureus, S.faecalis
-3	Corynebacterium sp., N.subflava
-1	S.zooepidemicus
3	A.calcoaceticus
6	Bacillus sp., Streptococcus sp.
10	A.lignieresii
13	A.calcoaceticus
17	Micrococcus sp., S.aureus
20	Aerococcus sp., Moraxella sp.
24	Neisseria sp., S.aureus
26	Bacillus sp.

(ii) Tissues

Site	Agent(s)
NC	Flavobacterium sp., S.aureus
TON	Bacillus sp., E.coli
RTLN	Flavobacterium sp., S.zooepidemicus
TR	A.lignieresii
RC	-
RM	-
RD	S.zooepidemicus
BRLN	A.calcoaceticus

Appendix 3,18.

Calf no.61; Control; slaughtered day 35.

(i) Nasopharyngeal swab isolates

Day	Agent(s)
-14	M.(B.)catarrhalis, Staphylococcus sp.
-10	A.viridans
-8	Flavobacterium sp., Micrococcus sp.
-3	Bacillus sp., E.coli
-1	Corynebacterium sp.
3	Proteus sp.
6	N.subflava
10	Micrococcus sp.
13	Bacillus sp.
17	S.aureus
20	Proteus sp.
24	N.subflava
26	Bacillus sp.
30	S.equinus
33	A.liquefaciens

(ii) Tissues

Site	Agent(s)
NC	A.liquefaciens
TON	A.liquefaciens, E.coli
RTLN	S.equinus
TR	Bacillus sp., S.equinus
RC	Micrococcus sp.
RM	S.equinus
RD	Proteus sp.
BRLN	Micrococcus sp.

Appendix 3,19.

Calf no.68; Test; slaughtered day 10.

(i) Nasopharyngeal swab isolates

Day	Agent(s)
-14	Micrococcus sp., S.aureus
-10	Bacillus sp., S.aureus
-8	N.subflava, Micrococcus sp.
-3	Micrococcus sp., Pseudomonas sp.
-1	Bacillus sp., Moraxella sp.
3	Aeromonas sp., Bacillus sp.
6	Corynebacterium sp., E.coli

(ii) Tissues

Site	Agent(s)
NC	Neisseria sp., S.aureus
TON	A.salmonicida, E.coli
RTLN	A.salmonicida, E.coli
TR	-
RC	Micrococcus roseus, Moraxella sp.
RM	Moraxella sp., S.aureus
RD	A.faecalis
BRLN	Corynebacterium sp., S.bovis

Appendix 3,20.

Calf no.65; Test; slaughtered day 20.

(i) Nasopharyngeal swab isolates

Day	Agent(s)
-14	A.viridans
-10	N.subflava
-8	A.faecalis
-3	Bacillus sp.
-1	Moraxella sp.
3	S.aureus
6	Micrococcus roseus
10	S.epidermius
13	E.coli
17	A.lignieresii

(ii) Tissues

Site	Agent(s)
NC	Bacillus sp.
TON	E.coli
RTLN	E.coli
TR	A.calcoaceticus
RC	A.lignieresii
RM	-
RD	-
BRLN	A.lignieresii

Appendix 3,21.

Calf no.64; Test; slaughtered day 25.

(i) Nasopharyngeal swab isolates

Day	Agent(s)
-14	Corynebacterium sp.
-10	Bacillus sp., S.aureus
-8	N.subflava
-3	Moraxella sp.
-1	Flavobacterium sp.
3	Aerococcus sp., Micrococcus sp.
6	A.lignieresii
10	A.calcoaceticus
13	E.coli, Proteus sp.
17	N.subflava
20	P.haemolytica
24	Bacillus sp.

(ii) Tissues

Site	Agent(s)
NC	-
TON	P.haemolytica
RTLN	E.coli
TR	Corynebacterium sp.
RC	-
RM	-
RD	-
BRLN	S.aureus

Appendix 3,22.

Calf no.69; Test; slaughtered day 30.

(i) Nasopharyngeal swab isolates

Day	Agent(s)
-14	Bacillus sp., S.aureus
-10	N.subflava
-8	Bacillus sp., Moraxella sp.
-3	E.coli, Micrococcus sp.
-1	N.subflava
3	A.viridans
6	Micrococcus sp., S.aureus
10	N.subflava
13	N.subflava
17	E.coli
20	S.pneumoniae
24	P.haemolytica
26	Bacillus sp.

(ii) Tissues

Site	Agent(s)
NC	Bacillus sp., S.equinus
TON	A-D group, E.coli
RTLN	Aerococcus sp., Micrococcus sp.
TR	N.subflava
RC	N.subflava
RM	N.subflava
RD	-
BRLN	E.coli, Micrococcus sp.

Appendix 3,23.

Calf no.71; Test; slaughtered day 35.

(i) Nasopharyngeal swab isolates

Day	Agent(s)
-14	Bacillus sp.
-10	Proteus sp.
-8	S.aureus
-3	Moraxella sp.
-1	N.subflava
3	E.coli
6	A.pyogenes
10	A.faecalis
13	Flavobacterium sp.
17	S.epidermius
20	S.aureus
24	S.aureus
26	P.multocida
30	Bacillus circulans
33	A-D group

(ii) Tissues

Site	Agent(s)
NC	Bacillus sp., S.aureus
TON	E.coli, N.subflava
RTLN	A.liquefaciens
TR	P.multocida, Staphylococcus sp.
RC	Flavobacterium sp., Micrococcus sp.
RM	A.pyogenes
RD	P.haemolytica
BRLN	S.aureus

APPENDIX 4,1-10

(Individual clinical findings, investigation reported in Chapter 5)

APPENDIX 4,1-10

(Individual clinical findings, investigation reported in Chapter 5)

Appendix 4.1. Clinical data, Calf no.14

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
0	23	101.2	0
1	21	102.4	0
2	20	102.4	0
3	18	102.4	1
4	20	102.2	1
5	24	102.2	1
6	19	101.6	1
7	24	102.0	1
8	23	102.4	1
9	42	101.8	1
10	20	102.2	1
11	30	101.4	1
12	22	102.8	1
13	24	101.0	1
14	25	102.2	0
15	22	101.9	0
16	24	101.4	0
17	22	100.4	1
18	25	101.2	1
19	45	102.0	0
20	25	102.2	1
21	33	102.6	0
22	48	101.6	0
23	34	101.6	0

Appendix 4.1. contd.

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
24	37	102.0	1
25	36	102.0	0
26	40	103.4	1
27	42	103.0	1
28	45	103.9	1
29	50	104.9	1
30	43	105.2	0
31	45	105.0	1
32	43	103.2	1
33	40	103.9	1
34	42	103.9	1
35	40	103.0	1

Appendix 4.2. Clinical data, Calf No.15

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
0	20	102.8	0
1	25	101.9	0
2	30	99.9	0

Appendix 4.3. Clinical data, Calf No.17

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
0	20	101.0	3
1	20	102.2	0
2	20	101.2	0
3	16	102.0	0
4	32	106.0	0
5	20	103.0	2
6	40	103.8	3
7	20	102.9	3
8	28	104.6	2
9	24	103.8	3
10	24	104.8	2
11	48	106.2	1
12	60	103.8	1
13	32	103.4	1
14	24	102.0	0
15	20	101.2	0
16	36	102.0	2
17	48	103.8	1
18	32	106.0	1
19	60	105.0	1
20	60	104.2	1
21	60	105.2	0
22	60	103.2	2
23	72	104.6	0

Appendix 4.3. contd.

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
24	68	104.6	1
25	47	104.1	0
26	39	104.2	1
27	40	103.9	1
28	45	103.7	1
29	45	103.9	1
30	35	103.2	1
31	30	102.5	2
32	51	102.5	1
33	39	102.9	3
34	34	103.0	2
35	31	103.2	1
36	-	-	-
37	33	104.2	0
38	36	102.2	1
39	35	103.2	1
40	34	103.0	2
41	35	102.9	2
42	35	102.5	1
43	34	102.4	1
44	30	103.1	1
45	36	102.1	1
46	30	102.2	1
47	30	102.4	2

Appendix 4.3. contd.

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
48	38	102.8	1
49	39	103.8	4
50	40	101.7	1
51	42	103.2	4
52	44	104.1	1
53	44	104.2	1
54	46	104.0	1
55	50	104.2	4
56	60	105.4	1
57	76	104.8	1
58	55	104.0	1
59	56	104.2	1
60	65	103.0	1
61	40	102.9	3
62	39	103.0	2
63	42	102.8	1
64	36	102.8	2
65	38	103.4	1
66	-	-	-
67	59	104.0	2
68	48	103.0	1
69	49	103.0	1

Appendix 4.4. Clinical data, Calf No.18

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
0	26	102.8	0
1	24	103.0	0
2	20	101.9	0
3	24	102.2	0
4	32	104.0	0
5	28	103.6	0
6	48	105.7	0
7	46	103.9	1
8	24	105.0	2
9	22	104.6	1
10	24	104.2	2
11	36	104.2	1
12	60	103.8	1
13	61	104.4	2
14	36	104.2	1
15	60	104.0	2
16	82	105.0	1
17	44	103.2	1
18	44	103.6	1
19	80	105.4	1
20	82	104.8	1
21	60	102.4	1
22	80	103.9	1
23	60	102.4	2

Appendix 4.4. contd.

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
24	80	104.0	2
25	71	104.3	1
26	78	103.9	2
27	79	103.8	2
28	78	104.5	2
29	75	105.9	2
30	65	104.0	2
31	62	104.0	2
32	70	105.9	1
33	60	105.2	1
34	79	105.3	1
35	69	104.9	1

Appendix 4.5. Clinical data, Calf No.19

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
0	21	102.2	0
1	24	102.1	0
2	20	102.1	0
3	22	102.7	1
4	60	105.4	1
5	23	103.3	1
6	40	103.0	2
7	61	105.0	0
8	32	105.2	1
9	60	106.1	2
10	62	106.1	1
11	80	105.7	1
12	81	106.0	2
13	68	105.7	1
14	60	105.3	1

Appendix 4.6. Clinical data, Calf No.20

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
0	20	103.2	0
1	24	102.2	0
2	20	102.2	0
3	28	102.1	0
4	46	104.1	1
5	24	103.0	1
6	36	103.8	1
7	24	103.3	1
8	34	101.8	1
9	56	102.4	1
10	36	102.9	2
11	44	103.0	1
12	80	105.0	2
13	80	105.0	1
14	68	104.6	3
15	60	105.2	1
16	85	104.6	1
17	40	104.9	2
18	82	105.0	1
19	82	104.6	1
20	81	104.2	2
21	80	104.6	1
22	62	103.3	1
23	60	104.8	1

Appendix 4.6. contd.

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
24	58	104.8	1
25	79	103.9	1
26	80	103.8	1

Appendix 4.7. Clinical data, Calf No.22

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
0	22	102.0	0
1	20	102.3	0
2	24	103.2	0
3	20	102.8	0
4	21	103.0	0
5	23	102.2	1
6	21	103.2	2
7	36	103.0	1
8	32	102.4	3
9	81	103.2	1
10	62	105.6	1
11	80	105.4	1
12	62	105.4	1
13	60	105.0	2
14	32	106.2	1
15	60	104.0	1
16	61	105.2	-
17	72	105.6	2
18	60	104.1	1
19	53	104.0	1
20	68	104.2	1
21	66	105.6	1
22	80	103.8	1
23	81	103.4	1
24	68	103.2	1

Appendix 4.8. Clinical data, Calf No.23

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
0	20	104.0	0
1	23	103.4	0
2	40	103.2	2
3	32	103.4	1
4	65	104.0	3
5	44	104.8	2
6	64	106.8	1
7	56	104.2	1
8	44	103.9	1
9	32	103.4	1
10	46	104.8	2
11	48	104.0	1
12	34	102.8	3
13	48	102.2	2
14	36	103.2	1
15	60	101.6	2
16	35	101.6	1
17	60	105.1	2
18	48	102.4	1
19	36	103.0	1
20	60	103.2	2
21	61	103.6	1
22	94	103.8	1
23	84	103.8	1
24	56	104.2	1

Appendix 4.8. contd.

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
25	47	104.1	1
26	43	104.9	1
27	45	103.9	0
28	48	103.9	0
29	49	103.7	0
30	44	103.9	0
31	42	103.9	0
32	45	103.9	0
33	46	103.4	0
34	49	103.2	0
35	48	103.2	0
36	-	-	-
37	59	104.0	1
38	57	103.4	1
39	40	103.3	0
40	44	103.2	0
41	44	103.5	2
42	46	103.3	1
43	37	102.9	3
44	41	103.2	1
45	55	102.8	0
46	60	103.6	0
47	70	102.9	3
48	80	102.9	2

Appendix 4.8. contd.

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
49	80	103.0	2
50	82	103.0	2
51	73	103.9	2
52	-	-	-

Appendix 4.9. Clinical data, Calf No.24

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
0	20	101.4	0
1	24	103.4	0
2	22	103.4	0
3	20	102.8	1
4	20	103.6	2
5	24	102.4	0
6	20	103.0	3
7	24	103.2	3
8	20	103.5	2
9	28	104.4	1
10	28	102.6	0
11	32	104.1	0
12	36	103.8	1
13	40	103.5	2
14	28	103.4	3
15	24	102.2	4
16	24	104.4	2
17	22	101.8	1
18	52	104.8	2
19	36	102.5	1
20	60	105.0	2
21	46	104.2	2
22	80	105.0	1
23	72	104.8	1

Appendix 4.9. contd.

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
24	66	104.9	1
25	70	105.9	0
26	66	105.4	0
27	59	106.0	0
28	49	104.9	0
29	80	104.9	0
30	60	103.0	0
31	44	102.9	1
32	45	102.9	2
33	37	102.0	1
34	44	103.0	2
35	45	103.0	0
36	43	102.9	1
37	49	104.0	2
38	48	103.3	3
39	38	103.0	4
40	50	103.1	2
41	51	103.2	2
42	49	103.4	1
43	44	103.0	2
44	48	103.2	4

Appendix 4.10. Clinical data, Calf No.25

Day of experiment	Respiratory rate	Rectal temperature	No. of coughs /5mins.
0	24	102.1	0
1	38	102.3	0
2	20	102.5	0
3	28	103.1	0
4	24	102.4	0
5	27	102.5	1
6	100	103.8	1
7	84	103.6	1
8	101	97.6	1

APPENDIX 5,1-7

(Individual clinical and pathological findings, mycoplasmal and bacterial isolations; investigation reported in Chapter 6)

APPENDIX 5, 1. Case no. 96141.

Admitted 19.9.84; slaughtered 20.11.84.

Subject

7 y.o. Hereford X cow.

History

Calved in spring. Whole group failed to thrive through summer but over last few weeks this individual was noted to be breathing heavily. Moved from bare pasture to hay aftermath one week prior to admission. Marked deterioration over last few days.

Presenting signs

Bright, thin, respiratory distress.

Clinical findings

Bright, eating well, rectal temperature 100.8^{o}F.
Tachypnoea (60/min.), hyperpnoeic, occasional cough.
Low-pitched squeaks and crackles cranioventrally; no thoracic pain.
Tachycardia (120/min.), exercise intolerance.

Significant laboratory findings

None.

Subsequent developments

Slight fever associated with increased dullness over last few days of life.

Clinical diagnosis

Diffuse fibrosing alveolitis.

Pathological findings

Heavy lungs (8.2 kg); some froth and mucus in trachea and major airways.
Widespread fibrosis and alveolar epithelialisation.

Final diagnosis

Diffuse fibrosing alveolitis.

APPENDIX 5, 1. (96141). Bacterial isolations from a total of ten nasopharyngeal swabs obtained from Case no. 96141.

Bacterial species*	Total no. of isolates	(%)
A.calcoaceticus	9	(39)
A viridans	2	(9)
B.coagulans	2	(9)
Flavobacterium sp.	2	(9)
Micrococcus sp.	2	(9)
M.(B.)catarrhalis	2	(9)
B.lentus	1	(4)
C.bovis	1	(4)
S.aureus	1	(4)
S.bovis	1	(4)
Total	23	

(* No mycoplasmas were isolated)

APPENDIX 5.1. (96141). Bacterial isolations from upper and lower respiratory tract and associated lymphatic tissues

Bacterial species*	Sampling sites**														
	NC_a	NC_p	NP	L	TR_a	TR_p	To	RTLN	RC_b	RC_L	RM_b	RM_L	RD_b	RD_L	BR
M.(M.)nonliquefaciens	-	-	+	-	-	-	-	-	-	+	+	+	+	-	-
Micrococcus sp.	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-
N.subflava	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Proteus sp.	+	+	-	+	-	-	+	-	-	-	-	-	-	-	-
Staphylococcus sp.	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-

(* No mycoplasmas were isolated)
(** a = cranial, p = caudal, b = bronchus, L = lobe)

APPENDIX 5, 2. Case no. 96737.

Admitted 9.11.84; slaughtered 6.12.84.

Subject

6 y.o. Ayrshire X cow.

History

Used as a suckler (beef) cow. Whole herd brought down off hill one week ago for tuberculin test. This one showed exercise intolerance and deteriorated rapidly over next three days.

Presenting sign(s)

Bright, eating well, rectal temperature 100.9°F.
Tachypnoea (52/min.), hyperpnoea, occasional cough; expiratory grunt.
Copious bilateral mucopurulent nasal discharge.
Squeaks cranioventrally left; crackles high on right side; total silence high on left side.
Thoracic pain (?) on right side.

Significant laboratory findings

None.

Subsequent developments

Gradual deteriorations.

Clinical diagnosis

Diffuse fibrosing alveolitis.

Pathological findings

Heavy lungs; lesions particularly severe in caudal lobes; widespread fibrosis and alveolar epithelialisation. Foci of eosinophils throughout lung substance; abundant globule leukocytes in the epithelium of the larger airways.

Final diagnosis

Diffuse fibrosing alveolitis.

APPENDIX 5, 2. (96737). Bacterial isolations from a total of seven nasopharyngeal swabs obtained from Case no. 96737.

Bacterial species*	Total no. of isolates	(%)
A.calcoaceticus	3	(19)
A.lignieresii	2	(12)
B.coagulans	2	(12)
M.(M.)liquefaciens	2	(12)
P.haemolytica A1	2	(12)
C.bovis	1	(6)
Flavobacterium sp.	1	(6)
Moraxella sp.	1	(6)
N.subflava	1	(6)
P.haemolytica A2	1	(6)
Total	16	

(* in addition, one isolation of M.bovirhinis)

APPENDIX 5, 2. (96737). Bacterial isolations from upper and lower respiratory tract and associated lymphatic tissues

Bacterial species*	Sampling sites**														
	NC_a	NC_p	NP	L	TR_a	TR_p	To	RTLN	RC_b	RC_L	RM_b	RM_L	RD_b	RD_L	BR
Micrococcus sp.	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
Bacillus sp.	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Corynebacterium sp.	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Moraxella sp.	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Staphylococcus sp.	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-

(* No mycoplasmas were isolated)
(** a = cranial, p = caudal, b = bronchus, L = lobe)

APPENDIX 5, 3. Case no. 06203.

Admitted 28.9.84; slaughtered 8.11.84.

Subject

7 y.o. Friesian cow.

History

Calved two weeks pre-admission. Developed milk fever - when found, lying in lateral recumbency with its head downhill. Subsequently developed pneumonia which only partially responded to antibiotic therapy. Very marked loss of condition.

Presenting sign(s)

Dull, thin, respiratory distress.

Clinical findings

Dull, inappetent, rectal temperature 102.8^{o}F.
Tachypnoea (60/min.), frequent cough, bilateral purulent nasal discharge. Expiratory grunt. Occasional crackles on right side. Dull area on percussion of right side; procedure also painful.
Abscess cranial to right prescapular lymph node.

Significant laboratory findings

Leukocytosis (17.3, 14.8x10^{9}/L); neutrophilia 73.0-77.5%.

Subsequent developments

Gradual deteriorations and weight-loss.

Clinical diagnosis

Aspiration pneumonia.

Pathological findings

Very severe, unilateral chronic suppurative pneumonia. Widespread fibrosis and abscessation on left side.

Final diagnosis

Chronic suppurative pneumonia subsequent to inhalation pneumonia following milk fever.

APPENDIX 5, 3. (96203). Bacterial isolations from a total of six nasopharyngeal swabs obtained from Case no. 96203.

Bacterial species*	Total no. of isolates	(%)
A.calcoaceticus	5	(36)
A.viridans	2	(14)
Micrococcus sp.	2	(14)
E.coli	1	(7)
Flavobacterium sp.	1	(7)
M.(M.)catarrhalis	1	(7)
Moraxella sp.	1	(7)
N.subflava	1	(7)
Total	14	

(* no mycoplasmas were isolated)

APPENDIX 5, 3. (96203). Bacterial isolations from upper and lower respiratory tract and associated lymphatic tissues

Bacterial species*	Sampling sites**														
	NC_a	NC_p	NP	L	TR_a	TR_p	To	RTLN	RC_b	RC_L	RM_b	RM_L	RD_b	RD_L	BR
Proteus sp.	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+
A.lignieresii	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+
A.calcoaceticus	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
B.lentus	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
E.coli	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
K.oxytosa	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
S.aureus	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-

(* in addition, one isolation of M.bovis)
(** a = cranial, p = caudal, b = bronchus, L = lobe)

APPENDIX 5, 4. Case no. 96887.

Admitted 21.11.84; slaughtered 31.12.84.

Subject

One year old Simmental X heifer.

History

Bucket-reared from purchase as newborn calf. Had calf-hood pneumonia. Uneventful first summer at grass but dull and coughing since brought indoors in October, 1984. Given "Ivomec" treatment but coughing persisted.

Presenting sign(s)

Dull, thin, respiratory distress.

Clinical findings

Dull, thin, poorly-developed, rectal temperature 104.2^{o}F.
Tachypnoea (36/min.), hyperpnoea, frequent cough, mucoid nasal discharge.
Exercise intolerance.
Loud squeaks over most of both lung fields.

Significant laboratory findings

Leukocytosis (16.6x10^{9}/L); neutrophilia (83%).

Subsequent developments

Gradual deterioration.

Clinical diagnosis

Chronic suppurative pneumonia.

Pathological findings

Widespread areas of chronic suppurative and non-suppurative (consolidated) pneumonia. Extensive fibrosis. Evidence of previous parasitic bronchitis; tracheitis, tonsillitis.

Final diagnosis

Severe chronic suppurative/non-suppurative pneumonia.

APPENDIX 5, 4. (96887). Bacterial isolations from a total of nine nasopharyngeal swabs obtained from Case no. 96887.

Bacterial species*	Total no. of isolates	(%)
N.pharyngisatus	3	(21)
A.viridans	2	(14)
C.bovis	2	(14)
M.nonliquefaciens	2	(14)
B.circulans	1	(7)
C.murium	1	(7)
Micrococcus sp.	1	(7)
P.multocida	1	(7)
S.bovis	1	(7)
Total	14	

(* no mycoplasmas were isolated)

APPENDIX 5, 4. (96887). Bacterial isolations from upper and lower respiratory tract and associated lymphatic tissues

Bacterial species*	Sampling sites														
	NC_a	NC_p	NP	L	TR_a	TR_p	To	RTLN	RC_b	RC_L	RM_b	RM_L	RD_b	RD_L	BR
A.lignieresii	-	-	-	-	-	+	+		+	+	-	+	-	-	-
Micrococcus sp.	+	-	+	+	-	-	-	+	-	-	-	-	+	-	+
A.viridans	-	-	-	-	-	-	+	+	-	-	-	-	-	-	+
Flavobacterium sp.	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
C.bovis	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
C.hofmonnii	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
C.murium	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Lactobacillus sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Neisseria sp.	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Staphylococcus sp.	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S.lactis	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-

(* no mycoplasmas were isolated)
(** a = cranial, p = caudal, b = bronchus, L = lobe)

APPENDIX 5, 5. Case no. 97439.

Admitted 18.1.85; slaughtered 6.2.86.

Subject

10 month old Friesian heifer.

History

Non-responsive chronic respiratory problem.

Presenting sign(s)

Bright, thin, respiratory distress.

Clinical findings

Bright, thin, poor appetite, rectal temperature 102.4°F.
Tachypnoea (48/min.), hyperpnoea, frequent cough, exercise intolerance.
Bilateral squeaks over large area.

Significant laboratory findings

Leukocytosis (14.0, 18.2x10^9/L); neutrophilia (55.5%, 610%); eosinophilia (16.5%, 12.5%).

Subsequent developments

Gradual deterioration.

Clinical diagnosis

Chronic suppurative pneumonia.

Pathological findings

Severe chronic suppurative pneumonia in anterior lobes. Lesions also in caudal lobes. From history and histopathological appearance (peribronchial lymphocyte accumulations, globule leukocytes in airways epithelia etc.) it appeared that there had been an earlier parasitic bronchitis.

Final diagnosis

Chronic suppurative pneumonia.

APPENDIX 5, 5. (97439). Bacterial isolations from a total of nine nasopharyngeal swabs obtained from Case no. 97439.

Bacterial species*	Total no. of isolates	(%)
P.haemolytica Al	3	(21)
Bacillus sp.	2	(14)
N.subflava	2	(14)
S.aureus	2	(14)
A.calcoaceticus	1	(7)
A.lignieresii	1	(7)
Micrococcus sp.	1	(7)
M.(B.)catarrhalis	1	(7)
Proteus sp.	1	(7)
Total	14	

(*no mycoplasmas were isolated)

APPENDIX 5, 5. (97439). Bacterial isolations from upper and lower respiratory tract and associated lymphatic tissues

Bacterial species*	Sampling sites**														
	NC_a	NC_p	NP	L	TR_a	TR_p	To	RTLN	RC_b	RC_L	RM_b	RML	RD_b	RD_L	BR
A.calcoaceticus	-	-	-	-	+	+	+		+	+	+	+	-	+	+
P.haemolytica Al	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Bacillus sp.	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Micrococcus sp.	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
M.(B.)catarrhalis	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
P.haemolytica A2	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
S.aureus	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-

(* no mycoplasmas were isolated)
(** a = cranial, p = caudal, b = bronchus, L = lobe)

APPENDIX 5, 6. Case no. 97669.

Admitted 7.2.85; died 5.3.85.

Subject

6 weeks old Friesian bull calf.

History

Suffered an acute episode of pneumonia three weeks pre-admission. Not thriven since.

Presenting sign(s)

Bright, thin, ringworm, respiratory distress.

Clinical findings

Fairly bright, eating, rectal temperature 101.9^{o}F.
Tachypnoea (49/min.), hyperpnoea, occasional cough.
Bilateral mucopurulent nasal discharge.
Bilateral squeaks cranioventrally.
Exercise intolerance.
Ringworm, lice, bovine papular stomatitis.

Significant laboratory findings

Leukocytosis (18.2x10^{9}/L), neutrophilia (61%).

Subsequent developments

Became duller, febrile and inappetant.

Clinical diagnosis

Chronic suppurative pneumonia.

Pathological findings

Severe consolidation involving cranial lobes and cranial parts of caudal lobes. Right lung-chronic non-suppurative pneumonia with marked fibrosis; left lung - softer lesions with fibrinous adhesions to pericardium and dilatation of interlobular septa. Also, severe acute exudative reaction in all lobes.

Final diagnosis

Severe widespread chronic non-suppurative pneumonia with a terminal acute exudative pneumonia.

APPENDIX 5, 6. (97669). Bacterial isolations from a total of six nasopharyngeal swabs obtained from Case no. 97669.

Bacterial species*	Total no. of isolates	(%)
Bacillus sp.	3	(18)
M.(B.)catarrhalis	3	(18)
A.calcoaceticus	2	(12)
Aerococcus sp.	2	(12)
Micrococcus sp.	2	(12)
N.subflava	2	(12)
Flavobacterium sp.	1	(6)
S.aureus	1	(6)
Streptococcus sp.	1	(6)
Total	17	

(*no mycoplasmas were isolated)

APPENDIX 5, 6. (97669). Bacterial isolations from upper and lower respiratory tract and associated lymphatic tissues.

	Sampling sites									
Bacterial species*	NC	NP	L	TR	To	RTLN	RC	RM	RD	BR
A.calcoaceticus	-	+	-	+	-	-	+	+	-	+
C.xerosis	-	-	-	-	-	+	-	+	-	-
N.subflava	-	-	+	-	-	+	-	-	-	-
A.viridans	-	-	-	-	+	-	-	-	-	-
E.coli	-	-	-	-	-	-	-	-	-	+
S.aureus	+	-	-	-	-	-	-	-	-	-

(* no mycoplasmas were isolated)

APPENDICES 5,7. Bacterial isolations from nasopharyngeal swabs obtained from seven pneumonic cattle

Case Nos.	100427	101904	102045	102215	103105	103393	104016	104254
No.of Nph* swabs	(15)	(4)	(7)	(4)	(15)	(11)	(12)	(3)
Admitted	19.11.85	23.4.86	6.5.86	13.2.86	18.9.86	21.10.86	19.1.87	10.2.87
Breed	Charolais	Friesian	Charolais	Friesian	CharolaisX	HerefordX	Simmental	HerefordX
Sex	F	F	M	M	F	M	F	M
Age	3 wks.	3 yrs.	1 yr.	3 mths.	9 mths.	1 yr.	1 yr.	6 wks.
Clinical diagnosis	Laryngeal abscess	Chronic pneumonia	Chronic suppurative pneumonia	Chronic pneumonia	Chronic suppurative pneumonia	Chronic pneumonia	Chronic suppurative pneumonia	Chronic pneumonia
Outcome	Slaughtered	Died	Slaughtered	Slaughtered	Slaughtered	Slaughtered	Slaughtered	Slaughtered
Post-mortem date	27.2.86	23.5.86	3.6.86	2.4.86	25.10.86	17.1.86	6.3.87	17.2.87
Final diagnosis	Laryngeal abscess, necrotising pneumonia	Necrotising pneumonia	Chronic pneumonia, bronchiectasis	Chronic non suppurative pneumonia	Chronic suppurative pneumonia	"Cuffing" pneumonic	Chronic pneumonia, bronchiectasis	Acute pneumonia

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