AN ASSESSMENT OF METHODS FOR THE QUANTITATION OF LUNG LESIONS IN SHEEP AND GOATS

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ABSTRACT

Although pneumonia is one of the most common diseases of ruminants worldwide, there is a wide variation in the way research workers have assessed the severity of pneumonic lesions. The problem is further complicated by the variable accuracy observers may have in judging the proportions of pneumonic areas in affected lungs.

The work reported here was undertaken to evaluate the methods available for quantitation of pneumonia in livestock killed in slaughterhouses. Some of the methods were then used to investigate the prevalence and variety of pneumonic lesions in the lungs of 4284 goats killed in a North Island slaughterhouse during the winter months.

A preliminary study of the *postmortem* change in lung volume demonstrated that the greatest decrease occurred from 3 to 24 hours *postmortem*, at which time there was an average loss of volume of 10%. A measurable decrease in lateral area occurred after 8 hours *postmortem*, and peaked at 96 hours with an average decrease of 8%. Image analysis was efficient in detecting changes in lung area, but the positioning of the lungs at the time of photography was a source of measurement error.

In assessing pneumonic surface areas there was no advantage in photographing a mid-sagittal section of the lungs over a dorso-lateral view of the whole lungs, but lungs separated into left and right sides before photography gave less distortion than if left attached to the trachea.

An image analysis technique proved to be more accurate than a paper silhouette technique for measuring lung areas.

While measuring the densities of the lungs, it was found that the density of the non-pneumonic portions of lung varied markedly between animals. This variation should be taken into account by using a formula to calculate the proportion of pneumonic tissue within each lung. A formula was derived which can be applied in all species when an objective estimate of the amount of pneumonic tissue is necessary, or a volumetric rather than an area value is required; but the need to sample and measure a non-pneumonic portion makes this technique inappropriate for routine work.

It was concluded that even though the percentage of lung volume impaired by pneumonia may be theoretically more important than the percentage of affected area, its measurement is too time consuming for routine use. Simple measurement of whole lung density is a poor indicator of the extent of pneumonia, while the measurement of pneumonic areas tended to overestimate the volume of pneumonic tissue. This is compounded by the irregular shape of the lungs and differences in spatial distribution of lesions.

A survey of 4284 goat lungs revealed only ten cases of bronchopneumonia. Forty-one percent of the lungs had lesions compatible with *Muellerius capillaris*; 33% had lesions compatible with *Dictyocaulus filaria*, and 8% had both types simultaneously. The prevalence of parasitic lesions increased with age. There was a statistical correlation between the severity of dictyocaulus lesions, the presence of nodular muellerius lesions, and low carcass weight. The carcasses of goats with mild to severe *Dictyocaulus filaria* lesions were from 0.81 to 1.52 kg lighter than those of animals without these lesions. The carcasses of goats with more than 10 nodular (*Muellerius capillaris*) lesions were 0.75 kg lighter than those of animals without these lesions.

Twelve sets of lungs had lesions of chronic bronchiectasis. Parasite larvae were seen in the bronchial lumina of three of them. The microscopic appearance varied from a moderate dilatation of occluded bronchi which retained an intact epithelium, to large foreign-body granulomas where the remaining bronchial outlines were barely discernible.

Subpleural lymph nodes were a common finding in more than 4% of goat lungs. Their frequency varied between flocks from as little as 2% to as much as 27%. Their distribution, architecture, and differentiation from the pulmonary lymphoid nodules described in cattle with dictyocaulus reinfection syndrome are discussed.

Multifocal fibrous pleural plaques were found in two cases of the over 4000 goat lungs sampled. They have not been described previously in the veterinary literature.

Pleural adhesions were found in 350 cases (8.16%). The relatively higher frequency of pleurisy *versus* pneumonia suggests that pneumonia in goats in the North Island of New Zealand completely resolves in most cases and probably has a seasonal occurrence, with a much lower prevalence than chronic non-progressive pneumonia of sheep.

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INTRODUCTION

Need for the development of quantitative methods

Pneumonia is recognised as one of the most common diseases of ruminants (Kirton *et al*, 1976; Trigo, 1988) and pigs (Muirhead, 1979) worldwide. Acute pneumonias often result in mortalities and chronic pneumonias cause loss of production in affected animals (Kirton *et al*, 1976; Muirhead, 1979; Alley, 1987a, 1987b). When it has been studied, the prevalence of pneumonic lesions within a herd has been found to be related to the average extent of lesions in individual lungs (Goodwin, 1971; Morrison *et al*, 1985). Many researchers have found that the degree of production loss correlates with the severity of pneumonia (Huhn, 1970; Jones *et al*, 1982; Alley, 1987), but some studies have found little (Kirton *et al*, 1976) or no (Goodwin, 1971; Jericho *et al*, 1975) relationship between the extent of pneumonia at slaughter and weight gain. Failure to demonstrate a correlation could be the result of a mis-interpretation of old healed pneumonias (Goodwin, 1971) or the occurrence of recent lesions which have had insufficient time to affect growth. The absence or severity of pneumonic lesions at slaughter is not necessarily a measure of the pneumonic history of a particular animal, since many pneumonias are self-resolving. Thus animals described as "pneumonia-free" may consist of two groups which are indistinguishable; (a) those that have never had pneumonia and (b) those that were affected early enough in life for resolution to have taken place (Harris & Alley, 1977).

While most researchers agree on the importance of pneumonia, the assessment of its severity remains a difficult issue, and different workers have taken different approaches to quantitating the extent of pneumonia in domestic animals. Because of this, some of the differences found between studies may only reflect differences in scoring technique (Morrison *et al*, 1985). Therefore, there is a need for a simple, accurate and reliable method for assessing the severity of pneumonia in production animals at the time of slaughter.

Types of lesion to be assessed

For a lesion to be quantitated, it must be possible to ascertain its extent. Single, large, localized, well-demarcated gross lesions are easily identified whereas multiple, small, diffuse lesions are not. Some of the lesions that have been described in lungs of sheep and goats are:

Well-demarcated lung lesions

Because consolidated and collapsed tissue has a different colour and density from normal lung, its surface area can be measured from a photographic image, or the amount of tissue involved can be calculated from weight and volume measurements.

Grey hepatisation of cranioventral portions of lung, which may have superimposed pleurisy, focal necrosis and abscessation, is found in sheep with chronic non-progressive pneumonia (Alley, 1975; Manktelow, 1984).

Dark red solid lung tissue with a thick layer of fibrin covering the pleural surfaces is found in acute fibrinous pneumonia of sheep associated with *Pasteurella haemolytica* type A (Salisbury, 1957; Manktelow, 1984).

Red purple consolidation corresponding to an acute exudative necrotizing broncho-pneumonia was found in an outbreak of caprine pneumonia associated with caprine herpesvirus and *Pasteurella haemolytica* (Buddle *et al*, 1990b).

Alveolar collapse without evidence of active pneumonia is sometimes a sequel to bronchitis (Kirton *et al*, 1976).

Diffuse lung lesions

The surface area of such lesions is not as easily measured from a photographic image because of their indistinct appearance, or multifocal or diffuse nature. These lesions may be best quantitated by their effect on the density of the lung:

Green-grey nodules, ranging in size from 1 mm to several cm, projecting above the pleura, represent lesions of multifocal interstitial pneumonia produced by *Muellerius capillaris* (Dungworth, 1985). Cases of microscopic *Muellerius* associated pneumonia can be found in the absence of gross lesions in goats (Ellis *et al*, 1988).

Small white foci corresponding to infiltrations of inflammatory cells can sometimes become confluent, giving rise to **large white areas** (Daoust, 1989). These

large white areas are often difficult to distinguish from **tan coloured areas** suggestive of parenchymal necrosis (Daoust, 1989).

Areas of consolidation and/or emphysema in the caudolateral aspects of one or both caudal lobes are associated with verminous bronchitis caused by *Dictyocaulus* sp. (Kirton *et al*, 1976).

Small, hard, elevated, **grey nodules** with congestion and consolidation of the surrounding lung tissue have been reported in zygomycotic pneumonia in sheep and goats (Chattopadhyay & Parihar, 1988).

Chronic interstitial pneumonia in caudal or cranio-ventral locations is characteristically found in goats infected with the caprine arthritis-encephalitis retrovirus (Ellis *et al*, 1988).

Diffuse lymphoproliferative pneumonia occurs in sheep with ovine progressive pneumonia and maedi-visna (Oliver *et al*, 1981; Dawson, 1987), a disease not present in New Zealand. It is synonymous with **lymphoid interstitial pneumonia**, and consists of thickening of alveolar septa due to interstitial accumulation of lymphocytes, plasma cells and mononuclear phagocytes. There are also prominent peribronchial or parenchymal lymphoid follicles (Lairmore *et al*, 1986).

Pleural lesions

Some cases of **pleural adhesions** in sheep are associated with pulmonary abscessation and pneumonia, but others are seemingly unrelated to pulmonary pathology (Pfeffer, 1986).

Pleural and interlobular fibrin deposition is usually found in acute fibrinous pneumonia, but is also found in some chronic pneumonias. Conversely, absence of gross evidence of fibrin does not rule out a microscopic diagnosis of fibrinous pneumonia (Daoust, 1989).

Species Differences in Pulmonary Anatomy and Pathology

There are species related differences in pulmonary anatomy and disease, even though the basic mechanisms of normal respiration and production of pulmonary disease are the same. There may therefore be important differences in the nature and distribution of lesions between species. Pulmonary function and pathology have been extensively studied in pigs (Talanti, 1959; Huhn, 1970; Jericho *et al*, 1975; Robinson, 1982; Morrison *et al*, 1985; Curtis *et al*, 1987; Buttenschon, 1989), and feedlot cattle (Veit & Farrell, 1968; Rybicka *et al*, 1974; Mariassy *et al*, 1975; Selman *et al*, 1977; Lekeux *et al*, 1984, 1985a, 1985b; Daoust, 1989; Castleman & Lay, 1990), but less so in sheep (Stamp & Nisbet, 1963; Kirton *et al*, 1976; Jones *et al*, 1982; Alley, 1987a, 1987b) and goats (Ojo, 1977; Robinson & Ellis, 1984; McSporran, 1985; Bakima, 1990).

It is known that the weight of the lung is an approximately constant fraction of body mass, even though the resting oxygen consumption increases with the 3/4 power of body mass (Taylor & Weibel, 1981). Animals with similar body size but with different metabolic activities have roughly comparable lung volumes, but different diffusing surface areas (Tenney & Remmers, 1963). Alveolar size is largely a function of animal size (Tenney & Remmers, 1963), but the alveolar surface density remains constant for animals of sizes as different as a 3.5 kg suni and a 100 kg wildebeest (Gehr *et al*, 1981). The greatest differences in alveolar surface densities are found between wild and domesticated animals; with domestic animals having a tendency towards smaller alveolar surface densities.

Within certain wide limits anatomy dictates function and patterns of disease (McLaughlin *et al*, 1961; Veit & Farrell, 1978). Species differences include degrees of lobation (International Comittee on Veterinary Anatomical Nomenclature, 1973) and lobulation, the thickness of the pleura, the presence or absence of terminal and respiratory bronchioles, the relative size of alveolar ducts and alveoli, the branching of the bronchial artery, distribution of mucus-secreting cells (Breeze *et al*, 1976), and the presence of bronchial artery-pulmonary artery anastomoses (McLaughlin *et al*, 1961; Kuhn, 1985).

Objectives of Current Study

The purpose of this research was to evaluate methods designed to quantitate the percentage of the lungs affected by pneumonia in livestock killed in a slaughterhouse. An important aspect of any quantitation technique is that it can be easily implemented in the location it is intended to be used most often. A very laborious and precise approach requiring specialized equipment may be ideally suited for a research laboratory, but impractical for a slaughterhouse survey or routine diagnostic pathology.

In the second part of the study some of the methods evaluated were used to investigate the prevalence and variety of pneumonic lesions in goat lungs during the winter months. The effects of breed and age on the frequency of pneumonia, and the relationship between the presence and extent of pneumonic lesions and the weight of the carcass was also studied.

LITERATURE REVIEW

Slaughterhouse Surveys of Pneumonia in Sheep

The first recorded slaughterhouse survey of pneumonia in lambs was that of McGowan *et al* (1957) who examined the lungs of 12495 fat lambs in California, and found that the frequency of bronchopneumonia varied between different groups of lambs from 20% to 90%. No lungworms were found in this survey.

In New Zealand, Alley (1975) carried out a slaughterhouse survey of lamb pneumonia but made no attempt to quantitate lesions. Lesions were categorised into:

1=red, ventral areas of alveolar collapse.

2=red-grey, ventral areas of bronchopneumonia.

3=grey-red, cranial areas of (consolidation) pneumonia.

4=severe, grey, cranioventral (consolidation) predominantely proliferative pneumonia.

A more extensive survey was undertaken by Kirton *et al* (1976), who investigated the seasonal frequency of chronic pneumonia and its effect on the growth of lambs. Gross pulmonary lesions were coded in a scale of one to six:

1=no gross lesions;

2=mild pneumonia, small discrete areas of consolidation in one or both cranial lobes;

3=moderate pneumonia, consolidation of the cranial lobes, ventral portion of the middle lobes and the adjacent cranio-ventral aspect of the caudal lobes, with less than one third of the lung parenchyma involved;

4=severe pneumonia, consolidation of cranial, middle and cranial aspect of the caudal lobe such that an estimated one third or more of the lung parenchyma was involved;

5=atelectasis (alveolar collapse) without evidence of active pneumonia. Lesions were found in all lobes but most commonly as discrete lobular patches or linear streaks in the cranial and middle lobes. These were associated with bronchiectasis which was a sequel to bronchitis;

6=areas of consolidation and/or emphysema associated with *Dictyocaulus* bronchitis in the caudo-dorsal aspect of one or both caudal lobes.

Lesions of *Muellerius* were not considered important unless they contributed to other lesions.

The prevalence of pneumonia was high, with a mean of 60%, varying from 41% in the lambs born in 1966 to 79% in the lambs born in 1967. The seasonal prevalence of pneumonia increased from January to April.

In spite of the high frequency of pneumonia, its severity was low: 20% of lambs had a mild pneumonia, 6% had a moderate pneumonia, 0.5% had a severe pneumonia, 24% had atelectasis, and 10% had *Dictyocaulus* pneumonia. Occassional downgrading of carcasses was associated with pleural lesions secondary to enzootic pneumonia or *Muellerius* infection (*sic*).

Kirton *et al* (1976) estimated that moderate and severe pneumonias reduced carcass weight on average by 0.45 kg per lamb. It is interesting to note the significant decrease in carcass weight observed in spite of the loose quantitation of pneumonia in moderate cases. It seems likely that a more accurate grouping of lesions might have resulted in different and perhaps more useful carcass weight data. In this survey, the effect of *Dictyocaulus* was not mentioned, and it is not apparent from the published results whether or not these lesions were also associated with a diminished carcass weight.

Pfeffer *et al* (1983), while studying the prevalence of pneumonia in a flock of sheep, classified pneumonic lesions into:

1) **Small lesions** of less than 3 cm^2 surface.
ii) **Large lesions** of more than 3 cm^2 surface.
iii) **Mottled lesions** with multiple interconnecting small lesions
iv) Lesions in the **caudal** regions of the **caudal lobes**.

These were further subdivided on the basis of their microscopic features and/or the presence of specified bacteria, parasites or viruses.

Pfeffer *et al* (1983) concluded that:

I) Large lesions were associated with *Pasteurella haemolytica* and *Mycoplasma* spp. The prevalence of these large lesions was inversely related to the average carcass weight of the lambs.
II) The prevalence of mottled lesions was associated with infections with adenovirus, *Pasteurella haemolytica* and *Mycoplasma* spp.
III) Lesions in the posterior caudal lobes were typical of infection with *Dictyocaulus filaria.*

Experimental Research on Pneumonia in Sheep

Jones *et al* (1982) compared the growth rate, feed consumption and carcass composition of normal lambs with those of lambs with an experimentally-induced chronic pneumonia. All growth parameters were significally lower in the pneumonic group. The depression in appetite and growth rate was highest in the first 35 days after inoculation and the growth rates of pneumonic lambs continued to be lower than the controls throughout the experiment. At slaughter, a significant correlation was found between the extent of lung lesions and total liveweight gain in individual lambs.

Jones *et al* (1982) found a difference of 7.8 kg between liveweight of normal lambs and that of lambs with a severe experimental chronic pneumonia after 108 days.

Alley (1987b) induced a chronic non-progressive pneumonia in pasture-fed lambs, and found that pneumonic lambs were 1.74 kg lighter than non-pneumonic lambs after 30 days, and 2.19 kg by 60 days. He concluded that a lamb may loose 1kg per month in weight gain for every 10% of lung surface area affected by pneumonia.

Slaughterhouse Surveys of Pneumonia in Pigs

Huhn (1970) assessed the incidence of enzootic pneumonia in pigs and its effect on the rate of body weight gain. He found that the expected effect of pneumonia on rate of body weight gain was often greater than differences attributed to herd of origin, sex, or feed type. The presence of pneumonia of all degrees of severity (from very mild to very severe) caused a 7% reduction in average daily body weight gain.

Jericho *et al* (1975) examined pig pneumonias by using standard cards depicting the limits of the bronchopulmonary segments of the dorsal and ventral aspects of the pig lung as described by Talanti (1959). The amount of visible macroscopic consolidation was recorded by shading on each card. The amount of consolidation was calculated by determining the percentage of squares of a standard transparent grid

which covered the shading of the diagram. The pigs were allocated into four groups showing:

i) no consolidation
ii) 0-5% consolidation
iii) 5-10% consolidation
iv) greater than 10% consolidation.

No effect of the percentage of pneumonia on either growth rate or food conversion efficiency was demonstrated. The authors suggested that possibly the range of the amount of pneumonic tissue in these pigs was insufficient to show a significant correlation between lung consolidation and performance. This was one of the few studies which has failed to demonstrate an effect of pneumonia in growth efficiency. Although the level of severity was lower than that encountered in other studies, the negative findings may have been due to other factors such as faulty experimental design or assesment methods, or time lag effects (Harris & Alley, 1977).

Morrison *et al* (1985) compared four techniques for assessing the prevalence and severity of enzootic pneumonia in market weight swine. These techniques were:

i) A detailed method for assessing pneumonia in which the proportion of each lobe involved is evaluated and the total percentage of lung affected is calculated. Groups of animal may be compared by means and standard deviations. The main constraint of this technique is that each lung must be scored in detail, which can be inconvenient and time-consuming.

ii) A simpler technique for evaluating pneumonia was proposed by Goodwin (1971) which involves counting only the number of pneumonic lungs in the sample,. Muirhead (1979) suggested that the ranges of 0-10%, 30-50%, 50-70%, 70-100% of the animals having pneumonia represented subclinical, mild, moderate and severe herd problems respectively. A constraint of this method is that the investigator must decide what minimum amount of lung involved qualifies as a case of pneumonia. In the study by Morrison *et al* (1985), the highest positive correlation between the mean amount of pneumonia in the sample and the percentage of animals with pneumonia was found for animals with more than 5% of lung tissue affected. An important prerequisite of this technique is that the investigator must be able to consistently and accurately recognise percentage of lung involvement, a skill which is not easily achieved.

iii) A third method involved scoring in detail the percentage of pneumonic lung involvement as in (i), but only in the most severely affected lung in the herd sample. Herds can be compared by non-parametric statistical techniques. Morrison *et al* (1985) suggested this technique could be used for evaluating specific-pathogen-free herds where it is important to be very confident that the pigs are free from pneumonic lesions.

iv) The categorical allocation of lungs in groups (eg:no lesions, mild, moderate or severe) according to the percentage of lung involvement (eg:0%, >0-5%, >5-10% or >10% respectively). This allows chi square analysis to be used for comparing results between different herds, provided the expected frequency cells are greater than 5 cases. If only two categories are scored, Fisher's exact test can be used even if one or more expected cells have a value of less than 5 cases.

The work of Morrison *et al* (1985) can be summarized as follows:

When limited by time or small sample size (less than 15 cases), scoring the maximally affected lung as well as categorically grading lungs may be the most practical method.

Larger samples (around 30 cases) are more conducive to calculating the percentage of animals with a preset minimum amount of pneumonia, together with scoring the maximally affected lung.

When detailed information is required, it may be necessary to score the percentage involvement of each lung.

The more detailed the scoring system and the larger the sample size, the greater will be the degree of confidence in the statistical interpretation of comparisons.

Slaughterhouse Surveys of Pneumonia in Cattle

In a morphological study of bacterial pneumonia in feedlot cattle, Daoust (1989) followed the method of Jericho & Langford (1982), estimating visually and by palpation the proportion of tissue involved in the pneumonic process for each of the eight lobes of cattle lungs. Afterwards, each lobe was sliced longitudinally from its hilar to its distal region. Only the cranial regions of the caudal lobes were sliced in this manner; their caudal regions were not examined. The distal region of each lobe and its affected region closest to the hilus were then examined grossly for the presence of 22 different types of lesion, and the severity of each of these was rated on a scale of 0 to 4+ (0=no lesion; 1+=slight; 2+=clearly present but not conspicuously so; 3+=marked; 4+=severe). In this manner, 16 pulmonary sites per case were examined in detail. Unfortunately, Daoust did not mention what the 22 types of lesion were, nor the location of the 16 sampling sites.

Pneumonia in Goats

There is little information in the literature about pneumonia in goats, though there is an increased awareness that pneumonia is a major goat health problem (McSporran, 1985).

An acute exudative bronchopneumonia causing low to moderate morbidity and high mortality occurrs in goats of all ages (McSporran, 1985). A subacute pneumonia similar to the chronic non-progressive pneumonia of sheep, associated with high morbidity and low to moderate mortality, occurrs in goat kids in their first year (McSporran, 1985). Chronic pneumonia, often caused by *Muellerius capillaris*, may be seen in goats of all ages (McSporran, 1985).

Pasteurella pneumonia was ranked 7 as a diagnosed cause of death in goats of all ages in a 15 month survey by Buddle *et al* (1988). In goats older than 6 months pasteurella pneumonia ranked as the third most common cause of death, while in flock over 500 animals, pasteurella pneumonia was the most commonly diagnosed cause of death (Buddle *et al*, 1988).

A caprine herpes virus (Bovid herpesvirus type 6) and *Pasteurella haemolytica* (serotype A7) were isolated from the lungs of adult goats dying in a severe outbreak of an acute exudative necrotising bronchopneumonia in the North Island of New Zealand (Buddle *et al*, 1990b).

An experimental inoculation of *Pasteurella haemolytica* alone produced pneumonia in goats, while inoculation with caprine herpes virus resulted in a clinical catarrhal rhinitis (Buddle *et al*, 1990a).

Pasteurella haemolytica and *Pasteurella multocida* are frequently isolated from the nose and lungs of goats in France (Richard *et al*, 1989). The serotypes A1, A2 and A6 of *Pasteurella haemolytica* are commonly isolated from ovine and caprine pneumonic lungs in Mexico (Trigo, 1986). The serotypes seen in pneumonic lungs in New Zealand are *Pasteurella haemolytica* A2, A7 and A11 (Midwinter *et al*, 1985).

In Asia and Africa the most important classical pulmonary disease of goats is contagious caprine pneumonia (Ojo, 1977) caused by *Mycoplasma mycoides* subsp. *mycoides*. This disease does not occurr in New Zealand.

Parasitic pneumonias are common in goats and will be discussed in detail in the following section.

Dictyocaulosis

Dictyocaulus filaria is probably the only lungworm of economic importance in sheep in Britain (Thomas, 1973). It is also thought to be the principal agent responsible for heavy lamb mortality in hilly and temperate regions in India (Bhat *et al*, 1986). In Australia, deaths of lambs are sometimes associated with dictyocaulus infection, but they are rarely heavy (Seddon, 1967). The frequency of dictyocaulosis ranges from very low (1.2%) in the Northern Terrritory, to very high (88%) in Gippsland (Seddon, 1967).

There is different susceptibility between sheep breeds to *Dictyocaulus filaria*; Soay sheep develop heavier burdens than Blackface sheep (Al-Sammarrae & Sewell, 1977), and yearling and adult sheep usually have higher rates of infection than lambs (George & Sullivan, 1973; Dikov & Nekipelova, 1984).

Dictyocaulus filaria is reputed to be more pathogenic to goats than to sheep (Wilson, 1964; Altaif, 1971), and infection of both sheep and goats is common in Iraq, where it seems likely that goats play a prominent role in the dissemination of *Dictyocaulus filaria* infection in areas grazed by sheep and goats simultaneously (Altaif, 1971).

In spite of the common belief that lungworms predispose to bacterial pneumonias in cattle (Pointer & Peacock, 1973; Yates, 1988), sheep (Epstein, 1975; Yates, 1988) and goats (Soulsby, 1982), no experimental data has been found to support this, except by extrapolation from studies in pigs, where the presence of ascarid larvae in the lungs was associated with impaired pulmonary bacterial clearance (Curtis *et al*, 1987). Nevertheless, Argentinian sheep attacked by *Dictyocaulus* bronchopneumonia were reported to be simultaneously affected by septicaemic *Pasteurella* sp. infections, and showed acute viral diseases caused by parainfluenza type III and foot and mouth disease (Epstein, 1975).

Pathogenesis of Dictyocaulosis

Susceptible animals eat infective third stage larvae, which pass through the wall of the intestine to the mesenteric lymph nodes within 18 hours (Anderson & Vester, 1971), where they moult. From there, fourth stage larvae travel via the lymph and bloodstream into the lungs. The size of each larva is such that embolization in the blood passing through the pulmonary arteries tends to deposit larvae in the ventral parts of the caudal lung lobes, which gives rise to the typical distribution of lesions in the patent phase (Breeze, 1985). The final moult occurs in the bronchioles a few days later (Urquhart *et al*, 1973).

The fifth larval stage of *Dictyocaulus filaria* is reached in the mesenteric lymph node 6-8 days *post*-infection. Migration to the lungs occurs from 7 to 13 days *post*-infection. Adult females begin laying eggs from day 28. (Reinecke *et al*, 1971).

During the early patency phase (weeks 3-10 after infection), serum lactate dehydrogenase levels in blood are elevated because of lung damage caused by the parasite (Bhat *et al*, 1986).

Verminous bronchitis may induce a localised airway obstruction which allows the hypoxic pulmonary vasoconstriction to redirect blood flow away from hypoxic to normoxic regions in the lung (Lekeux *et al*, 1985).

Lung disease can adversely affect productivity, and impair physiological function, because of a reduction in respiratory function. It seems more likely, however, that it is the regenerative process following lung disease which causes the production deficit (Morris, 1988).

Cattle with dictyocaulus infection have increased difficulty in breathing and an associated higher energy use in respiration; but because of the low amount of energy used in respiration, the growth depression frequently observed during this disease seems to be mainly due to diminution of feed intake (Lekeux *et al*, 1985).

Experimental infections of rats and guinea pigs with *D.filaria* results in larvae migrating to the lungs, but they do not complete the life cycle (Casarosa, 1975; Duk, 1975). In guinea pigs, lesions in the bronchial wall produce cystic emphysema in bronchial lymph nodes (Casarosa, 1975).

Tomanek (1971) reported that in guinea pigs the inoculation by subcutaneous, intramuscular or intraperitoneal routes produces a similar proportion of larvae reaching the lungs as oral administration, but Duk (1975) stated that the subcutaneous route was more infective for rats than the oral route.

Immunology of Dictyocaulosis

Dictyocaulosis was the first parasitic disease of cattle (Urquhart *et al*, 1981) and sheep (Al-Saadi *et al*, 1984) to be successfully and economically (Breeze, 1985) controlled by an attenuated (irradiated) vaccine, proving the enormous importance of the immune system in the development of nematode-caused disease, about which so little is known.

A low-level, slowly escalating pattern of infection with *Dictyocaulus filaria* in sheep in a temperate climate results in a mild, stable infection which prevents a severe, sudden, massive infection (Al-Sammarrae & Senell, 1978).

Eosinophil adherance to larvae of *Dictyocaulus viviparus* is mediated by both complement and antibodies (Knapp & Oakley, 1981).

Hyperimmune serum against *Dictyocaulus viviparus* when injected intraperitoneally into calves conferred a considerable degree of immunity to infection (Jarret *et al*, 1955).

Irradiated *D. filaria* larvae are used as a vaccine in sheep (Al-Saadi *et al*, 1984).

Diagnosis of Dictyocaulosis

The systemic immunity developed against *Dictyocaulus* spp. can be evaluated by introducing larvae into the teat canal of sheep. An eosinophil-rich exudate is observed in immune, but not in control animals, and extensive destruction of the larva occurs over a one to four hour period (Butterworth, 1984).

The enzyme-linked immunosorbent assay can be used to detect and quantify antibodies to whole worm extract antigen of *D. filaria* (Joshi *et al*, 1984).

At necropsy, the adult and juvenile lungworms can be recovered from lungs by applying water at a high pressure into the pulmonary vascular system (Oakley, 1980; Eysker *et al*, 1990), though this renders the lungs unsuitable for histopathology or other studies.

The clinical signs and lesions of dictyocaulosis relate mainly to obstruction of the small bronchi by adult worms and exudate (Yates, 1988).

The characteristic gross features of infection are catarrhal bronchiolitis (Sangster, 1990). The affected lung looks depressed and consolidated, with pinkish-gray patches, especially along the borders of the caudal lobes (Seddon, 1967; George & Sullivan, 1973; Stockdale, 1976). There is frequently atelectasis and "compensatory" emphysema in unconsolidated parts of the lungs (Soulsby, 1982; Jones & Hunt, 1983). Collapse of alveoli in the dorsal caudal lobes is caused by occlusion of bronchi or bronchioles by parasites, mucus and inflammatory exudate (Stockdale, 1976). Pulmonary oedema may be be the only lesion seen in the prepatent phase of recent infections (Poynter & Selway, 1966; Seddon, 1967).

Histologically, there is mucus-producing goblet cell metaplasia of the bronchial and bronchiolar epithelium (Stockdale, 1976). and the bronchioles are frequently infiltrated by globule leukocytes (Munro, 1983). Lymphoid hyperplasia may be seen peribronchiolar or in the lamina propria of the affected bronchus or bronchiolus (Stockdale, 1976); it may bulge into and partly occlude the lumen of the air pathway. There may also be ulceration of the epithelium, infiltration of the epithelium and lamina propria by eosinophils, lymphocytes and neutrophils and hyperplasia of the epithelium and bronchial-associated lymphoid tissue (Stockdale, 1976).

The type II cell hyperplasia and interstitial emphysema seen frequently in bovine dictyocaulosis (Jarret *et al*, 1957) have not been described in ovine or caprine dictyocaulosis.

Muelleriosis

Muellerius capillaris is a common inhabitant of the lungs of sheep and goats and is found in almost every goat examined at *postmortem* in Australia (Robinson, 1990), where it is regarded as a subclinical infection. Many different levels of infestation have been reported in various northern hemisphere countries. In 20 of 24 goats flocks examined in U.S.A., 83% of the goats were infected (Lloyd & Soulsby, 1978), whereas in Mexico, 6.2% of sheep and 5.7% of goats surveyed in a slaughterhouse had *Muellerius capillaris*. It has been found in 8-22% of sheep in hilly Slovakia (Filo *et at*, 1986). Lungworm disease in Pakistani sheep is seasonal, with the highest (50%) prevalence in October, and the lowest (3%) in June; *Muellerius capillaris* occurrs only in February and March (Hayat *et al*, 1986).

In Germany, one study found that the rate of infection of *Muellerius capillaris* (61%) was very similar to that of *Protostrongylus* spp. (61%) in old sheep in stationary flocks, but in the shepherded herds 95% of old sheep had lungworms, which were: *Muellerius capillaris* 74%, *Dictyocaulus filaria* 42%, *Protostrongylus* spp. 36%. Overall, *Cystocaulus* spp. had a 2-5% frequency (Kandels, 1984).

In Iraqi sheep with a high (53%) prevalence of lungworms, 16% of the sheep had mixed infections. *Muellerius* was more common (18%) than *Dictyocaulus* (11%), *Cystocaulus* (4%), *Protostrongylus* (2%), and *Neostrongylus*(1%) (Al-Alousi *et al*, 1986).

McSporran (1985) considered *Muellerius capillaris* to be more important than *Dictyocaulus* in goats in New Zealand, but no supporting evidence was given.

Cabaret (1984) emphasized the need for further studies on goat infections to evaluate whether the differences in prevalence and severity of the disease in sheep are related to susceptibility or grazing ethology, as goats are browsers which have only recently been exposed to grazing, and thus, lack protective responses to internal parasites (Sangster, 1990).

In a mixed grazing situation, goats have heavier worm burdens than sheep (Sangster, 1990), and this can result in a severe disease, rather than the mild infection associated with shot-like nodules usually seen in sheep (Sangster, 1990).

In sheep and goats, muelleriosis has been reported to cause reduced weight gain, increased secondary infections, severe respiratory distress and ultimately death

(Li, 1946; Rose, 1959; Poynter & Selway, 1966; Lloyd & Soulsby, 1978; Benakhla, 1981; Soulsby, 1982). However, there is little evidence to support these claims in Australia and New Zealand (George & Sullivan, 1973; Robinson, 1990).

Life cycle of *Muellerius capillaris*

Impregnated females lay eggs which hatch into mobile first stage larvae (L1). These L1 are swallowed and excreted in faeces. The L1 measure 300-320 *um* and have a curved tail with a characteristic dorsal spine (Benakha, 1981). This dorsal spine distinguishes them from lungworms belonging to the genus *Protostrongylus* (Soulsby, 1982). The larvae then go through an intermediate stage in slugs or snails (*Agriolimax, Arion, Helix, Limax, Maracha, Succinea, Trichia* spp.) before becoming infective (Poynter & Selway, 1966; Benakha, 1981). Infection of the primary host is achieved by ingestion of an infested slug or snail (Poynter & Selway, 1966).

The proportion of *Muellerius capillaris* larvae reaching the lungs varies inversely with the number of larvae ingested (Rose, 1959). Once in the lung many female *Muellerius capillaris* fail to produce fertile eggs if they do not find a male within the same nodule (Rose, 1958, 1959).

Experimental infections of mice, Guinea-pigs, rats and rabbits with third stage larvae of *Muellerius capillaris* progressed up to a fourth stage larvae which reached the lungs, but were destroyed by eosinophil and granulomatous response (Beresford-Jones, 1966; Casarosa, 1975).

No explanation has been put forward to explain the tendency for *Muellerius capillaris* to establish pulmonary nodules in such close proximity to the pleura. Although some nodules are present within the lungs, they are less numerous than the superficial nodules, which comprise 80-90% of the total (Rose, 1959). Possible explanations are that this could be the effect of local pulmonary micro-environment influences, a mere mechanical embolism (Rose, 1958) as in *Dictyocaulus* (Breeze, 1985), or due to pulmonary lymphatic drainage (Albertine *et al*, 1982).

The pulmonary lesions of *Muellerius capillaris* in goats are more commonly of the diffuse interstitial type, and therefore significantly different and more severe than the nodular lesions usually observed in sheep (Nimmo, 1979). Because the lesions are more diffuse, goats may release more *Muellerius capillaris* larvae than sheep (Cabaret, 1984).

Rose (1959) found that lambs inoculated with *Muellerius capillaris* had slightly smaller weight gains (average 9.4 pounds), despite a lack of obvious clinical signs, but he did not attach much importance to this, as the experiment was not designed specifically to study weight gain.

Anthelmintic treatment may lead to immature forms (not affected by treatment) resuming development to maturity and laying of first stage larvae upon destruction of the adult population (McCraw & Menzies, 1988). This phenomenon is highest in females of three years and older, which suggests an accumulation of inhibited larvae as a result of repeated infection as animals become older (McCraw & Menzies, 1988).

Some of the heaviest *Muellerius capillaris* infections in goats necropsied at the Ontario Veterinary College have been found in older animals up to 9 years of age (McCraw *et al*, 1981). Older goats were found to excrete more larvae of *Muellerius capillaris* in the spring (Richard *et al*, 1990). The increased larval output of *Muellerius capillaris* in the last month of gestation seemed to be related mostly to reinfection in spring (Richard, 1990).

Lesions of Muelleriosis

The histopathological changes caused by *Muellerius capillaris* and *Protostrongylus* are similar to those caused by *Dictyocaulus* (Stockdale, 1976).

Beresford-Jones (1967) described 3 different types of lesions in experimental muelleriosis of sheep:

Type A: Reddish to purple blebs, 1-3 mm in diameter, circumscribed, soft to touch and raised slightly above the level of the lung pleura, are associated to presence of L4 larvae. The lesions consist of either haemorrhagic foci and a few scattered eosinophils, or diffuse areas of alveolar haemorrhage and thickening of alveolar walls as well as areas of eosinophils mixed with lymphocytes and macrophages associated with bronchioles.

Type B: Yellow, raised, circumscribed nodules 1-3 mm in diameter, appearing as a hard pellet with a calcified centre, are developing granulomas with a central parasite surrounded by eosinophils, macrophages and lymphocytes. The worms are either males, immature females and occassional L4 larvae.

Type C: These lesions are associated with adult females, ova and L1 larvae. The lung surface has a patchy appearance because of irregularly distributed yellow to grey, raised patches covering irregular areas, visible above the lung pleura and penetrating into the parenchyma. The lesions are of diffuse character and often there are no eosinophils, but patches of lymphocytes and macrophages. The tissue shows emphysema, thickening of the alveolar walls and haemorrhage.

The commonest lesion described for sheep and goats with *Muellerius* is that of the parasite living within the alveoli but enclosed in visibly greyish nodules from 1 mm to several cm in diameter, which are mostly subpleural in location and generally on the dorsal aspect of the caudal lobes (George & Sullivan, 1973; Dungworth, 1985). These nodules represent lesions of multifocal interstitial pneumonitis (Dungworth, 1985). Calcification of the nodules is common (Seddan, 1967). The small hard nodules produced by *Muellerius* in sheep usually contain an area of bronchiolitis (Stockdale, 1976).

Histologically, adults, eggs and L1 larvae of *Muellerius* are seen surrounded by inflammatory reactions (Seddon, 1967). Granulomas are produced around larvae in letharsis, around discarded cuticles of moulting larvae, around nests of eggs and developing L1, and around dead adult and larval lungworms (Stockdale, 1976).

Fibromuscular hyperplasia of alveolar and alveolar duct septa is the common consequence of the resolution of both focal interstitial pneumonitis and granulomas (Stockdale, 1976).

The presence of fertilized females together with masses of eggs and L1 larvae produces large sized lesions greater than 5 mm in diameter. The eggs of *Muellerius* often become surrounded by leucocytes and epithelioid cells, forming smaller separate foci, with the adjacent pulmonary tissue becoming hyperaemic and the alveoli filled with round cells. After the eggs hatch the reaction subsides and the lesion may heal (Poynter & Selway, 1966; Soulsby, 1982).

Mediastinal lymph nodes may contain parasitic debris within foreign body granulomas (Seddon, 1967).

Goats may develop a generalized interstitial pneumonia around adult worms, larvae and eggs (Sangster, 1990). This diffuse interstitial pneumonia is seen grossly as widespread resilience, firmness and failure of the lung to collapse (Nimmo, 1979). No subpleural nodules are seen. Microscopically, there is diffuse thickening of alveolar septa with a mononuclear cell infiltrate plus fibromuscular hyperplasia (Nimmo, 1979).

In goats affected with *Muellerius* there can be a large variation in the severity of lesions, with the mildest case showing only small peribronchiolar lymphocyte aggregations and some fibrin, lymphocytes and macrophages around the parasites. The severest reaction may be a marked, generalized interstitial pneumonia with many alveolar macrophages, polymorphonuclear leucocytes and mononuclear inflammatory cells and much fibrin within the alveoli, plus marked type II cell hyperplasia of alveolar epithelium (Nimmo, 1979).

Dungworth (1985) is cautious in his appraisal of *Muellerius capillaris* in extensive diffuse interstitial pneumonia of goats, and points out that it is often impossible to assess the possible role of concurrent infection with *Mycoplasma* spp.or caprine arthritis-encephalomyelitis virus.

Methods Used to Quantitate Pneumonia to Date

Weight

Weighing of the lungs is part of the routine autopsy procedure performed on human corpses (Webel *et al*, 1973). However, this method has not been widely used in assessing the severity of pneumonia in livestock in spite of it being objective, easy, fast and inexpensive. The only reported use is that of Jones *et al* (1982) who found that lungs experimentally affected by proliferative exudative pneumonia were approximately 1.5 to 2 times heavier than an equivalent volume of normal lung tissue.

Although weighing the lungs is a fast, in-expensive, and simple method for evaluating changes in lung tissue, there are several drawbacks:

Firstly, the weight of normal lungs removed by standard methods must be accurately known. Normal lung weight is highly correlated to carcass size (*vide* part I B). There is also a small difference recorded in the rate of growth of lungs between rams and ewes, as well as between different breeds of sheep (Earle, 1990).

While most, if not all, pneumonias bring about a noticeable increase in the weight of the lungs, the reverse is not always true. That is, not all increases in lung weight are due to pneumonia. Some of the non-pneumonic causes of increased lung weight are oedema and blood aspiration. Interstitial oedema of the lung is a common consequence of the electrical stunning of animals. Oedema also commonly occurs when animals are killed with barbiturates. The varying severity of this oedema limits the possibility of mathematically correcting for its occurrence. Blood aspiration can also occur if the trachea is sectioned while severing the major neck blood vessels as is a common practice in slaughter-houses throughout the world.

Surface Area of Pneumonic Lesions

The quantitation of the area of lung visibly affected by pneumonia seems a simple and logical way to grade its severity. Many veterinary pathologists in different laboratories routinely estimate the proportion of the lung which seems to be affected by pneumonia in routine diagnostic cases. In the author's experience, many pathologists

tend to underestimate the extent of pneumonia, and sometimes an estimate made by one individual for one particular lung may be substantially different to that made by another colleague (*vide* APPENDIX 4).

Surface area has been the most widely used method for assessing severity in both experimental and naturally occurring cases. It has been used by Huhn (1970), Jericho *et al* (1975), Jones *et al* (1982), Alley (1987b), and Buddle *et al* (1990a).

Huhn (1970) categorized gross lesions of pneumonia in pigs into seven groups according to the number of lobes affected and the extent of lobe involved:

0) No gross lesions.

1) Very mild pneumonia, involving less than 5% of any lobe.

2) Mild pneumonia, involving multiple lobes but less than 10% of any lobe.

3) Moderate pneumonia, involving as much as 30% of one or two lobes, but not involving other lobes.

4) Moderate to severe pneumonia, involving more than 50% of at least two lobes but involving other lobes less.

5) Severe pneumonia, involving 100% of at least two lobes and involving other lobes less, but still extensively.

6) Very severe pneumonia, involving 100% of cranial and middle lobes and involving as much as 40% of caudal lobes.

A disadvantage of a complicated grading system such as this is that a reference table or chart is needed, and the time needed to classify one lung into the correct category could hamper the examination of the following lungs.

The grid method developed by Jericho *et al* (1975) was adapted to sheep lungs by Jones *et at* (1982). These authors concede that the assessment of the extent of pneumonia by lung lesion scores has the drawback that only the lung surfaces are considered. Because the lungs have a complex irregular shape, the proportion of lung areas do not necessarily translate into true proportion of lung tissue affected.

Alley (1987b) studied the effect of chronic non-progressive pneumonia on weight gain of pasture-fed lambs. He estimated the percentage of pneumonic lung from tracings made of projected colour transparencies of both the dorsal and ventral aspect of the pneumonic lungs. These tracings were then placed on a digitising tablet linked to a microcomputer and the areas of the pneumonic surfaces were measured together with total lung surface area. The percentage of pneumonic lung for each animal was then calculated.

Buddle *et al* (1990a) used **pneumonic lesion scores** for assessing an experimentally induced pneumonia in goats. The pneumonic lesion score was defined as the highest lesion score in any lobe of a lung where the scores represented consolidation of the following proportions of a lobe:

- = lesion not present
1 = < 25%
2 = 25-49%
3 = 50-74%
4 = 75-100%

Proportion of Pneumonic Volume

Since the lung is a three-dimensional organ, a volumetric assessment of its state would seem a better approach than a two-dimensional measurement.

Jericho & Langford (1982) calculated the extent of pneumonia in calf lungs by estimating the percentage of pneumonia in each lobe, and multiplying these figures by the percent contribution of each lobe to total lung weight mass. The normal contribution of each lobe to total lung weight mass was derived from the weighing of four healthy adult cattle lungs, and it was found to vary less than 1.5% between lungs. The average ratio of each lobe to total lung mass, expressed in percentage was: left cranial = 5%, left posterior cranial = 6%, left caudal = 32%, intermediate = 4%, right cranial = 6%, right posterior cranial = 5%, right middle = 7%, and right caudal = 35%

The detailed method used by Morrison *et al* (1985) for assessing enzootic pneumonia in pigs, involved visually assessing the percentage of each affected lobe macroscopically. These proportions were then multiplied with each lobe's relative weight and summed to equal the total percentage, by weight, of each lung affected by macroscopic pneumonia. The normal relative weight of each lobe was calculated from blunt dissection along interlobular fissures of eleven lungs without gross lesions.

The morphological study of bacterial pneumonia of feedlot cattle undertaken by Daoust (1989) followed the method of Jericho & Langford (1982), estimating visually and by palpation the proportion of tissue involved in the pneumonic process for each of the eight lobes in cattle lungs. Daoust (1989) considered that this method of estimating

the proportion of pulmonary tissue involved on a lobar basis was more accurate than if the lungs had been examined as a whole. However, he was aware that both methods may lead to an overestimated value, since areas affected by bacterial pneumonia, especially in acute cases, are often expanded by exudate and may therefore, appear to represent a larger proportion of the pulmonary tissue than normal. Daoust (1989) suggested that it would probably be easier to correct for this problem if only smaller portions of the organ were examined, as it would be more accurate to estimate a proportion of an area within a simple, triangular or trapezoidal shape such as a pulmonary lobe, than within a complete lung.

Measurement of Pulmonary Volume

Measurement of lung volumes for morphometric studies usually involves the fixation *in situ* of the lungs by instillation of a fixative via the trachea at a constant pressure (Weibel *et al*, 1981). The lungs are kept overnight at low temperature, after which lung volume is measured by the water displacement method of Scherle (1970). Large lungs may loose some volume (around 5%) during the first hours after the instillation procedure, probably due to some residual elastic recoil, or because of air trapped in the most cranial parts of the cranial lobes (Weibel *et al*, 1981). Weiber *et al* (1981) stated that fixation by instilling the fixative solution into the airways removes the alveolar surface lining layer and thus opens up the many folds and pleats formed to smooth the surface. Whether this is indeed the case remains unknown, as it would be technically cumbersome to prove or dis-prove.

Silicone rubber casts have also been used by Perry (1978) to determine the lung volume of one red-eared turtle on an *in situ* fixed lung using the technique described by Duncker & Schluter (1964).

Other Methods

i) GRAVITY POINT CALCULATION:

Buttenschon (1989) described five distribution patterns in pig pneumonias through gravity point calculation on the lesions of abattoir samples of pig lungs, using the gravity points as the matrix for a statistical characterisation of the type of pneumonia. The patterns observed distinguish all groups except pleuropneumonia and

verminous pneumonia. The patterns reflected the pathways of disease dispersion: bronchogenic or haematogenous. The two major dispersion pathways could be distinguished with high levels of confidence from each other by calculating the number of lesions in the cranial and middle lobes and expressing them as a percentage of the total number of lesions.

The method of gravity point calculation as described by Buttenschon (1989) divides the lungs into three shadow planes or projections; a longitudinal left and right (the shadow of either lung seen from the side), and a horizontal plane (seen from above). Normal lungs were used to draw shadow templates and these templates were fitted with an arbitrary scale in a three axis coordinate grid; a (X) cranio-caudal axis, a (Y) ventro-dorsal axis, and a (Z) latero-lateral axis. Each lung lesion was mapped in the appropriate projections, and its coordinates in the grid were read. The gravity point of a case with (n) number of lesions was calculated by averaging all X,Y,Z points respectively. From the resulting coordinates in combination with the gross pathological appearance, the case was designated to the appropriate category of pneumonia.

In the description of this method Buttenschon (1989) gave no details of how the lesions were located by the coordinate grid points, and it is not clear whether they were identified by their centre, their periphery, or all points encompassing a lesion.

ii) LUNG DENSITY:

a) Floatation test:

Observing whether or not pieces of lung placed in formalin or water float or sink has long been used by pathologists as an approximate indicator of lung density (Thomson & Cotton, 1968). Unfortunately, not all pneumonia cases are sufficiently consolidated to consistently sink. Collapsed lungs also sink (Dungworth, 1985), so this method is of little value in differentiating lesions.

b) Lung density was studied by Bertram (1985) at the alveolar level using both light and electron microscopy by taking samples (2 to 6 for light microscopy and 10-20 for EM) from four sagittal sections from the caudal lobe and two sagittal sections from the cranial lobes of lungs fixed by intratracheal infusion. Even though this method does evaluate the relative proportion of alveolar diffusing area *versus* pulmonary tissue, it is only useful for investigating pulmonary structure, not for assessing pulmonary damage.

PART I: A: MEASUREMENT OF POSTMORTEM PULMONARY COLLAPSE IN SHEEP

Introduction

Lungs have a tendency to collapse when they lose the negative pressure of the thoracic cavity (Dungworth, 1985), be it as a pneumothorax in a living animal or as *postmortem* change in eviscerated lungs. Pulmonary autolytic collapse may confound the gross and microscopic image, and may be a severe handicap when lungs are obtained at a place which is distant from the laboratory where they are examined.

No quantitative data was found in the literature regarding the *postmortem* collapse of lungs.

The aim of this study was to measure the degree of autolytic collapse of lungs over a period of time in order to find the artifactual change in volume and area which occurs. The study also provided an opportunity to compare a simple method of measuring lung areas using a paper silhouette with a more sophisticated image analysis technique using a computer video camera.

Materials and Methods

Source of Lungs

Ovine lungs were obtained from Romney ewes slaughtered at the Feilding meatworks. Both pneumonic and non-pneumonic lungs were selected, placed in individual plastic bags and taken to the laboratory where they were kept at 4 ^{o}C.

The bronchi were sectioned at the tracheal bifurcation and the lungs divided into right and left halves.

Measurements of Lung Area

1/ Measurement of Lung Area by Paper Silhouette Tracing Technique

Lateral areas of lungs were calculated by taking a transparent colour photograph *(Ektachrome* tungsten film) of the lungs on a flat surface at 3, 24, 48, and 120 hours *postmortem*. The transparency was later projected to a lifesize image on a

sheet of white paper. The outline of the lungs was traced onto the paper, cut out and weighed. Each A4 sheet of paper was weighed before use. One A4 sheet was used to cut a 10 cm^2 piece of paper, whose weight was used to convert silhouette weights into area measurements, thereby adjusting for variations in weight from one sheet of paper to another.

Lung length was taken as the longest possible longitudinal lung measurement from the photograph. Lung width (height) was taken as the greatest width at right angles to the line used for length measurement.

At 3 hours *postmortem*, the lungs were photographed while still joined by the trachea. All subsequent photographs were taken with the lungs separated into left and right (lateral view).

2/ Measurement of Lung Area by Image Analysis Technique

Photographs were taken of a ruler and the lateral views of both lungs floating in a pan with a 3 cm depth of water and a clear background at 2, 4, 8, 12, 24, 48, 96 and 240 hours *postmortem*, and these transparencies were analyzed with image analysis equipment. The lungs were kept at 4 ^{o}C from 2 to 240 hours *postmortem*.

For image analysis, an IBM PC-AT compatible computer with an image analysis card (Metrabyte IV) and matched software (Jandell Video Analysis) was connected to a (Panasonic) video camera with a 35 mm slide copying attachment. The digitised image was shown on a multisync analog monitor. The areas to be measured were outlined by connecting points of a polygon while moving a mouse around the image. A white square piece of paper of known area was included in the photographs of each lung as an inherent calibration area.

Measurement of Lung Weight and Volume

Lung weight was measured at 3 hours *postmortem* using an analogue balance with ten gram divisions.

Lung volume was calculated using a 2 L plastic jar with 100 cc graduations to visually estimate the volume of water displaced by submerging the lung with the main bronchus occluded by artery forceps. These measurements were made at 3, 24, 48 and 120 hours *postmortem*.

Statistical Analyses

The volumes, areas, lengths and widths of areas at different times were compared in a paired t test using the STATISTIX 3.0 analysis program (Analytical software, St Paul, Minnesota, U.S.A.) on a PC computer. The same program was used for calculating the simple (Pearson) correlation matrix and the scatter plot figures.

Results

Lung Volume

The lung volumes are shown in **Table IA.I**. In the first 24 hours the lung volume decreased an average of 43 cc ($p<0.001$), but the decrease was only 7 cc ($t=3.28$ $p=0.0095$) on the second day. No significant change occurred from 2 to 5 days ($p=0.16$).

The estimate of lung volumes by measuring the volume of water displaced was subject to a +/- 5% operator measurement error, as estimated by repeated measurements of the same lung. Complete occlusion of the main bronchi prior to immersion proved to be technically difficult and this may have contributed substantially to the measurement error.

Table IA.I. *POSTMORTEM* CHANGES OF OVINE LUNG VOLUME

Lung number	Volume at different times *post-mortem* 3 hours (cc)	24h (cc)	48h (cc)	120h (cc)	Weight (g)
1 left	380	330	320	270	273
1 right	440	450	430	430	292
2 left	300	270	260	270	175
2 right	330	300	300	300	220
3 left	440	370	360	360	240
3 right	500	450	450	440	320
4 left	380	340	330	330	218
4 right	500	470	460	460	340
5 left	470	400	400	390	250
5 right	570	500	500	480	310
MEANS	431_a	388_b	381_c	373_c	263

Means with a different subscript are statistically different ($p<0.05$).

Lung Area

1/ Measurement by Paper Silhouette Tracing Technique

The lung areas are presented in **Table IA.II**. The measurable lung area increased an average of 12 cm^2 from 3 to 24 hours ($p=0.002$), but remained constant from 24 to 48 hours ($p=0.12$) and 120 hours ($p=0.18$).

Table IA.II .*POSTMORTEM* CHANGES IN LUNG AREA AS MEASURED BY PAPER SILHOUETTES.

Lung number	Lateral area at different times *pm* 3hours (cm^2)	24 h (cm^2)	48h (cm^2)	120h (cm^2)	Change (cm^2)
1 left	168	172	172	175	+ 4, 0,+ 3
1 right	190	206	198	186	+16, -8,-12
2 left	133	152	152	152	+19, 0, 0
2 right	153	162	148	144	+ 9,-14,- 4
3 left	169	168	174	169	- 1,+ 6,- 5
3 right	200	201	184	191	+ 1,-17,+ 7
4 left	167	191	177	172	+24,-14,- 5
4 right	217	230	222	211	+13,- 8,-11
5 left	183	193	200	200	+10,+ 7, 0
5 right	181	206	207	207	+25,+ 1, 0
MEANS	176_a	188_b	183_b	180_b	+12,- 5,- 3

Means with a different subscript are statistically different ($p<0.05$).

Relationship Between Lung Volume and Lung Area

The matrix of simple correlation for measured variables is presented in **Table IA.III** and the lengths and widths of lungs at different time intervals are shown in **Table IA.IV** and **Figures IA.1** and **IA.2**.

There was a strong direct correlation between all volume measurements, between the area at 120 hours *postmortem* and all volumes, and between the areas at 48 and 120 hours *postmortem*.

Table IA.III. MATRIX OF CORRELATION OF VARIABLES

	Vol03	Vol24	Vol48	Vol120	Area3	Area24	Area48	Area120
Vol024	0.954							
Vol048	0.968	0.996						
Vol120	0.926	0.976	0.977					
Area3	0.799	0.866	0.857	0.833				
Area24	0.796	0.892	0.878	0.875	0.928			
Area48	0.857	0.897	0.883	0.862	0.871	0.934		
Area120	0.908	0.901	0.901	0.851	0.855	0.894	0.968	
Weight	0.836	0.896	0.891	0.820	0.921	0.855	0.808	0.829

Table IA.IV. LENGTHS AND WIDTHS OF OVINE LUNGS FROM 3 TO 120 HOURS *POSTMORTEM* AS MEASURED FROM PHOTOGRAPHIC SILHOUETTES.

Case	Len3 (mm)	Len24 (mm)	Len48 (mm)	Len120 (mm)	Wid0 (mm)	Wid24 (mm)	Wid48 (mm)	Wid120 (mm)
1 left	227	215	213	224	107	114	114	115
1 right	266	261	270	271	97	115	108	127
2 left	188	191	197	190	100	101	107	110
2 right	240	250	230	242	85	93	90	92
3 left	227	200	200	202	104	100	100	100
3 right	267	255	261	260	97	101	97	100
4 left	220	214	223	210	108	146	131	115
4 right	296	262	275	259	117	127	114	127
5 left	220	223	228	230	113	144	145	120
5 right	262	261	250	245	99	114	125	127
MEANS	241_a	233_a	234_a	233_a	102_b	115_c	113_c	113_c

Means with a different subscript are statistically different ($p<0.05$).

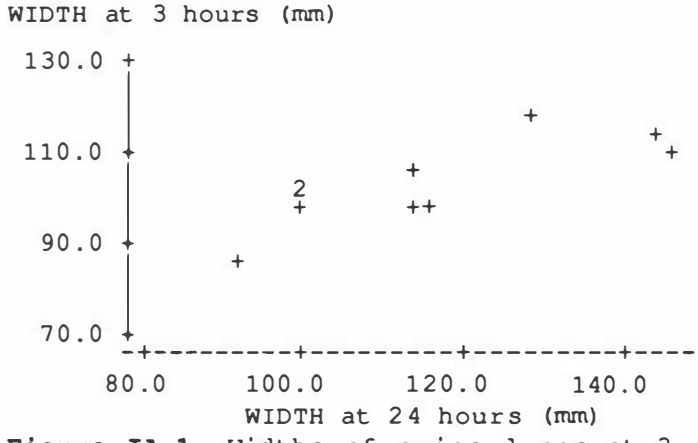

Figure IA.1. Widths of ovine lungs at 3 and 24 hours *postmortem* as measured from photographic silhouettes

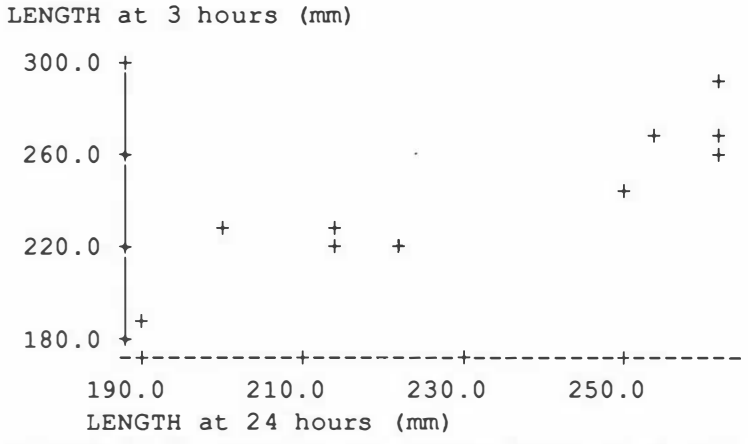

Figure IA.2. Lengths of ovine lungs at 3 and 24 hours *postmortem* as measured from photographic silhouettes

2/ Measurement by Image Analysis Technique

The lateral areas of lungs measured by image analysis are shown in **Table IA.V** and the matrix of correlation of areas at different times is presented in **Table IA.VI**.

The lateral areas did not vary from 2 to 4 hours *post-mortem* (p=0.35), nor from 2 to 8 hours (p=0.33), however there was an average decrease of 8.7 cm^2 at 12 hours, and 11 cm^2 at 96 and 240 hours.

Table IA.V. LATERAL AREAS OF LUNGS FROM 2 TO 240 HOURS *POSTMORTEM*

Case #	2 hrs (cm^2)	4 hrs (cm^2)	8 hs (cm^2)	12 hs (cm^2)	24 hs (cm^2)	48 hs (cm^2)	96 hs (cm^2)	240 hs (cm^2)	Weight (g)
61 Left	114	116	122	115	118	117	106	119	200
61 Right	148	149	152	147	153	147	143	141	260
62 Left	127	131	133	130	129	127	124	122	225
62 Right	151	149	156	137	141	148	138	126	310
63 Left	147	143	143	147	153	142	137	128	210
63 Right	153	156	157	142	155	138	145	138	285
64 Left	144	153	150	129	135	144	143	134	260
64 Right	162	162	166	155	148	151	152	144	350
65 Left	106	110	106	102	101	107	102	088	155
65 Right	115	117	122	111	115	114	113	109	200
66 Left	151	152	152	145	144	143	141	NT	225
66 Right	163	164	165	153	159	159	143	NT	300
67 Left	163	164	164	153	162	155	151	NT	235
67 Right	191	182	179	168	165	157	157	NT	310
68 Left	140	134	139	125	132	136	125	NT	230
68 Right	168	158	166	160	160	157	155	NT	310
69 Left	111	107	109	100	101	102	103	NT	200
69 Right	133	124	124	116	116	112	108	NT	255
70 Left	137	132	142	124	132	132	124	NT	240
70 Right	159	159	159	150	155	145	147	NT	320
MEAN	144_{a}	143_{ab}	145_{a}	135_{bcd}	138_{bc}	136_{c}	132_{de}	e	254

Nt = Not Tested.
Means with different subscripts are statistically different ($p<0.05$).

Table IA.VI. MATRIX OF CORRELATION OF VARIABLES

	Area2	Area4	Area8	Area12	Area24	Area48	Area96	Area240
Area4	0.983							
Area8	0.983	0.981						
Area12	0.946	0.903	0.915					
Area24	0.932	0.896	0.901	0.957				
Area48	0.968	0.955	0.959	0.922	0.898			
Area96	0.981	0.992	0.973	0.921	0.914	0.957		
Area240	0.892	0.901	0.927	0.901	0.903	0.889	0.905	
Weight	0.883	0.886	0.934	0.784	0.713	0.846	0.857	0.812

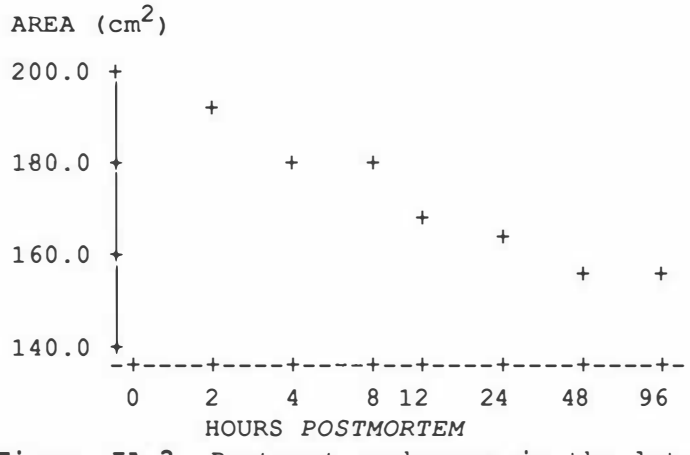

Figure IA.3. *Postmortem* changes in the lateral area of lung 67 (right) as measured by image analysis.

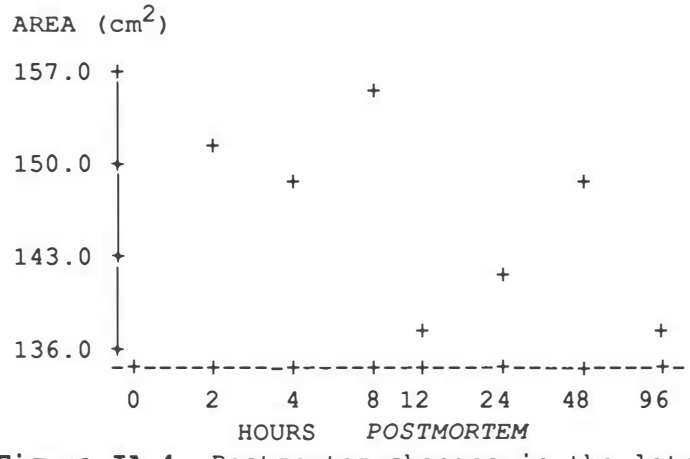

Figure IA.4. *Postmortem* changes in the lateral area of lung 62 (right) as measured by image analysis.

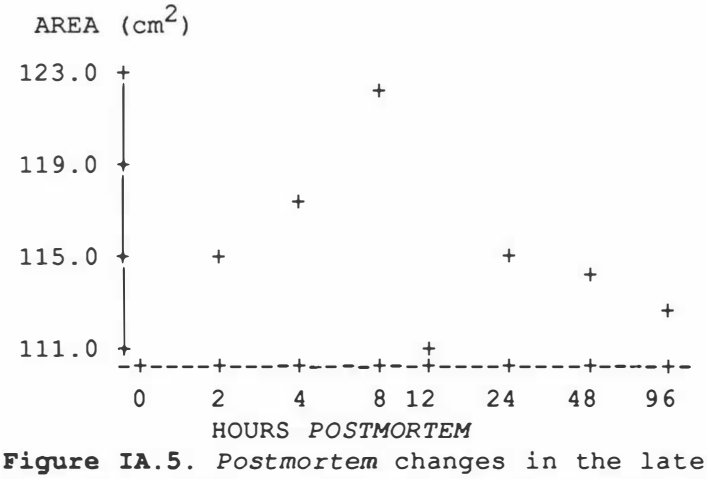

Figure IA.5. *Postmortem* changes in the lateral area of lung 65 (right) as measured by image analysis.

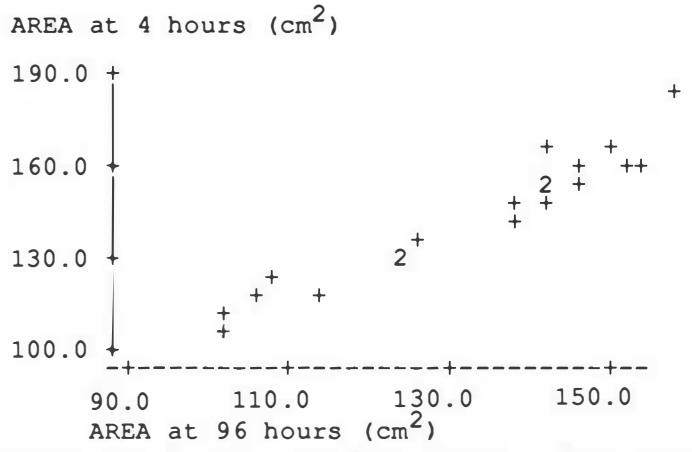

Figure IA.6. Relationship between lung areas at 4 and 96 hours *postmortem* as measured by image analysis.

Discussion

Lung Volume

The results indicate that the greatest artifactual change in lung volume occurs before 24 hours *postmortem*. This has serious practical consequences, since accurate measurements of lung volume can only be made soon after the death of the animal. Unless the slaughterhouse is near the laboratory, the measurement of lung volume should therefore be made within the slaughterhouse itself.

The amount of decrease in volume between 3-24 hours was inversely correlated to the decrease in volume from 24 to 48 hours ($r=-0.7980536$ $p=0.005$). This would indicate that those lungs which experienced the greatest amount of collapse in the first day would have the least subsequent collapse in the second day, as there would be less intact alveolar spaces left. It also accounts for the slightly greater correlation of volume at 3 hours with volume at 48 hours ($r=0.9682$ $p<0.001$), and not at 24 hours ($r=0.9548$ $p<0.001$). Finally, the change in volume from 48 to 120 hours *postmortem* was not significantly correlated to any variable, and the final volume at 120 hours had a poor correlation with weight and lateral area.

Lung Area Measured by Paper Silhouette

The lung lateral areas at 24 hours *postmortem* were on average, 12 cm^2 larger than those at 3 hours *postmortem* (p=0.002). This change was attributed to a bias caused by the differing placement of the lungs for photography. Due to the greater flexibility of the lungs as they began to autolyse, the lengths of lungs remained constant from 2 to 120 hours (p>=0.09), but the width of lungs at 24 hours were 13 cm greater than those at 3 hours (p=0.013), and remained constant from 24 to 48 hours (p=0.36) and from 48 to 120 hours (p=0.9612).

A marked change in the shape of the lung silhouettes from one photo to the next was noted but not quantitated. It was not technically possible to measure the proportion of overlapping of lung areas in consecutive photographs of the same tissue.

The failure to demonstrate a decrease in lung area with the paper silhouette technique may be partly due to photography of the lungs in two different positions. It was found that separating the lungs before photography produced higher correlation coefficients with most other variables.

Relationship Between Lung Volume and Lung Area

The different volumes of the lungs at varying times were better correlated to the area of the lungs after 24 hours than at 3 hours. That suggests that the first measurement of lung area was biased by the way the lungs were held together while the photograph was taken at this time. The change in the positioning of lungs resulted in an artifactual increase in measured lung area, in spite of a considerable decrease in lung volume. These results suggest that it would be better to have a picture of the lungs apart, rather than joined, for subsequent analysis. However, the correlation of lung weight was roughly the same for the lungs pictured together at 3 hours (r=0.851297 p=0.001) or separate at 24 hours (r=0.8581902 p=0.001).

Lung Area Measured by Image Analysis

It was expected that this technique would reveal a smooth tendency for lung area to decrease with time, as typically seen in the semi-logarithmic plot for lung 67 right in **Figure IA.3**. However, many lungs showed either an irregular fluctuation in area (**Figure IA.4**), or a middle peak (**Figure IA.5**). This phenomena could be explained by:

1) Inaccuracy of the PC-image analysis setup (*vide* part IC).
2) Changes in lung shape during cold storage. Decrease in area could be caused by compression, and a subsequent increase caused by elastic recoil.
3) Changes in the positioning of the lung lobes during photography which may have occurred although the lungs were free-floating in water. Unfortunately this could not be tested.

The findings that there was a considerable change in the shape of the lung silhouettes from one photo to the next highlights the importance of the way the lungs are placed for photographing, as minor changes in position result in fluctuations of up to 10% in the area measurements.

Comparision Between the Paper Silhouette and the Image Analysis Techniques

The matrix of correlation obtained using image analysis had considerablely higher values than that obtained for similar times with the paper silhouette method (**Table IA.III** *versus* **Table IA.VI**). Particularly interesting (**Figure IA.6**) are the high correlations between areas at 4 and 96 hours ($r=0.9922$ $p<0.001$) using image analysis. In the paper silhouette experiment, the highest correlation found was between areas at 48 and 120 hours ($r=0.97$ $p<0.001$).

The differing results between the paper silhouette and the image analysis methods can be ascribed to different accuracies. From a user's point of view, the computerized setup is easier to use as long as the areas to be measured have contrasting borders (*vide* APPENDIX 3).

Conclusions

i) The greatest rate of autolytic decrease of lung volume was seen from 3 to 24 hours *postmortem*. At this time lung volume decreased on average by 10%. Lung volume must therefore be sampled early in order to diminish the degree of variability caused by artifactual change in volume.

ii) The greatest decrease in lung volume was reached by 48 hours *postmortem*, and averaged 13% of the initial lung volume.

iii) The image analysis method was efficient in detecting autolytic decrease in lung area. Measurable autolytic decrease in lung lateral surface begins some time after eight hours *postmortem* and reaches a peak at 96 hours *postmortem*. The average decrease in lung lateral surface area was 8% of the initial area.

iv) The positioning of the lungs at the time of photography accounts for a moderately large measurement error.

PART I: B: ASSESMENT OF METHODS FOR MEASURING THE SEVERITY OF PNEUMONIA IN SHEEP

I.B.I. MEASUREMENT OF SURFACE AREAS

Introduction

No information is available on the value and accuracy of measuring the area of different lung surfaces or sections to assess the degree of pneumonic involvement.

The following study was undertaken to compare the areas of total and pneumonic lung surface obtained by photographing lungs joined together or separated into left and right sides. It also compared dorso-lateral and ventro-medial surface views with mid-sagittal sections.

Material and Methods

Ten pairs of ovine lungs with degrees of pneumonia varying from very mild to very severe were collected from the meatworks in Feilding. They were placed in plastic bags and taken to the laboratory to be photographed. The lungs were separated into left and right, and each lung was sectioned in a sagittal plane aimed at obtaining the largest possible cross-section. Photographs (*Ektachrome* tungsten transparencies) were taken of the dorso-lateral view, the ventro-medial view, and the sectional areas of both halves. Four pairs of lungs were photographed in this fashion, and the remaining six pairs of lungs had ventro-medial and dorso-lateral photographs taken while they were unsectioned, and still joined to the trachea.

The photographic transparencies were projected for the production of paper silhouettes as described previously (Part I A).

Results

The total and pneumonic surface areas measured using different photographic approaches are presented in **Table IB.I** and **Table IB.II**.

The correlations of the variables for the cases where left and right lungs were separated are shown in **Table IB.III**. The correlations of the variables for the lungs photographed while still joined together are shown in **Table IB.IV**.

Table IB.I. LUNG AREAS MEASURED BY DIFFERENT METHODS *

Number	Med (cm^2)	Medpne (cm^2)	Lat (cm^2)	Latpne (cm^2)	Sme (cm^2)	Smepne (cm^2)	Sla (cm^2)	Slapne (cm^2)	Weight (g)
31Left	170	3.25	224	4.7	154	0.75	189	2.13	340
31Right	180	0.0	227	2.4	150	1.50	197	0.00	280
32Left	161	11.0	190	5.0	134	1.82	145	5.03	220
32Right	205	6.17	250	7.2	170	2.63	204	9.20	300
33Left	119	18.8	134	47.5	116	25.0	105	31.4	290
33Right	174	27.6	186	45.0	128	17.5	143	33.9	290
34Left	158	0.0	202	1.5	143	0.0	177	0.0	200
34Right	216	78.0	271	103.0	195	47.0	229	89.0	430

Notes:
Med: area measured from a photograph of the medial aspect of the lung.
Lat: area measured from a photograph of the lateral aspect of the lung.
Sme: area of a sagittal section obtained by photographing the medial half of the lung.
Sla: area of a sagittal section obtained by photographing the lateral half of the lung.
pne: pneumonic part of lung area.
* For obtaining the lateral and medial values of Lungs 31-34, the lungs were photographed separately.

Table IB.II.LUNG AREAS MEASURED BY DIFFERENT METHODS *

Number	Med (cm^2)	Medpne (cm^2)	Lat (cm^2)	Latpne (cm^2)	Sla (cm^2)	Slapne (cm^2)	Weight (g)
35Left	157	27.5	149	28.2	163	29.5	210
35Right	184	12.2	182	10.4	193	3.3	230
36Left	161	0.9	158	1.8	171	8.9	260
36Right	195	0.4	184	3.8	180	7.3	320
37Left	159	0.0	148	4.0	144	10.7	200
37Right	198	4.2	208	9.2	197	18.0	270
38Left	183	2.1	158	2.0	NT	NT	220
38Right	178	2.8	172	3.5	NT	NT	230
39Left	132	6.8	140	1.5	136	0.0	170
39Right	177	4.2	183	5.4	197	3.4	250
40Left	193	1.3	189	1.8	171	0.6	240
40Right	252	28.9	212	2.8	223	0.0	340

Notes:
Med: area measured from a photograph of the medial aspect of the lung.
Lat: area measured from a photograph of the lateral aspect of the lung.
Sme: area of a sagittal section obtained by photographing the medial half of the lung.
Sla: area of a sagittal section obtained by photographing the lateral half of the lung.
pne: pneumonic part of lung area.
NT : not tested.
* For obtaining the lateral and medial values of Lungs 35-40, the lungs were photographed while still joined together.

Table IB.III.CORRELATIONS OF VARIABLES FOR PNEUMONIC LUNGS MEASURED SEPARATELY (Cases 31-34).

	Medial area	Sagittal lateral half	Sagittal medial half	Weight
Lat	0.9506532 p<0.001	0.98206 p<0.001	0.9548759 p<0.001	0.705557 p=0.05
Medial		0.8894729 p=0.003	0.8961319 p=0.002	0.718528 p=0.04
Sagittal of lateral half			0.9412469 p<0.001	0.6658876 p=0.07
Sagittal of medial half				0.7879924 p=0.02

Note: The matrix of correlations is symmetrical, only the upper right half is shown.

Table IB.IV. CORRELATION OF VARIABLES FOR PNEUMONIC LUNGS JOINED TOGETHER (Cases 35-40).

	Lateral	Latpne	Weight
Medial	0.8832071 p<0.001	n.s.	0.8511419 p<0.001
Medpne		0.6043806 p<0.001	n.s.
Lateral			0.7932217 p=0.002

Note: n.s. = Not significant (p>0.10).
The matrix of correlations is symmetrical, only the upper right half is shown.

When the lungs were cut sagittally into medial and lateral portions, the total lung areas obtained from the lateral photographs (mean=210cm^2) were significantly greater than the areas obtained from the medial photographs (mean=172cm^2) (p=0.015). The two areas were highly correlated (r=0.95 p=0.002).The regression equation for this is:

Lateral area = -23.65204 + 1.354459(Medial area)

The area of the sagittal sections was smaller than the original outer areas. The cross sectional area of the lateral half of the lung was on the average, 82% of the surface area of the dorso-lateral photograph. The regression equation for this is:

Sagittal section of lateral half = -21.55454 + 0.9272187(Lateral area)

The total sectioned areas of the sagittal sections were also different (p=0.014); the lateral part had a larger cross-section (mean=173cm^2) than the medial part (mean=148cm^2). The regression equation for this is:

Sagittal section of lateral half = -50.92848 + 1.509603(Sagittal section of medial half)

The pneumonic areas and the proportions of pneumonic surface measured by the different approaches were not statistically different (p>0.05).

Discussion

In spite of the larger size of the dorso-lateral surface area when compared to the ventro-medial surface area, the areas of pneumonic tissue measured by the different photograph approaches were not significantly different ($p=0.089$). The sagittal sections had the same amount of pneumonic tissue as the dorso-lateral or ventro-medial surface photographs ($p>0.05$). This indicates that sagittal sectioning of the lung does not increase the amount of pneumonic tissue that can be detected above that visible by means of a simple dorso-lateral surface examination.

The measurement of lungs 35-40, which had their dorso-lateral and ventro-medial photographs taken while the lungs were still joined together, before sagittal sectioning, produced striking results. The dorso-lateral total area of the lungs before sectioning was not statistically different ($p=0.10$) from the ventro-medial area, but the correlation of both variables was low ($r=0.8832071$ $p<0.001$). There was a much higher correlation ($r=0.9506532$) between the dorso-lateral and ventro-medial areas in the measurement when the lungs were separated into left and right before being photographed. This finding indicates that photographing the lungs while they are still connected by the trachea may result in uneven placing and distortion of the lung tissue, which in turn results in unreliable area measurements.

Conclusions

i) In assessing pneumonic surface area there is no advantage in photographing a mid-sagittal section of the lungs over a dorso-lateral view.

ii) Lungs should be separated into left and right sides before photographing them to measure their areas.

PART I: B: ASSESMENT OF METHODS FOR MEASURING THE SEVERITY OF PNEUMONIA IN SHEEP

I.B.II. MEASUREMENT OF LUNG WEIGHT AND DENSITY

Introduction

The inflammatory reaction in a pneumonic lung invariably increases the weight of the lung. The lung density, which is the quotient of lung volume divided by its weight, will change due to both the loss of alveolar lumina and the increase in weight brought about by infammation of the lung. While this is often subjectively assessed, and pneumonic lungs are often described as heavy, no objective data was found in the literature concerning the measurement of lung weight or density as an indication of the extent of pneumonia.

Material and Methods

Weight of Normal Lungs

Ten ovine lungs without obvious lesions of respiratory disease were collected from the Feilding meatworks and taken to the laboratory where they were kept at 4 ^{o}C for 17 hours. The carcass weight of each animal to the nearest 0.5 kg was recorded at the meatworks. The trachea was cut 15 cm from the bronchial bifurcation, and the lungs were weighed using a mechanical analogue balance, and perfused endotrachealy with tap water using an endotracheal tube at a water pressure of 30 cm. The lung capacity (ml) was estimated as the difference between the weights (g) of lung before and after filling endotracheally. The tracheal diameter was measured 15 cm from the bronchial bifurcation.

Density of normal and pneumonic lungs

Eleven ovine pneumonic lungs from lambs were collected from the Oringi meatworks and taken to the laboratory, where they were weighed using digital (electronical) and analogue (mechanical) balances, and their volume was calculated by water displacement as described by Scherle (1970). A portion of non-pneumonic lung was then removed and its weight and volume were measured.

Results

Even though the analogue (mechanical) balance gave very similar results to the digital (electronic) balance, the slight difference in accuracy had a profound effect on the calculated lung densities. Therefore, only the results of the digital balance are presented here. The details are given in the APPENDIX 2.

Weight of Normal Lungs

On detailed examination, several lungs were found to have small numbers of *Muellerius* lesions, mild interstitial oedema, and small areas of cranioventral collapse of less than 5 % of the lung area. The relationship between the measured variables is presented in **Table IB.V** , **Figure IB.1** and **Figure IB.2**.

The weight of the lungs was statistically correlated to the carcass weight ($r = 0.98$ $p<0.001$). For this sample, the regression equation relating lung and carcass weights was:

Lung Weight(g) = 28.5(**Carcass Weight**)(kg)

Even though the diameter of the trachea had a high correlation ($r=0.96$ $p<0.001$) with lung weight (**Figure IB.2**), it was not a significant addition to the former equation ($p=0.30$), as it was highly correlated to carcass weight ($r=0.99$ $p<0.001$).

The attempt to perfuse lungs was not successful, as the rate of lung dilatation was very small in the beginning, and before all the lung lobules were fully dilated, water leaked from many different places, in spite of no gross discontinuities.

The measurement of tracheal diameter was found to have a considerable subjective element due to flexibility of the tracheal rings, and an estimate of the horizontal inner tracheal ring diameter was recorded. In two cases the tracheal sampling site had been sectioned at the meatworks and no measurement was taken.

Table IB.V. RELATIONSHIP BETWEEN LUNG WEIGHT, CARCASS WEIGHT, LUNG CAPACITY AND TRACHEAL DIAMETER.

	Carcass weight (kg)	Lung weight (g)	Perfused weight (g)	Tracheal diameter (mm)	Notes
1	13	265	890	20	
2	16	410		19	m av
3	16	460		23	m e
4	16.5	535		22	m e
5	13.5	410		17	
6	17.5	630			e t
7	15	480		19	
8	17	530	3050	20	
9	18	465	3740	23	e
10	14.5	440			e

Notes: m: presence of small *Muellerius* lesions.
av: presence of small areas of cranio-ventral collapse.
e: pulmonary interstitial oedema.
t: endotracheal probe slipped off while attempting to perfuse.

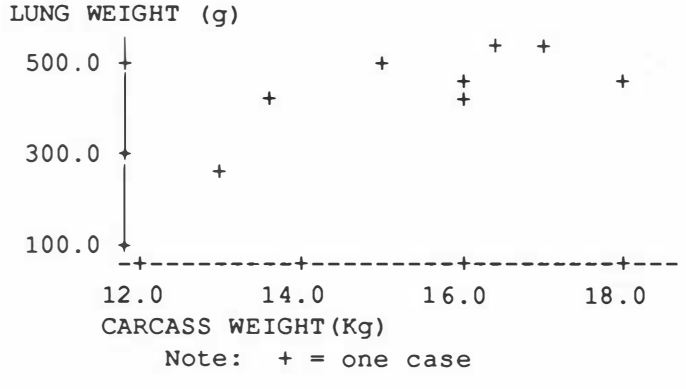

Figure IB.1. Lung Weight *versus* Carcass Weight (n=8)

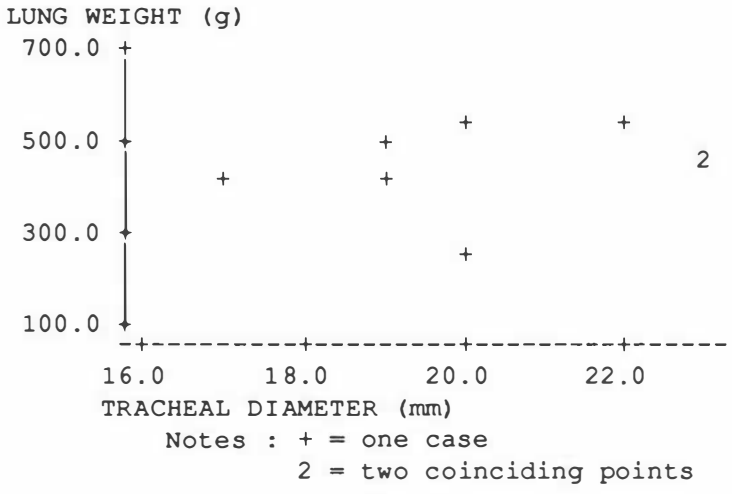

Figure IB.2. Lung Weight *versus* Tracheal Diameter (n=8)

Density of normal and pneumonic lungs

The calculated mean pneumonic volume was higher in the right lung than the left lung (68 cc *versus* 24 cc). The mean proportion of pneumonic tissue within each lung was also higher in the left lung (19 % *versus* 8 %).

The densities of both whole lungs and non-pneumonic parts are shown in **Figure IB.3** and **Figure IB.4**.

There was a large variation in density of the whole lung, which in most cases was linearly related to the proportion of pneumonic tissue in them (**Figure IB.5**), except for three cases (asterisks in **Figure IB.5**). These statistical outliers were due to a small sample weight (**Figure IB.6**) and balance accuraccy (*vide* APPENDIX 2).

The density of the non-pneumonic portions of lung was variable, and its value varied proportionally to the density of the whole lung (**Figure IB.7**).

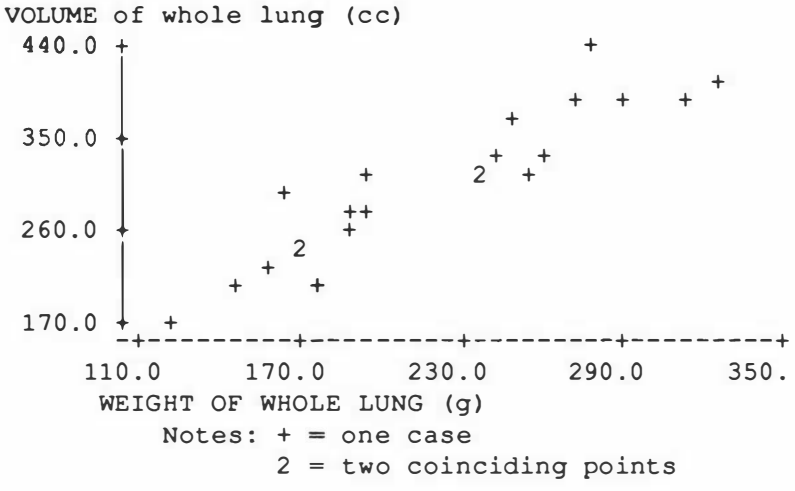

Figure IB.3. Density of Whole Pneumonic Lungs: Plot of Volume *versus* Weight (Electronic Balance)

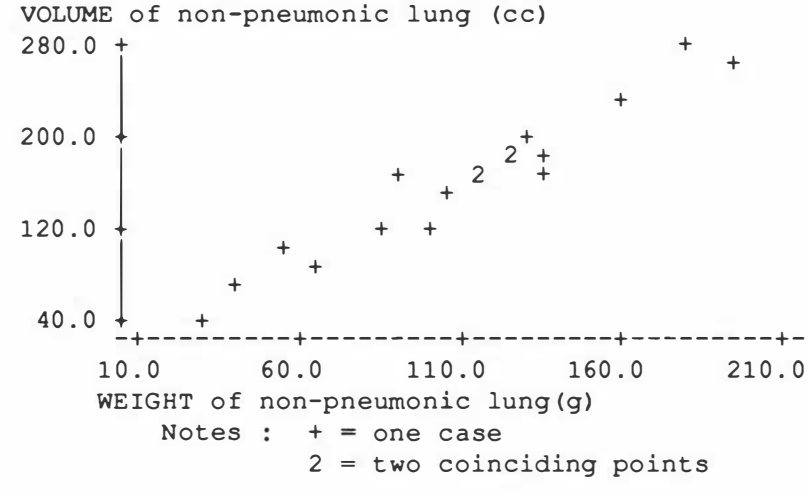

Figure IB.4. Plot of Volume and Weight for Non-Pneumonic Portions of Lung

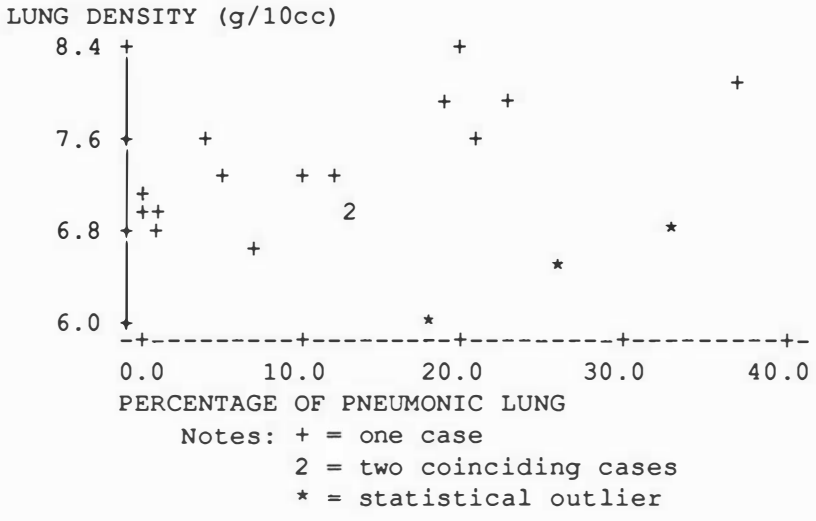

Figure IB.5. Relationship of Whole Lung Density to Extent of Pneumonia

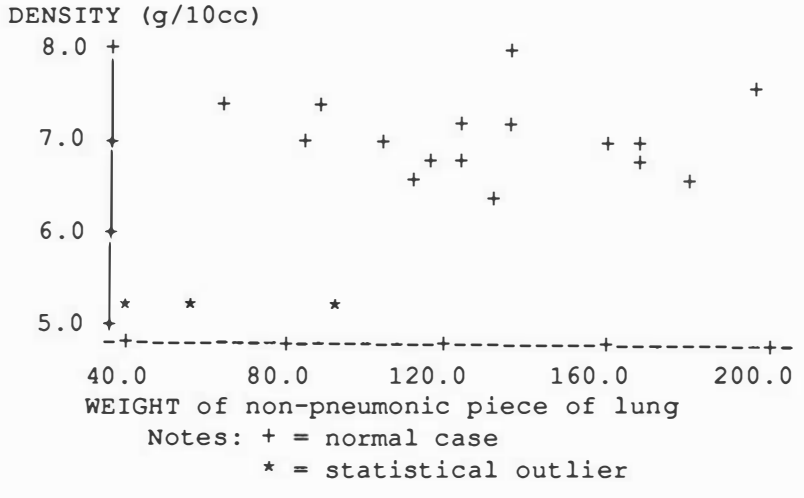

Figure IB.6. Density *versus* Sample Weight for Non-Pneumonic Portions of Lung

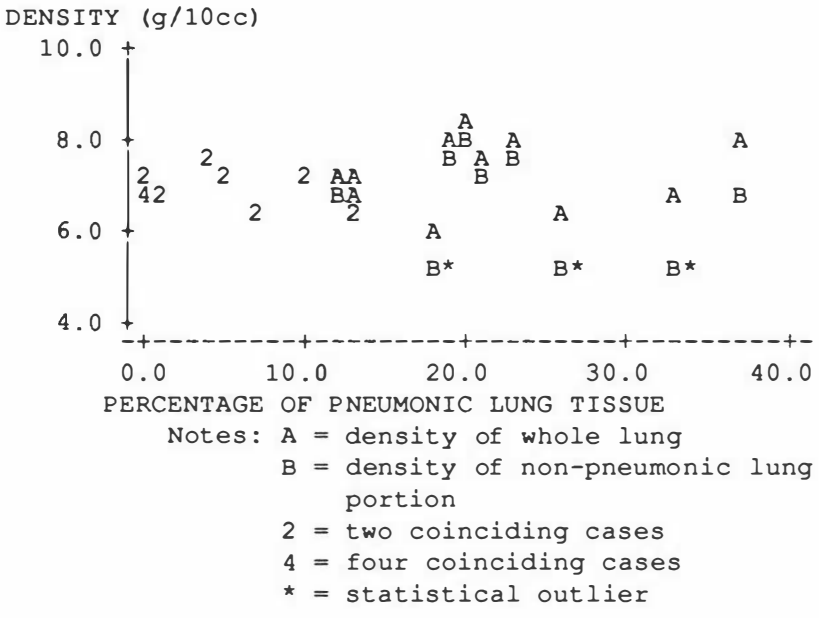

Figure IB.7. Density of Lung According to Proportion of Pneumonia

There was a very high correlation between the volume and the weight of the non-pneumonic portions of lung ($r=0.9889$ $p<0.001$). The volume of a non-pneumonic portions of lung can be calculated with the formula:

Volume of non-pneumonic lung = 1.4436(**Weight of non-pneumonic lung**)

The average volume of the whole right lung (349 cc) was larger than the left lung (263 cc), and the average density of the whole right lung (0.68 g/cc) was higher than for the left lung (0.66 g/cc).

The intact pneumonic lungs had variable densities (**Figure IB.8**). It was not possible to measure the density of portions of pneumonic lung because the lungs sank as their density was higher than that of water. The density of the non-pneumonic portions of lung was the same for left (0.68 g/cc) and right (0.66 g/cc) lung ($p>0.10$).

In many cases, the measured density of the non-pneumonic portions overlapped the values found for the density of whole pneumonic lungs (**Figure IB.8** *versus* **Figure IB.9**)

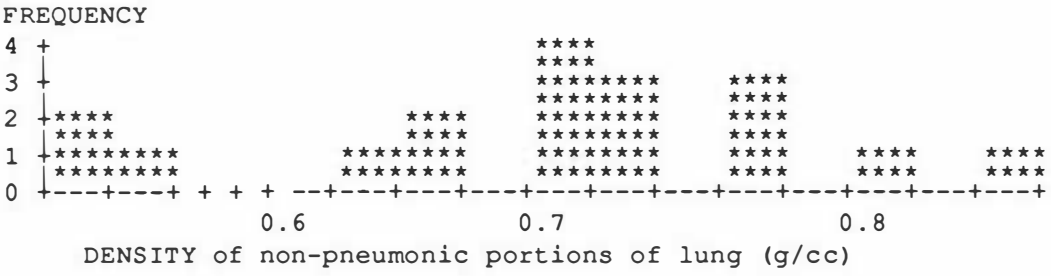

Figure IB.8. Densities of Non-Pneumonic Portions of Lung

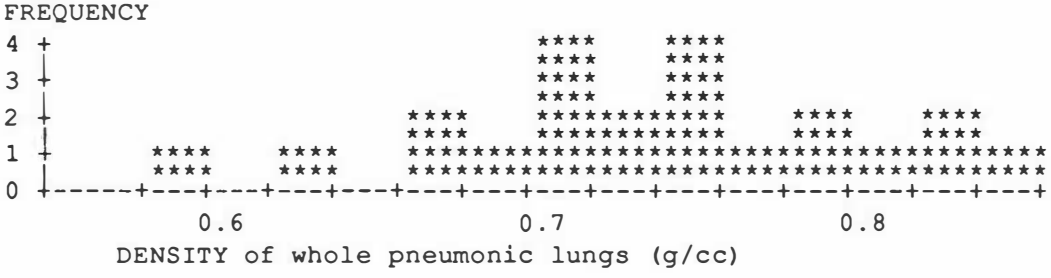

Figure IB.9. Densities of Whole Pneumonic Lung

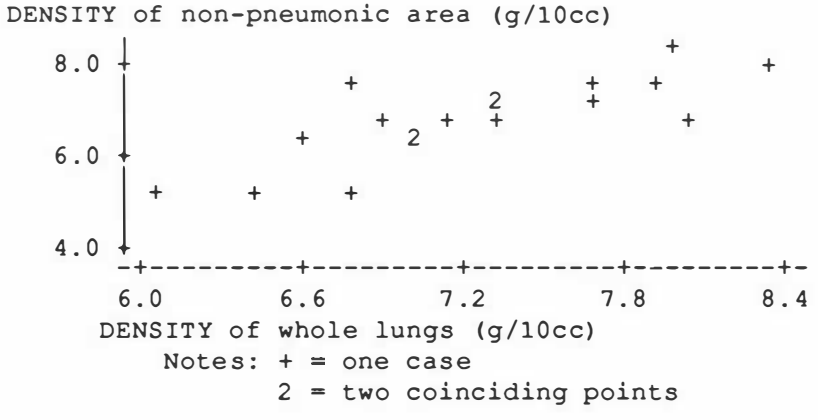

Figure IB.10. Comparison of Whole Lung Densities and the Density of Non-Pneumonic Pieces of Lung

Theoretical Basis for the Calculation of Volume of Pneumonic Portions by Lung Density

In order to estimate the amount of pneumonic tissue in a lung, the following equations were used:

(1) Lv = Pv + Nv
Lung volume = Volume of pneumonic part + volume of non-pneumonic part.

(2) Lw = Pw + Nw
Lung weight = weight of pneumonic part + weight of non-pneumonic part.

(3) Pd = Pw / Pv
Density of pneumonic portion = weight of pneumonic portion divided by its volume.

(4) Nd = Nw /Nv
Density of non-pneumonic portion = weight of non-pneumonic portion divided by its volume.

Re-arranging equation (1) produces:
(5) Nv = Lv - Pv

Re-arranging equation (2) produces:
(6) Pw + Nw - Lw = 0

Re-arranging equation (3) produces:
(7) Pw = (Pd) (Pv)

Re-arranging equation (4) produces:
(8) Nw = (Nd) (Nv)

Substituting Nw in equation (6) by its equivalence in (8) gives:
(9) Pw + (Nd)(Nv) - Lw = 0

Substituting Nv by its equivalent in (5) leads to:
(10) Pw + (Nd)(Lv -Pv) - Lw = 0

Substituting Pw by its equivalent in (7) produces:
(11) (Pd)(Pv) + (Nd)(Lv-Pv) - Lw = 0

Adding Lw to both sides of the equation results in:
(12) (Pd)(Pv) + (Nd)(Lv-Pv) = Lw

Expanding the multiplication results in:
(13) (Pd)(Pv) + (Nd)(Lv) - (Nd)(Pv) = Lw

Subtracting (Nd)(Lv) to both sides of the equation produces:
(14) (Pd)(Pv) - (Nd)(Pv) = Lw - (Nd)(Lv)

Factorizing (Pv) on the first side of the equation leads to:

(15) $(Pv)(Pd - Nd) = Lw - (Nd)(Lv)$

Dividing both sides of the equation by (Pd-Nd) finally produces:
(16) $Pv = (Lw - (Nd)(Lv)) / (Pd-Nd)$

Thus, the volume of pneumonic tissue can be calculated by:

(a) multiplying the volume of the lung by the density of the non-pneumonic portion,

(b) subtracting this amount (a) from the lung weight, and

(c) dividing the result (b) by the difference between the densities of pneumonic and non-pneumonic portions.

Discussion

The evaluation of tracheal diameters was discarded because it was not possible to consistently measure them accurately, and it gave no improvement on the predictive value of the regression equation for calculating lung weight from carcass weight.

The presence of a mild interstitial oedema and a few *Muellerius* lesions in these lungs hampered the estimation of normal lung parameters. Interstitial pulmonary oedema was probably the result of electric stunning prior to killing.

The different accuracy of both balances determined that the lung densities calculated using the data from the analogue and the digital balances was not identical. The details are given in the APPENDIX 2.

These mis-measurements , plus the correlation of densities of non-pneumonic and whole lungs (as seen in **Figure IB.10**), may be responsible for the poor relationship found between the proportion of lung which suffers pneumonia and the resulting lung density (**Figure IB.5**).

The moderate relationship found between the density of the non-pneumonic area of the lung and the density of the whole lungs (**Figure IB.10**) could be due to diffuse changes affecting all the lung, such as interstitial and/or alveolar oedema. This trend complicates the calculation of a simple mathematical model for estimating the extent of pneumonia in a lung from its known volume and weight, requiring a correction for the density of the non-pneumonic areas.

The formula **Pv = (Lw - (Nd)(Lv)) / (Pd-Nd)** can be interpreted as:

(a) Calculating the predicted weight for a non-pneumonic lung of known volume **((Nd)(Lv))**.

(b) Finding the difference between the calculated and actual weights **(Lw-(Nd)(Lv))**. This excess in weight is deemed to be caused by an unknown amount of heavier pneumonic tissue.

(c) The amount of pneumonic tissue can then be found by dividing the difference in weights (b) by the difference between the densities of pneumonic and non-pneumonic lung tissues **(Pd-Nd)**.

Using this formula with the individual values for the density of the non-pneumonic portions of lung, the calculated proportion of pneumonic lung had a mild linear association with lung density (**Figure IB.5** , **Figure IB.7**). Three data points (asterisks in **Figure IB.5**) were statistical outliers, and were related to very small size of normal lung portion being used for measurements and a relatively large experimental error (*vide* APPENDIX 2).

The non-pneumonic portions of left and right lungs had the same mean densities (0.68 g/cc *versus* 0.66 g/cc). The higher mean density of right lungs (0.74 g/cc *versus* 0.68 g/cc) can be explained by the greater proportion of pneumonic tissue which usually occurrs in the larger (mean volume = 349 cc *versus* 263 cc) right lobes.

Conclusions

i) The density of the non-pneumonic portions of lung varies markedly between lungs.

ii) This variation should be taken into account by using a formula to calculate the proportion of pneumonic tissue within each lung.

iii) The formula **Pv = (Lw - (Nd)(Lv)) / (Pd-Nd)**

where **Pv** = the volume of pneumonic tissue
Lw = the weight of the whole lung
Lv = the volume of the whole lung
Nd = the density of (a sample of) the non-pneumonic portion
Pd = the (estimated) density of the pneumonic portion

can be used to calculate the volume of pneumonic tissue present in a lung.

The calculation involves substracting the product of the lung volume and the density of the pneumonic portion from the lung weight and dividing the result by the difference between the densities of pneumonic and non-pneumonic lung.

iv) This formula can be applied in all species when an objective estimate of the amount of pneumonic tissue must be obtained, or a volumetric rather than an area value is required.

v) The need to sample and measure a non-pneumonic portion makes this technique inappropiate for routine work.

PART I C: THE RELATIONSHIP BETWEEN SURFACE AREA AND DENSITY MEASUREMENTS

Introduction

Having studied the feasibility of quantitative area and density methods for assessing the severity of pneumonia, the following experiment was undertaken to ascertain the relationship between area measurements as obtained by image analysis, and lung volumes calculated by density measurements.

Material and Methods

The data obtained on pneumonic lung volumes (Part IB) was compared to the area measurements of pneumonic tissue taken from photographs obtained using the image analysis system previously described (Part IA).

Statistical Analysis

The scatter plot diagrams and correlations between the different measured variables were obtained using the STATISTIX v3.0 analysis program (Analytical Software, St. Paul, Minnesota, U.S.A.) on an IBM-AT compatible computer.

Results

The values for examined variables are shown in **Table IC.I** and the correlation of measured variables in **Table IC.II**.

There was little relationship between the density of the whole lung and the proportion of lung surface area occupied by pneumonia (**Figure IC.1**).

In most cases, the proportion of pneumonic area was considerably higher than the proportion of pneumonic volume (**Figure IC.2**)

Table IC.I. COMPARISION OF IMAGE ANALYSIS AND DENSIMETRIC RESULTS

CASE ID	PA	PV	PA100	PV100	ND	LD
90 left	52.000	56.447	34.437	17.640	0.5215	0.6059
90 right	95.000	114.51	48.718	26.324	0.5164	0.6437
91 left	50.000	64.992	32.680	21.101	0.7025	0.7653
91 right	73.000	92.754	38.421	22.623	0.7415	0.8000
92 left	35.000	M	23.179	M	M	0.5759
92 right	81.000	121.77	42.632	32.912	0.5166	0.6757
93 left	35.000	M	18.325	M	M	M
93 right	96.000	M	43.439	M	M	M
94 left	33.900	20.237	20.798	7.0760	0.6313	0.6573
94 right	51.960	49.106	28.239	12.722	0.6557	0.6995
95 left	1.7800	M	1.3692	M	M	0.7227
95 right	26.300	33.735	16.335	10.285	0.7043	0.7348
96 left	64.500	37.156	39.091	11.611	0.6959	0.7312
96 right	83.600	142.50	42.653	36.538	0.6889	0.8026
97 left	0.0000	0.0000	0.0000	0.0000	0.7168	0.7168
97 right	26.700	43.000	20.382	20.188	0.7941	0.8357
98 left	0.0000	0.0000	0.0000	0.0000	0.6800	0.6800
98 right	0.0000	60.718	0.0000	18.915	0.7464	0.7944
99 left	28.800	35.492	20.426	13.001	0.6590	0.7033
99 right	55.500	M	32.081	M	M	0.7513
100 left	0.0000	1.5094	0.0000	0.7188	0.6882	0.6905
100 right	4.0000	13.019	2.6667	5.0073	0.7166	0.7308
101 left	0.0000	0.0000	0.0000	0.0000	0.6996	0.6996
101 right	0.0000	11.968	0.0000	3.5409	0.7577	0.7663

PA: Area of pneumonic portion as measured with image analysis.
PV: Volume of pneumonic portion calculated from densities.
PA100: Percentage of lung area occupied by pneumonia.
PV100: Percentage of lung volume occupied by pneumonia.
ND: Density of non-pneumonic portion of lung.
LD: Density of whole (left or right side) lung.
M: Measurement not taken.

Table IC.II. CORRELATIONS OF VARIABLES (n=19)

	Area	Lw	Ld	Pa	Pa100	Pv	Pv100	Lv
Area	1.000							
Lw	0.993	1.000						
Ld	0.986	0.974	1.000					
Pa	0.804	0.828	0.731	1.000				
Pa100	0.817	0.837	0.755	0.993	1.000			
Pv	0.812	0.846	0.750	0.942	0.926	1.000		
Pv100	0.837	0.865	0.792	0.926	0.926	0.987	1.000	
Lv	0.996	0.996	0.971	0.837	0.847	0.842	0.859	1.000
Nd	0.976	0.962	0.997	0.691	0.717	0.707	0.753	0.958

Area: Area of whole lung.
Lw: Weight of whole lung.
Ld: Density of whole lung
Pa: Surface area of pneumonic portion.
Pa100: Percentage of surface area occupied by pneumonic tissue.
Pv: Volume of pneumonic portion.
Pv100: Percentace of lung volume occupied by pneumonia.
Lv: Volume of lung.
Nd: Density of non-pneumonic portion of lung.

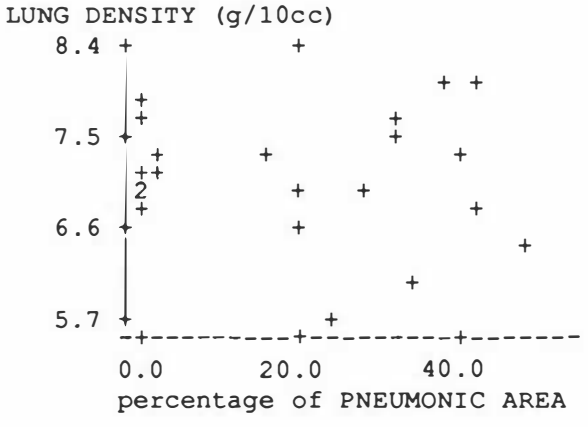

Figure IC.1. Relationship Between Whole Lung Density to Percentage of Pneumonic Area (n=19)

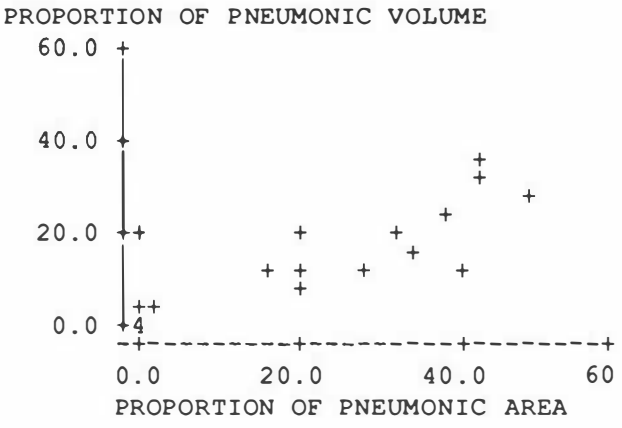

Figure IC.2. Relationship Between Proportion of Pneumonic Area and Proportion of Pneumonic Volume (n=19)

Discussion

The measurement of pneumonic areas tended to overestimate the extent of pneumonia, as can be seen in **Figure IC.2**.

There was a poor asociation between whole lung density and percentage of pneumonic area (**Figure IC.1**), even though both variables relate to the extent of pneumonia.

The correlation (0.9260) found between the proportion of pneumonic area and the proportion of pneumonic volume is not as high as expected. This can be explained in two ways:

i) The irregular shape of the lung lobes makes for a poor fit between a two-dimensional and a three-dimensional measurement, and the different cases did not have the same spatial distribuition (eg: they were not all cranio-ventral).

ii) Errors were made (*vide* APPENDIX 2) during the measurement of small volumes (non-pneumonic lung) which masked a good correlation. An improved procedure for measuring small volumes (using a firm stand instead of holding by hand) could correct this problem.

From a functional point of view, the percentage of lung volume impaired by pneumonia is the most important parameter, but from a practical point of view,

measuring this is too time consuming for routine use. The simple measurement of total lung density proved to be a method of little or no value in assessing the severity of pneumonia.

PART II : GOAT PNEUMONIA SURVEY

Introduction

Little information is available on the prevalence of pneumonia in goats in New Zealand although pneumonia has been reported to cause high mortality in goats of all ages (McSporran, 1985). Goat pneumonias can be acute, subacute or chronic. The pathogenesis of the first two diseases is not fully understood, while chronic pneumonia may be caused by *Muellerius capillaris* (McSporran, 1985). Recently Buddle *et al* (1990b) described a field outbreak of pneumonia in goats associated to an infection with caprine herpesvirus (bovid herpesvirus type 6) and *Pasteurella haemolytica*.

A slaughterhouse survey was undertaken to assess the characteristics and severity of pneumonias in goats, and to test the usefulness of the techniques developed in Part I of this thesis. The survey also aimed to identify any relationship between carcass weight, severity of pneumonia and lungworm lesions present in goats at slaughter.

Materials and methods

Animals

The lungs of 4284 goats killed in Mamaku meatworks at Rotorua were examined during July-August 1990.

Before slaughter, the breeds of the animals were classed as either Angora (phenotypically typical), feral, or crossbred. For lots of mixed breeds, each head was classified into one of the above groups while examining the incisors. The number of large permanent incisors on the head matching each set of lungs was recorded. When it was not possible to positively match a head with a set of lungs, the case was recorded as a missing case.

The owner and place of origin for each lot (line) of slaughtered animals were recorded. When a carcass could not be positively ascribed to one origin, it was treated as a missing case.

The weight of the warm carcass was recorded from the meat works scale, which was accurate to 0.5 kg. Carcasses that had some parts trimmed or condemned were treated as missing cases in regard to carcass weight data. Carcasses which could not be positively matched to a set of lungs were also treated as missing cases.

Scoring of lung lesions

Each lung was inspected and palpated on both lateral and medial sides. The size and number of nodular lesions, the severity of dictyocaulus lesions, the presence of pleural adhesions and other gross lesions were recorded for each lung. When one set of lungs was incomplete, it was treated as a missing case.

(i) Nodular lesions

The number of roughly circular, grey coloured, slightly elevated, hard nodules present in a 50 by 20 mm area on the caudo-dorsal aspect of the right lung were counted using a rectangular hole of these dimensions cut in water-resistant photographic paper. The lesions were recorded as **small** when all nodules had a diameter of < 2 mm, or **large** when at least one nodule was > 2 mm. Both types of nodular lesion were believed to be associated with *Muellerius capillaris* (Yates, 1988).

(ii) *Dictyocaulus filaria* lesions

The presence of either (a) areas of alveolar emphysema, or (b) areas of consolidation, both with a characteristic caudo-dorsal distribution (Kirton *et al*, 1976; Stockdale, 1976) which were associated with the presence of nematodes indistinguishable from *Dictyocaulus filaria* were arbitrarily scored into three grades on the basis of their size and number. Grade 1 was allocated to cases with emphysematous lesions involving < 20 cm^2 on both lungs, grade 3 for cases which had consolidation lesions involving more than half of the dorsal area of the caudal lobe and grade 2 for intermediate cases.

(iii) Pleurisy

The presence of either thin, filamentous or large, thick, dense bands of connective tissue on either the pulmonary or costal pleura was recorded while examining the lungs and weighing the carcasses. Severity was arbitrarily

scored into three grades, with grade 1 representing a few mild filamentous strands, grade 2 firm adhesions involving no more than two pulmonary lobes, and grade 3 severe extensive adhesions involving more than two pulmonary lobes.

(iv) Bronchopneumonias

Lungs with areas of consolidation other than those described in (i) and (ii) were placed in a plastic bag and removed to the laboratory where the weight, volume and density of the most severe cases of bronchopneumonia was obtained as described in Part I.

(v) Other lung lesions

When an un-familiar lesion or structure was found, the lungs were placed in a plastic bag and examined later in the laboratory. A written description was made, a gross photograph taken, and a sample collected for histology.

Histology

All cases with non-characteristic lesions had a sample of lung (from 4 to 10 mm thickness) fixed in 10% buffered formalin. In addition tissues from 3 to 6 characteristic cases of dictyocaulosis, muelleriosis, blood aspiration, and bronchopneumonia were also sampled. Fixed tissues were embedded in paraffin, and 4 um thick sections cut and stained with H.E. (Luna, 1968).

Identification of Parasites

Nematodes observed in histological sections were identified according to the methods of Chitwood & Lichtfels (1972), and Toft & Ekstrom (1980).

Formalin fixed specimens of *Dictyocaulus* sp. from a red deer (*Cervus elaphus*) were sorted into male and female specimens by examining their tail ends for the presence of spicules (Soulsby, 1982). These parasites were embedded in paraffin and 4 um thick perpendicular sections were cut and stained with H.E. for use as a reference set to identify parasites. Samples of sheep lung containing *Muellerius capillaris* were also processed for histology and used in the same manner.

Statistical Analysis of Data

The statistical analysis program STATISTIX 3.0 (Analytical Software, St. Paul, Minnesota, U.S.A.), was used to produce correlation, regression and chi squared tests on the data using an IBM-AT compatible computer with 2 Megabytes of expanded memory. The program deleted all cases with one or more missing values for any variable involved in regression or correlation analyses. It also deleted any variable which was highly correlated to a variable already included in the model for multiple linear regression analysis.

For the regression analyses, dummy variables were used for all levels of age, severity of dictyocaulus lesions, the presence or absence of subpleural lymph nodes (SLN), the presence of > 10 small or large nodular pleural lesions and each and every group of > 100 animals of the same place of origin.

Results

Of the 4684 cases recorded, 4398 cases had carcass weights recorded, 4284 cases had lung lesions recorded and 3969 cases had all records complete.

Bronchopneumonias

Only ten cases (0.23%) of antero-ventral consolidation were found (**Table II.I**). One case (No 1222) involved 98% of the lateral surface of the left lung (**Figure II.01**). Another case (No 863) had multiple abscesses of different sizes scattered throughout the lungs.

Histologically, an acute suppurative bronchopneumonia was seen in 6 cases (No 863, 1222, 1224, 1420, 2775, 3840), and in two cases moderate numbers of *Muellerius capillaris* (but no nodular lesions) were embedded within the lung parenchyma (No 1222, 1224). A moderate smooth muscle hyperplasia was present in another case (No 1420), but no lungworms were seen.

One case (No 3465) resembled the chronic non-progressive pneumonia of sheep (Alley, 1979), having a catarrhal proliferative bronchiolitis, type II cell hyperplasia,

desquamated macrophages within the alveolar lumina, and widespread nodular peribronchiolar lymphoid hyperplasia.

The case (No 863) with multiple abscesses contained colonies of Gram positive rods. No bacterial isolation was attempted.

There was no statistical association of bronchopneumonia with either line, age, breed or carcass weight.

Table II.I. CHARACTERISTICS OF CASES WITH BRONCHOPNEUMONIA

ID	Owner	Breed	Age	Weight (kg)	Group_W (kg)	(sd)	P_Area (cm^2)	Notes
863	5	Feral	6	10	8.9	1.7	M	a
1222	7	Feral	0	12.5	9.8	2.0	> 100	
1224	7	Feral	6	12.5	13.0	2.8	M	
1420	11	Feral	6	15.5	19.4	3.3	< 10	
2775	25	Feral	0	7.5	8.7	1.7	< 10	
3128	30	X-Bred	6	8.5	11.5	2.7	< 5	b
3197	M	M	M	M	M		M	b
3437	34	X-Bred	0	9	9.8	1.8	12	b
3465	35	X-Bred	0	6	6.9	1.2	< 2	
3840	35	X-Bred	6	14.5	10.7	2.1	M	c

Age : Number of permanent incisors
Weight : Weight of warm carcass
Group_W : Average weight and standard deviation (sd) for animals of the same age and the same breed within the same group
P_Area : Area of consolidated lung
M = Missing data
Notes : a = Abscesses with Gram positive rods
b = No sample was taken for histology
c = Intersitial oedema

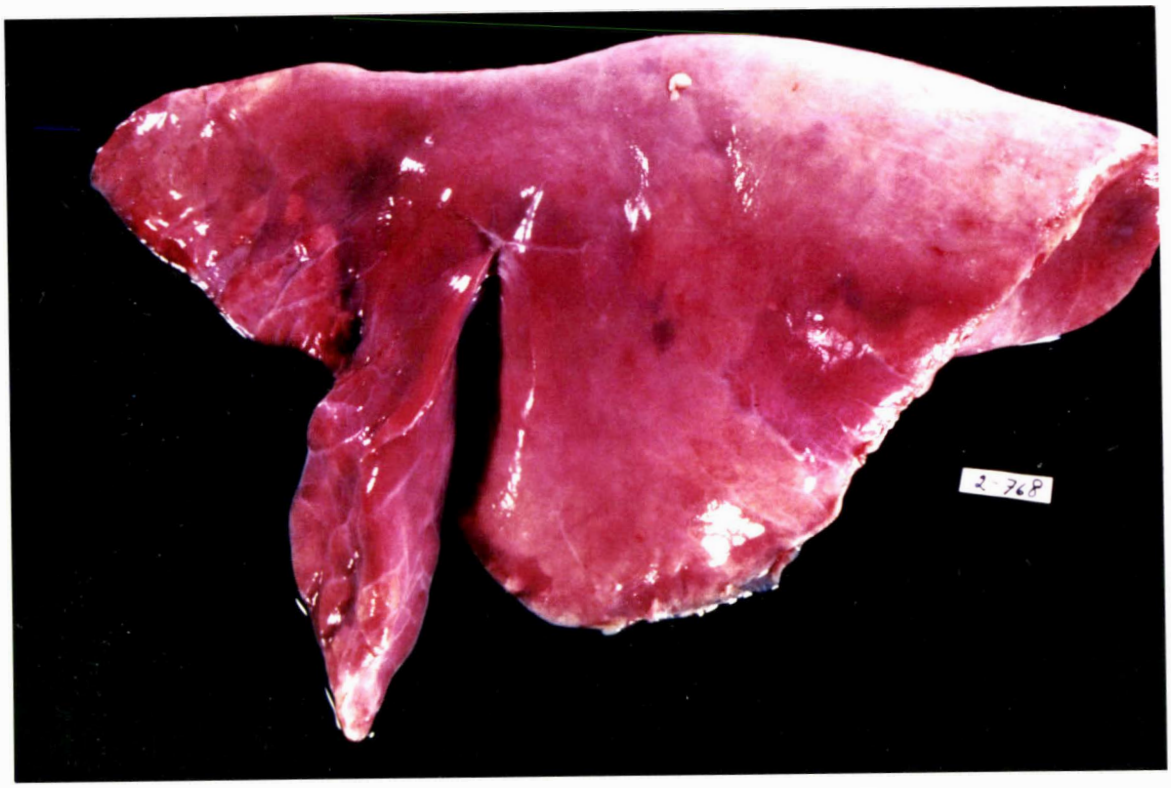

Figure II.01. Severe caprine bronchopneumonia involving more than 98% of the left lung. The right lung was less than 25% affected.

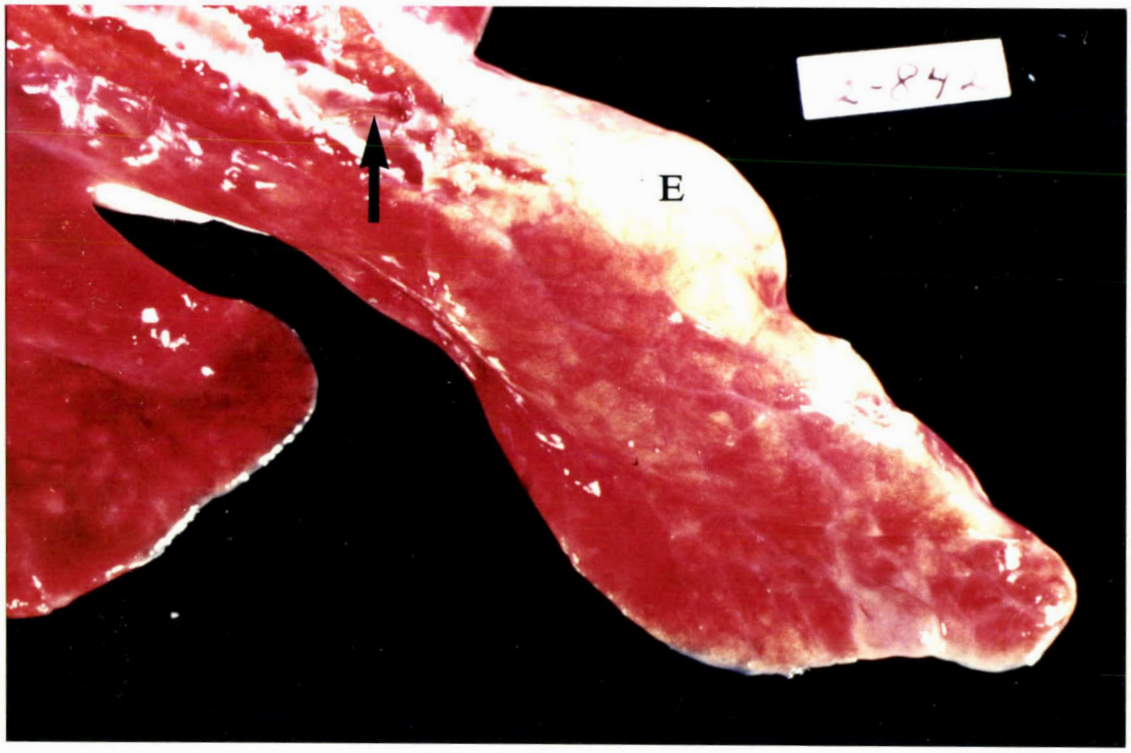

Figure II.02. Emphysematous lesions (E) associated with caprine dictyocaulosis. Arrow points to the location of a few *Dictyocaulus filaria* nematodes.

Parasitic lesions

Parasitic lesions were found in 3519 (82%) cases. 1756 (50%) were ascribed to *Muellerius capillaris* , 1418 (40%) to *Dictyocaulus filaria*, and 345 (10%) had both simultaneously.

a) *Dictyocaulus filaria*

A total of 1418 lungs had lesions compatible with dictyocaulosis (Kirton *et al*, 1976). Of these, 547 (39%) had mild lesions, 696 (49%) had moderate lesions and 175 (12%) had severe lesions.

Some lungs had large emphysematous areas in odd locations (eg: the middle of the intermediate lobe) (**Figure II.02**). Careful dissection revealed a few long, thin, nematodes indistinguishable from *D. filaria* within the bronchi of the affected area (**Figure II.02**).

Microscopically, adult worms in bronchioles were associated with a chronic catarrhal-proliferative bronchitis (**Figure II.03**). The bronchial epithelium was hyperplastic, and there was severe lymphoid hyperplasia in the submucosa and lamina propria. The eggs were surrounded by a mixed polymorph and macrophage inflammatory reaction which completely blocked the alveoli (**Figure II.04**). Some perivascular lymphoid aggregates of varying sizes were found scattered throughout the lung parenchyma. A moderate degree of bronchiolar smooth muscle hyperplasia was evident in some areas, though it was not always associated to the presence of parasites. Some small calibre bronchioles had very narrow lumina, due to hyperplasia of mucosal epithelium, lymphoid tissue and smooth muscle (**Figures II.03 II.04**)

The gastric epithelium of adult *Dictyocaulus filaria* showed a thick brush border (**Figure II.05**) characteristic of the suborder Trichostrongylina (Chitwood & Lichtenfels, 1972). Paired excretory gland cells (**Figure II.07**), a supposedly characteristic structure of the suborder Metastrongylina, were clearly seen in some *Dictyocaulus* specimens. The first stage larvae of *Dictyocaulus filaria* was recognized in host tissue by the presence of a rounded caudal end (Chitwood & Lichtenfels, 1972) (**Figure II.08**).

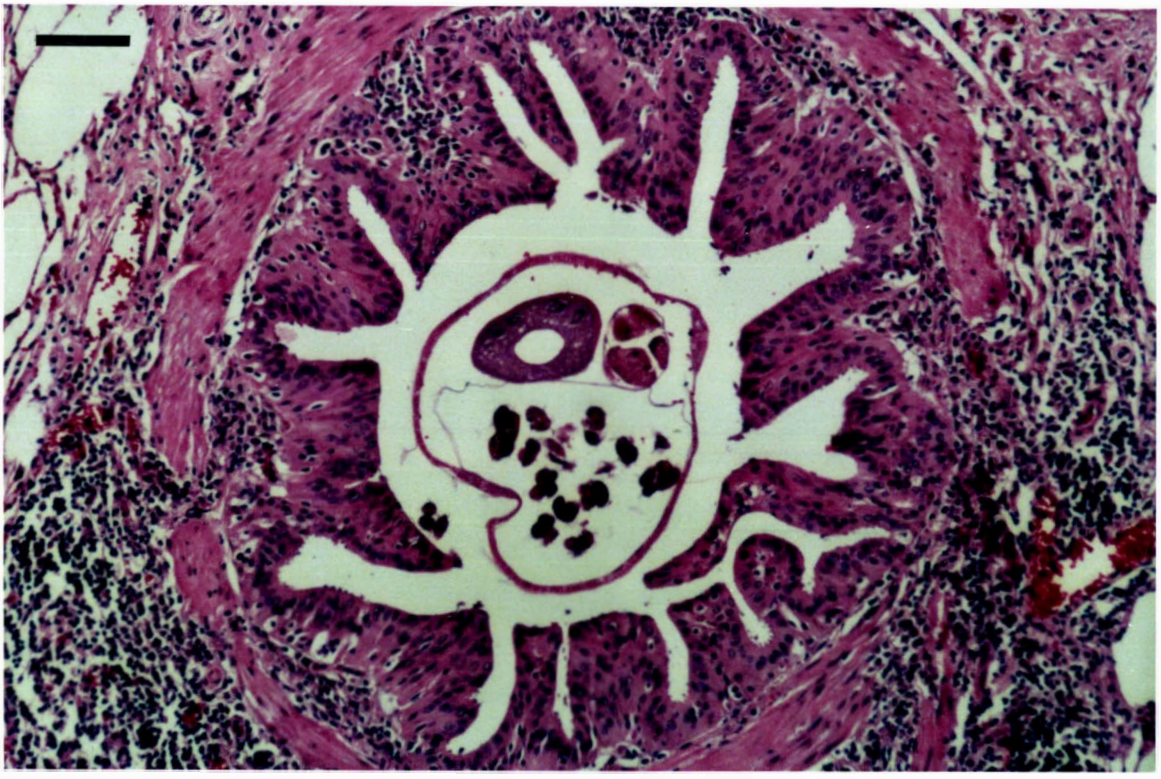

Figure II.03. Chronic bronchiolitis in a caprine lung associated with dictyocaulus nematodes. HE stain. Bar = 100 μm

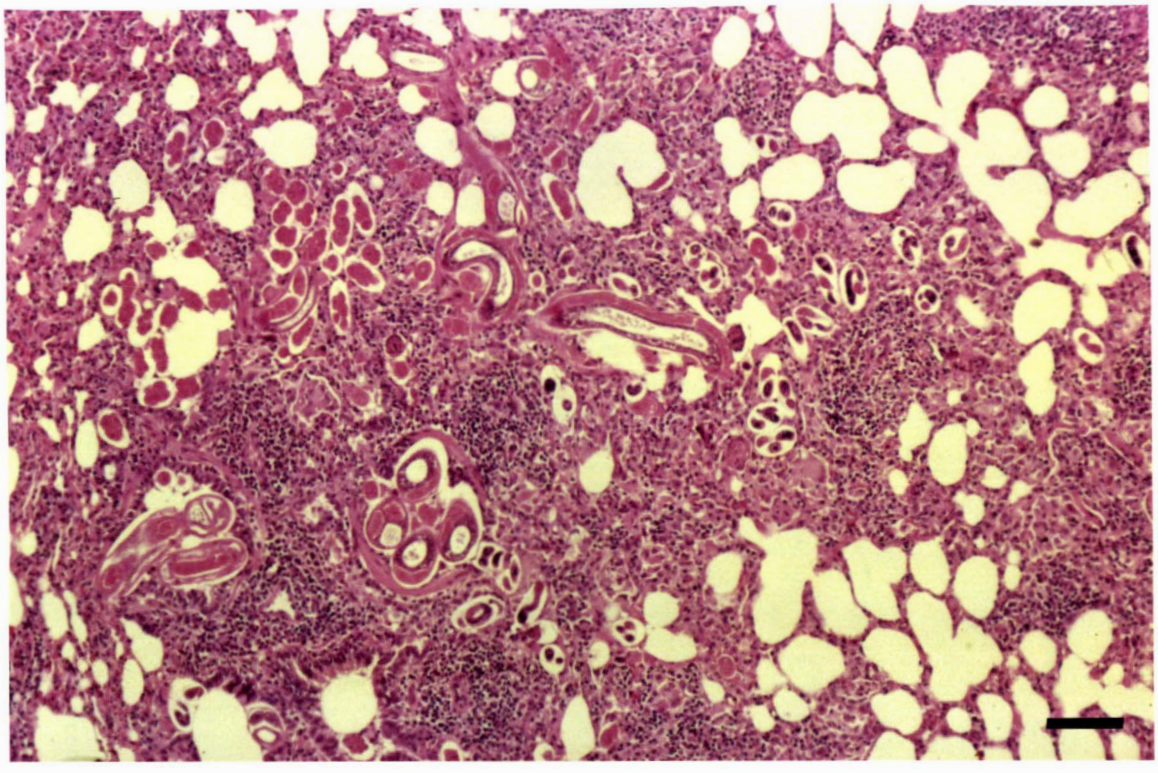

Figure II.04. A diffuse inflammatory reaction in the parenchyma of a caprine lung with dictyocaulosis. Adults and larvae present. HE stain. Bar = 100 μm.

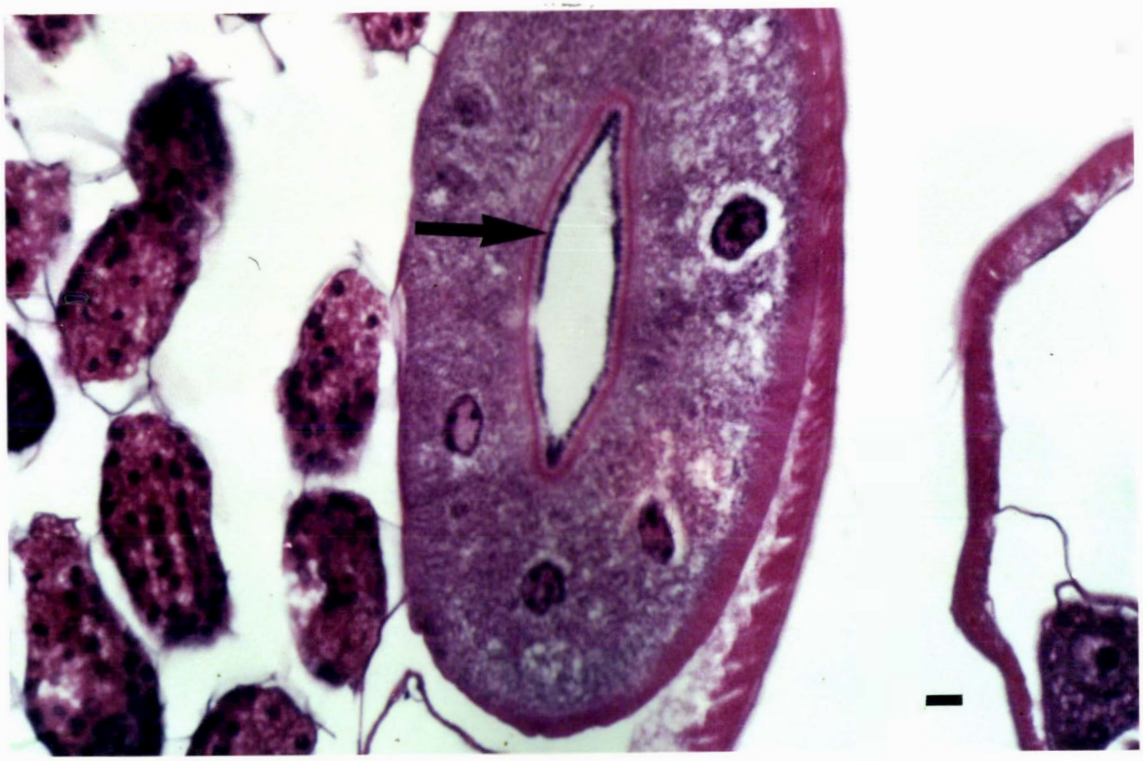

Figure II.05. Adult *Dictyocaulus filaria* showing the intestinal epithelium with a characteristic thick brush border (arrow). HE stain. Bar = 10 μm.

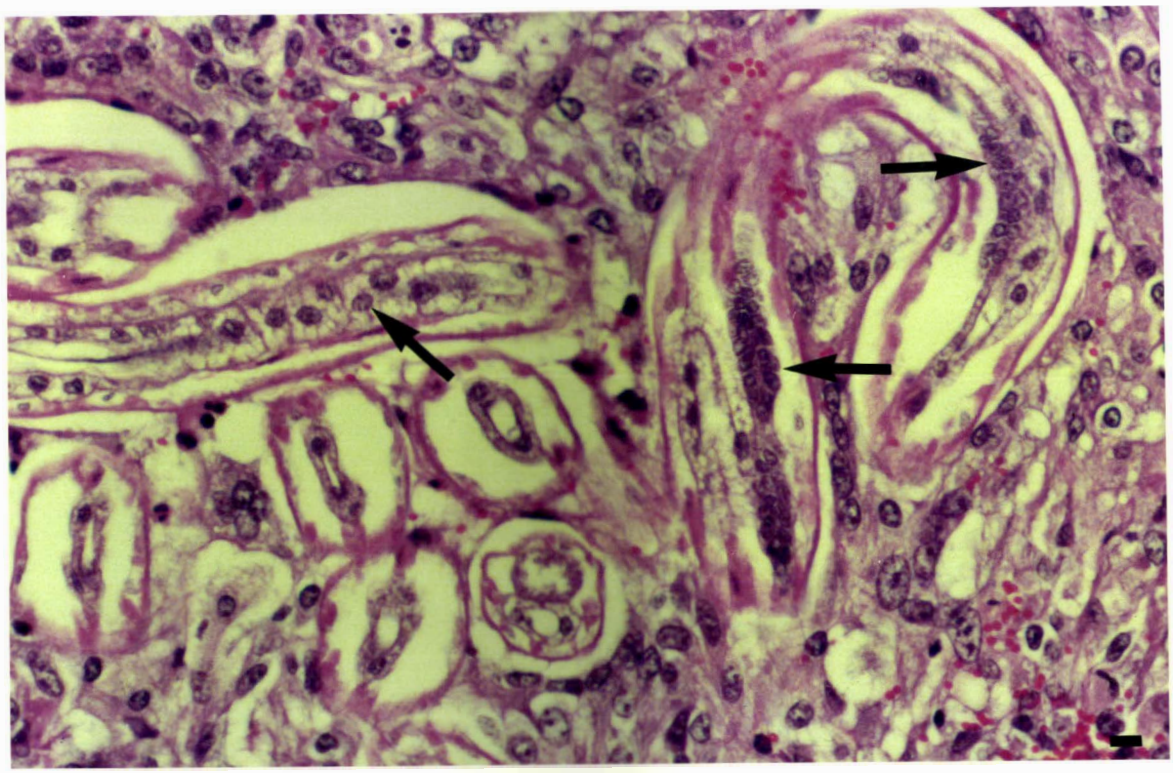

Figure II.06. Adult *Muellerius capillaris* showing characteristic multinucleated intestinal epithelium. HE stain. Bar = 10 μm.

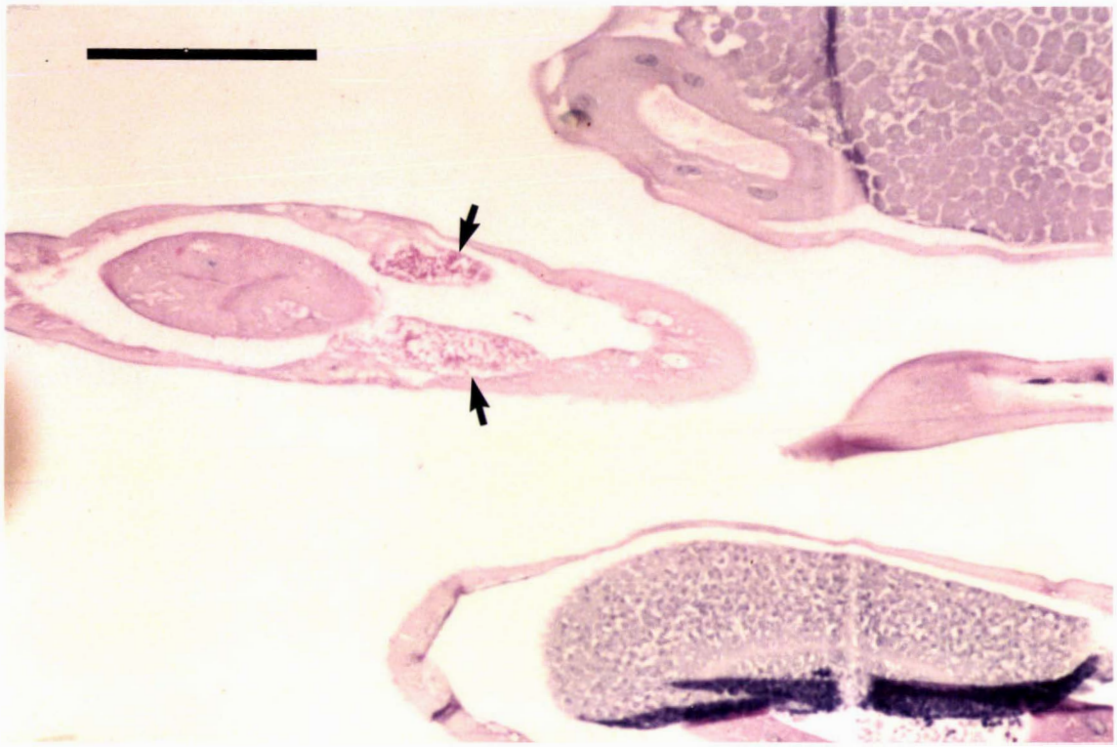

Figure II.07. Micrograph of *Dictyocaulus filaria* showing the excretory gland cells (arrows). PAS stain. Bar = 100 μm.

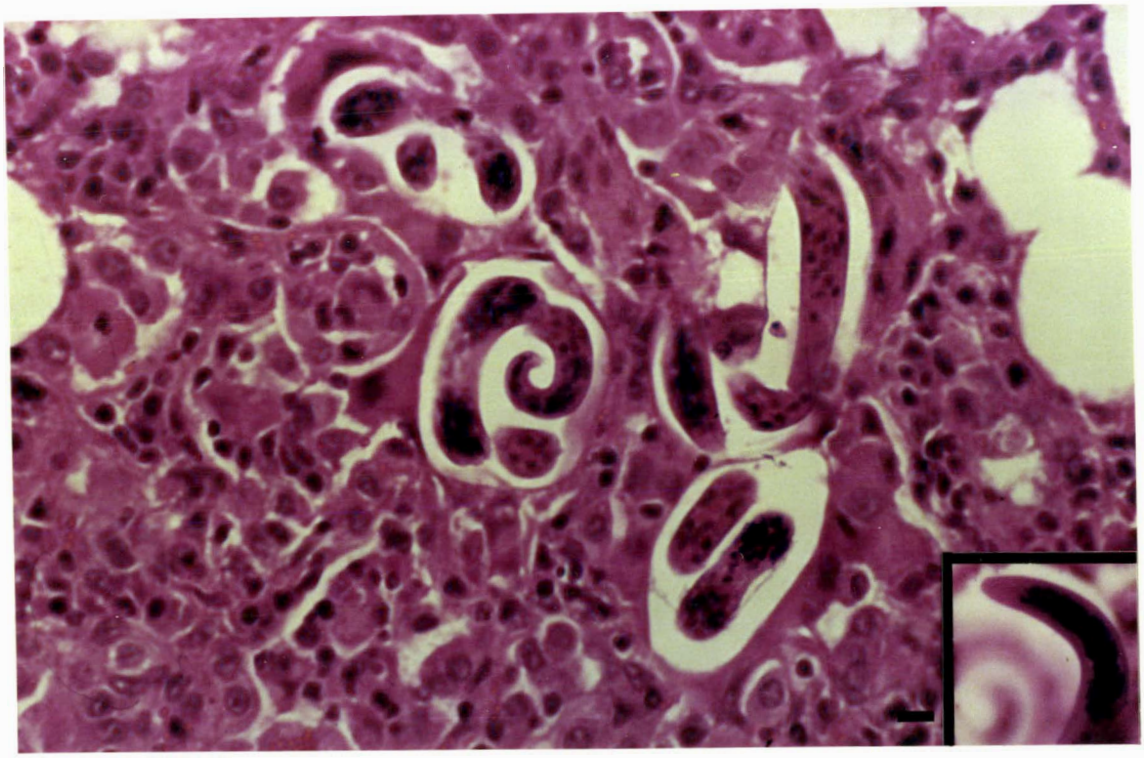

Figure II.08. Larvated eggs of *Dictyocaulus filaria*. Insert shows the rounded caudal end of a larva. HE stain. Bar = 10 μm.

b) *Muellerius capillaris*

A total of 1756 lungs had superficial pleural nodular lesions. Of these, 1182 (67%) were smaller than 2 mm, and 574 (33%) had at least one nodule larger than 2 mm (**Figure II.09**). The frequency distribution of these lesions is shown in **Table II.II**.

Microscopically, the lesions varied from the presence of parasites and their eggs in alveolar spaces with little inflammatory reaction but some smooth muscle hyperplasia (**Figures II.10 II.11**), to well-developed foreign body granulomas around remnants of necrotic parasites. (**Figure II.12**).

The gastric epithelium of *Muellerius capillaris* showed a characteristic multinucleated appearance (**Figure II.06**), common to the suborder Metastrongylina (Chitwood & Lichtenfels, 1972). The gubernaculum had neither crura nor capitulum and the distal end of the body was spirally coiled (Chitwood & Lichtenfels, 1972). The first stage larvae of *Muellerius capillaris* was recognized in host tissue by the presence of a caudal spine that is quite distinctive (Chitwood & Lichtenfels, 1972; Benakhla, 1981).

The number of small nodular lesions characteristic of *Muellerius* was associated with age ($p<0.001$). Animals two years old had almost twice as many cases of multiple (more than 10 nodules per 10 cm^2) small nodular lesions than expected ($p<0.001$). The number of large nodular lesions characteristic of *Muellerius* was also associated with age ($p<0.001$). Animals two years old had about half as many cases of multiple (more than 10 nodules per 10 cm^2) small nodular lesions than expected ($p<0.05$).

Table II.II. DISTRIBUTION OF CASES WITH NODULAR MUELLERIUS LESIONS (N=4284)

Number of Nodules	**Small** Nodules	**Large** Nodules
1	114	56
2	144	92
3	171	132
4	97	76
5	99	56
6	67	18
7	59	9
8	18	2
9	11	0
10	10	1
10-20	310	96
>20	82	36

The number of nodules were counted in a 20 x 50 mm area of the dorsal posterior right lung.
Small = nodules < 2 mm.
Large = at least one nodule > 2 mm.

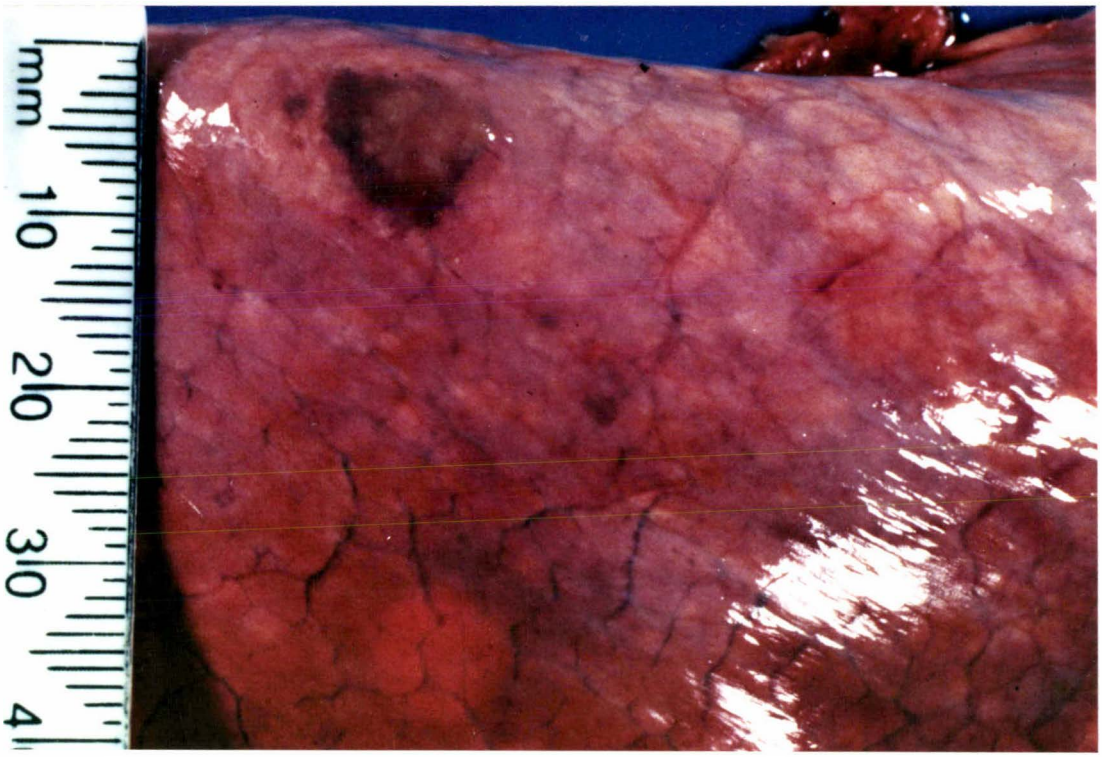

Figure II.09. A case of caprine muelleriosis showing a relatively large nodular lesion.

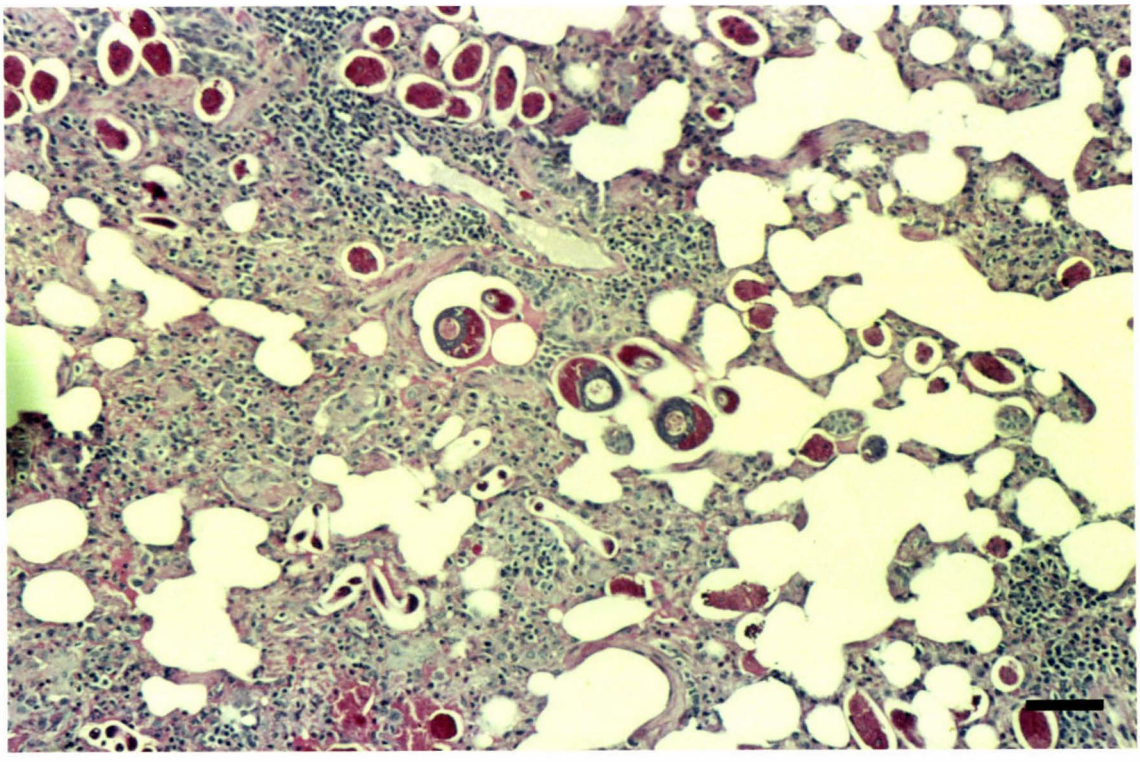

Figure II.10. A case of caprine muelleriosis with a diffuse inflammatory reaction. PAS stain. Bar = 100 μm.

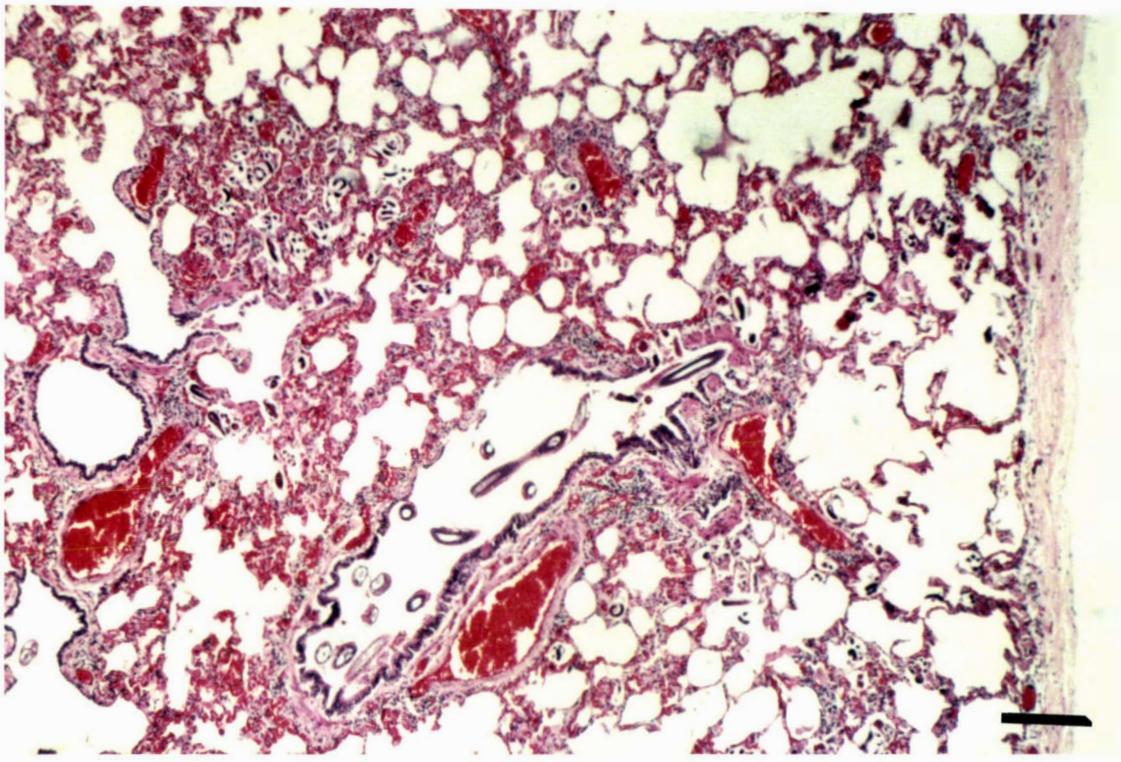

Figure II.11. Numerous muellerius nematodes present in a caprine lung with minimal inflammatory reaction. HE stain. Bar = 100 μm.

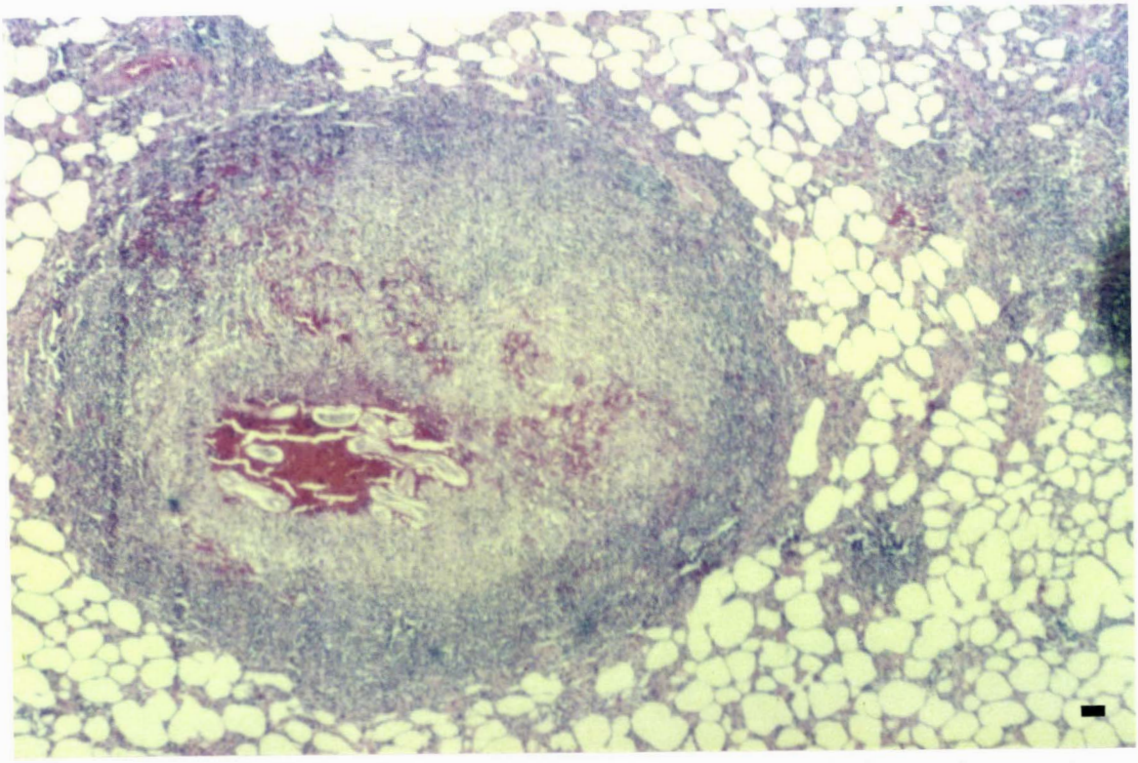

Figure II.12. A typical muelleriosis lesion in a caprine lung centred on a degenerating nematode. HE stain. Bar = 100 μm.

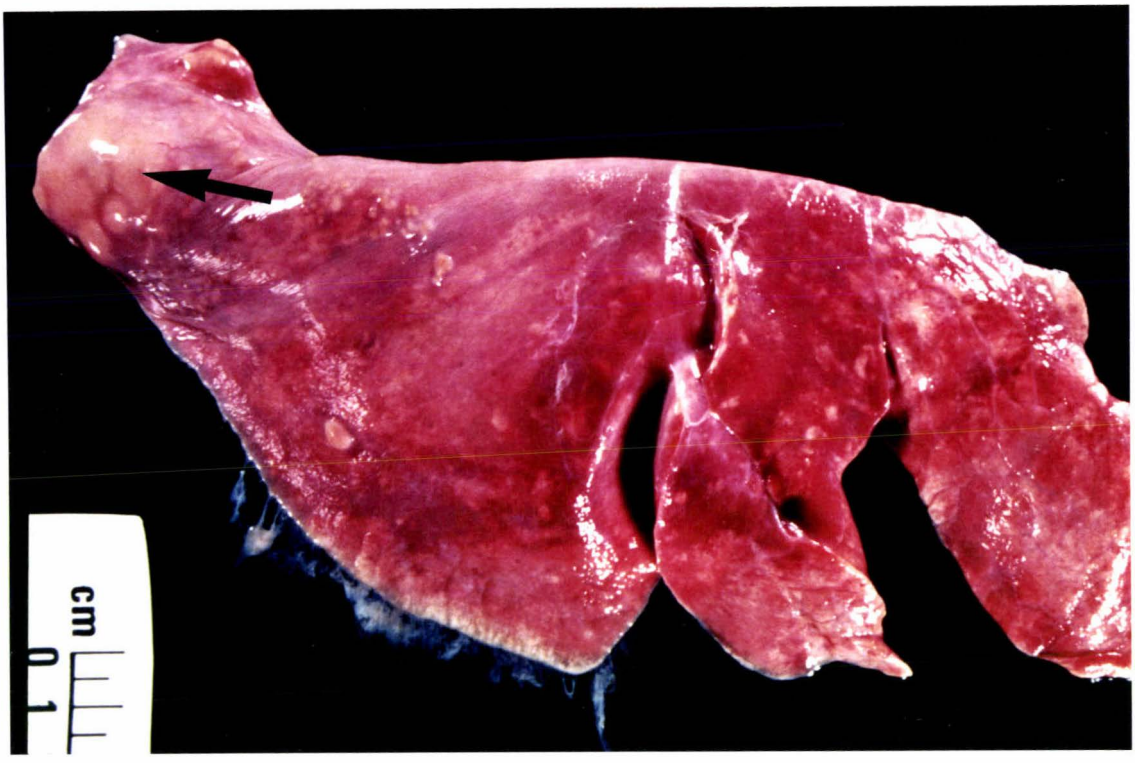

Figure II.13. Caprine lung with a lump (arrow) in the caudo-dorsal area in a case of severe bronchiectasis.

Other lesions

a) Bronchiectasis

Twelve sets of lungs (0.28%) had a lumpy appearance, and a firm nodular texture on palpation of the caudal lobes (**Figure II.13**). When dissected they contained several hard yellow branching casts within the bronchial tree (**Figure II.14**). On microscopic examination, these structures were seen to consist of a dense hyaline proteinaceous material within the lumina of bronchiectatic bronchi (**Figure II.15**). In several areas, the bronchial epithelium was absent, and replaced by epithelioid cells and foreign-body type giant cells, typical of a large foreign-body granuloma (**Figure II.16**). Parasite larvae were seen embedded in these casts in three cases (**Figure II.17**). In 3 cases there was peribronchial and peribronchiolar fibrosis. In 2 other cases there was cystic dilatation of submucosal glands. Most cases of bronchiectasis had mild to moderate nodular peribronchial lymphoid hyperplasia. A very small number of eosinophils were seen in the submucosa of one case.

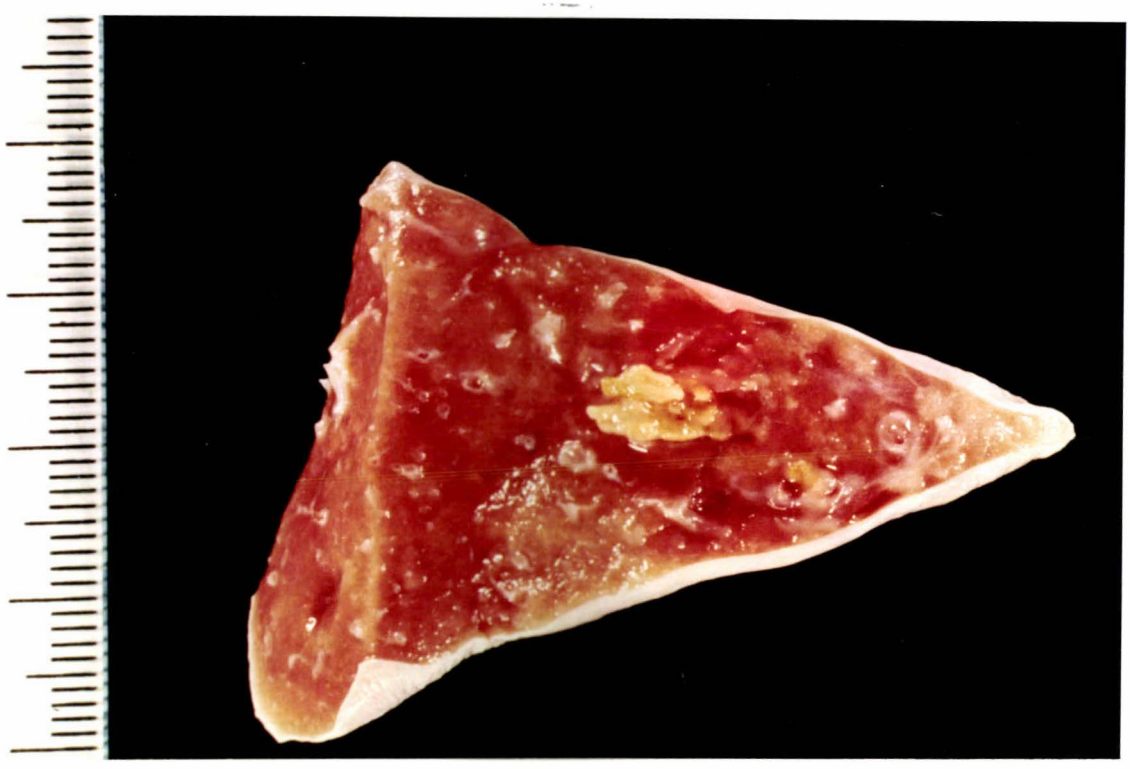

Figure II.14. Section of caprine lung with bronchiectasis showing bronchial cast of creamy coloured material.

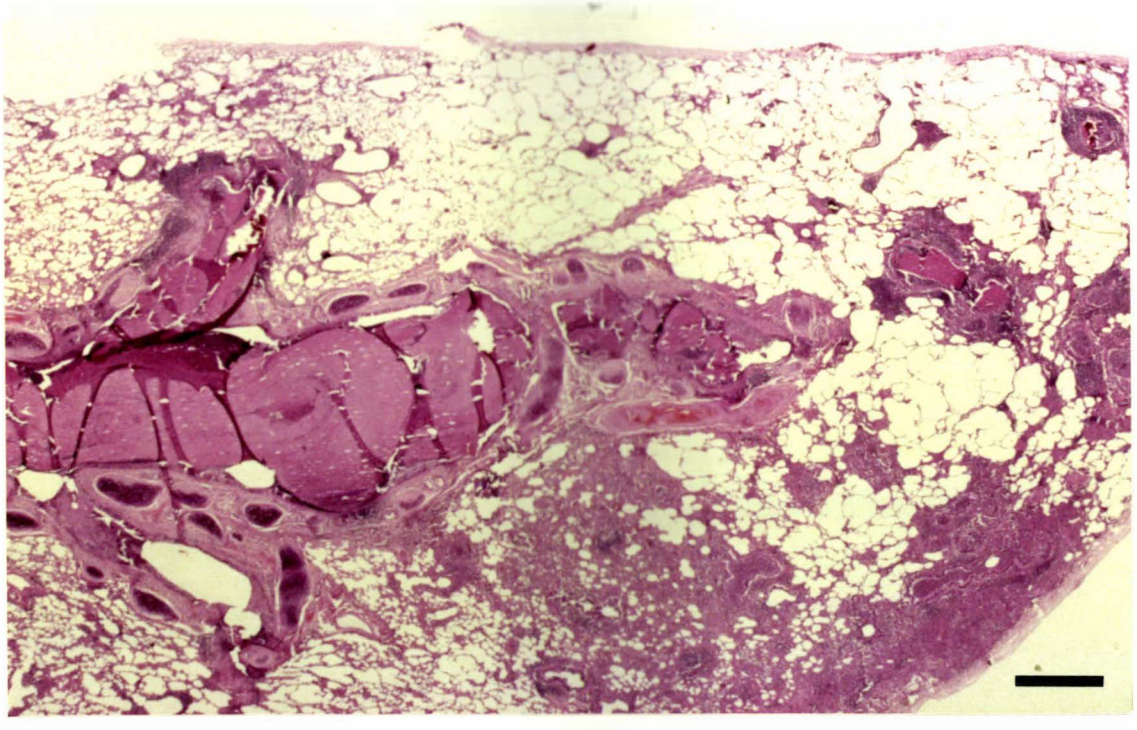

Figure II.15. Bronchiectasis in a caprine lung showing dense focally calcified hyaline proteinaceous matrix within the bronchial tree. HE stain. Bar = 1000 µm.

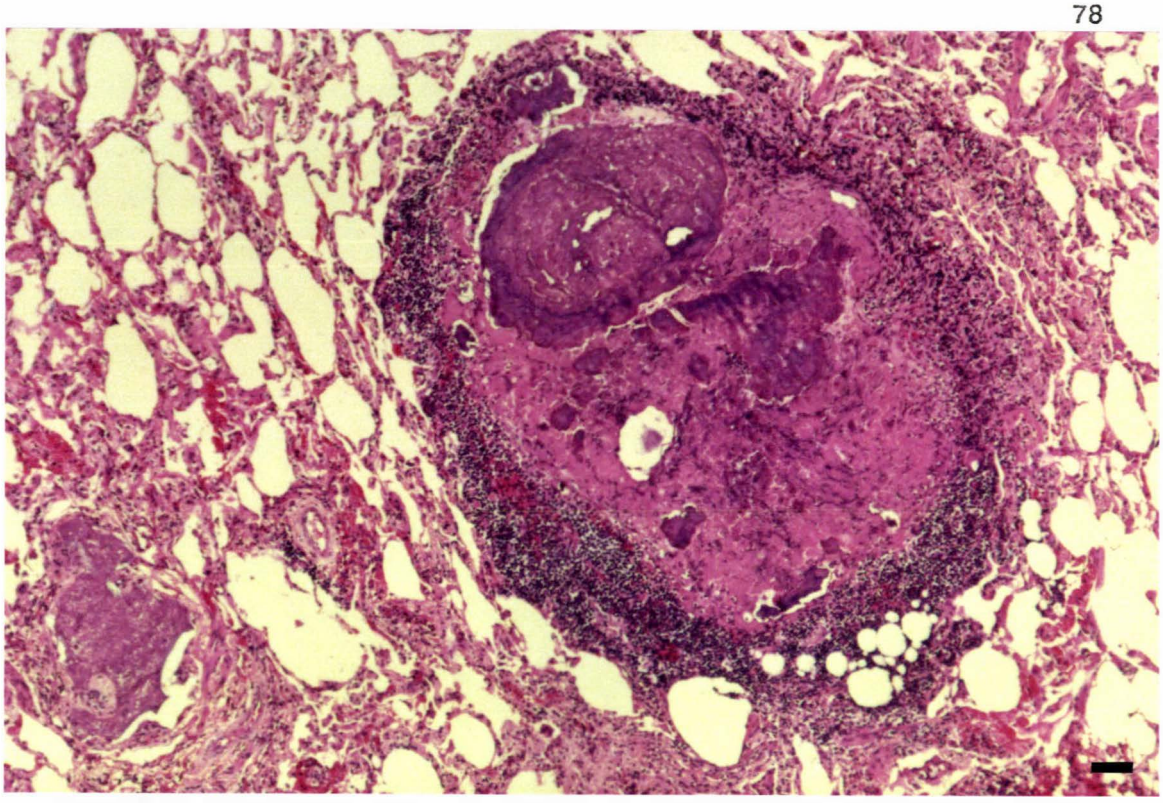

Figure II.16. Severe case of bronchiectasis in a caprine lung showing formation of large foreign-body granuloma and destruction of the bronchiolar wall. HE stain. Bar = 100 µm.

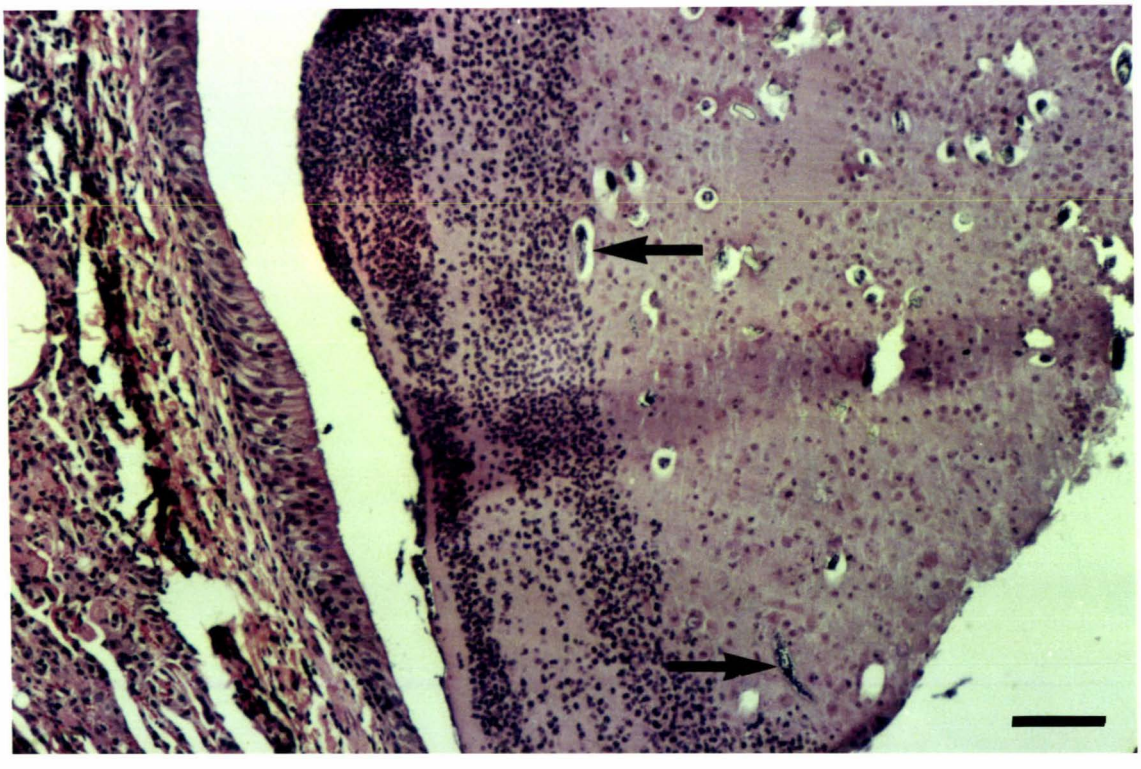

Figure II.17. Bronchiectasis in a caprine lung showing the presence of lungworm larvae (arrows) within proteinaceous material in the lumen. HE stain. Bar = 100 µm.

b) Pleural Adhesions

Fibrous pleural adhesions were found in 350 cases (8.16%). Of these, 80 (23%) were seen in both the pulmonary and costal pleura (**Figure II.18**), 184 (53%) in the costal pleura only, and 86 (24%) in the pulmonary pleura only. The distribution of severity of adhesions is shown in **Table II.III**.

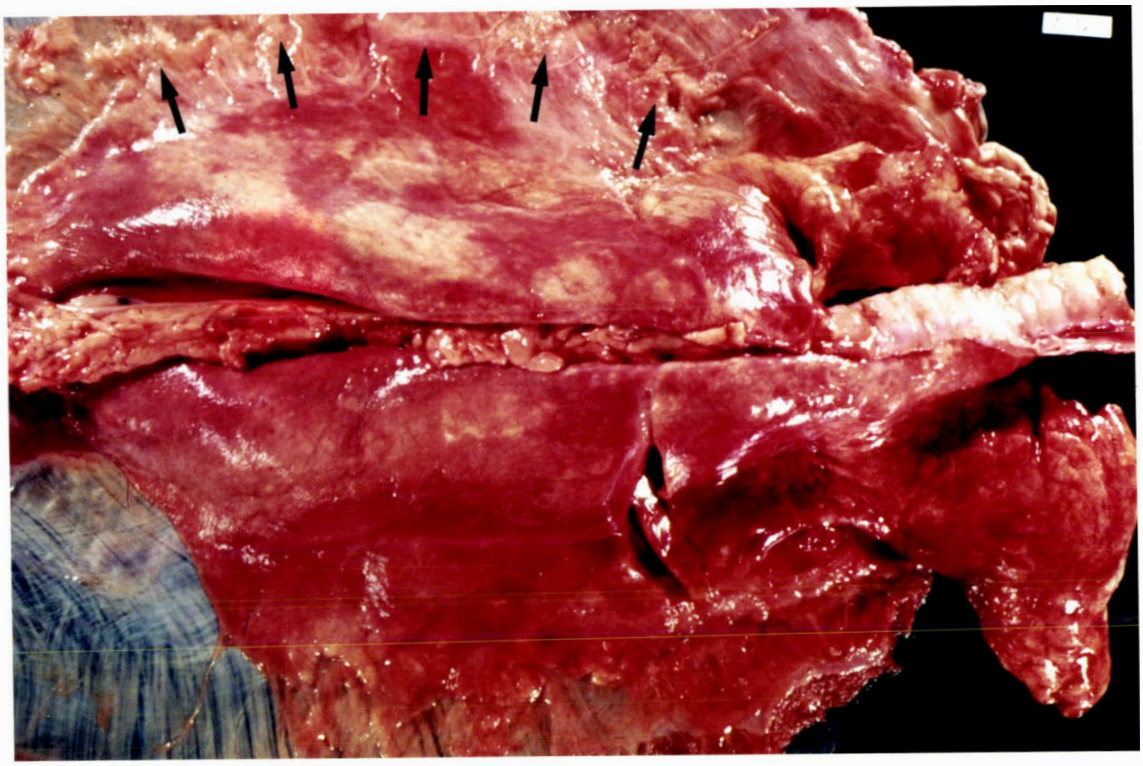

Figure II.18. Caprine lung with severe pleural adhesions.

Table II.III. Occurrence of Pleural Pulmonary and Costal Adhesions

Lung Adhesions	Costal Adhesions			
SEVERITY	Nil	Mild	Moderate	Severe
Nil	3965	99	52	33
Mild	48	12	7	6
Moderate	37	13	11	19
Severe	1	1	3	8

c) Pleural Plaques

Two cases had multiple oval, large (20-40 mm) thick (1-2mm), hard, white coloured plaques on the surface of the lung (**Figure II.19**).

On microscopic examination, a dense regular deposit of collagen containing a few fibroblasts was seen on the pleural surface (**Figure II.20**). The outer zone of each plaque had a basophilic mucinous appearance, whereas the innermost zone consisted of compact lamellated collagen. In one case the mid-zone contained occasional plump cells resembling chondrocytes. Some plaques overlayed zones of the lung which showed smooth muscle hyperplasia, while others did not.

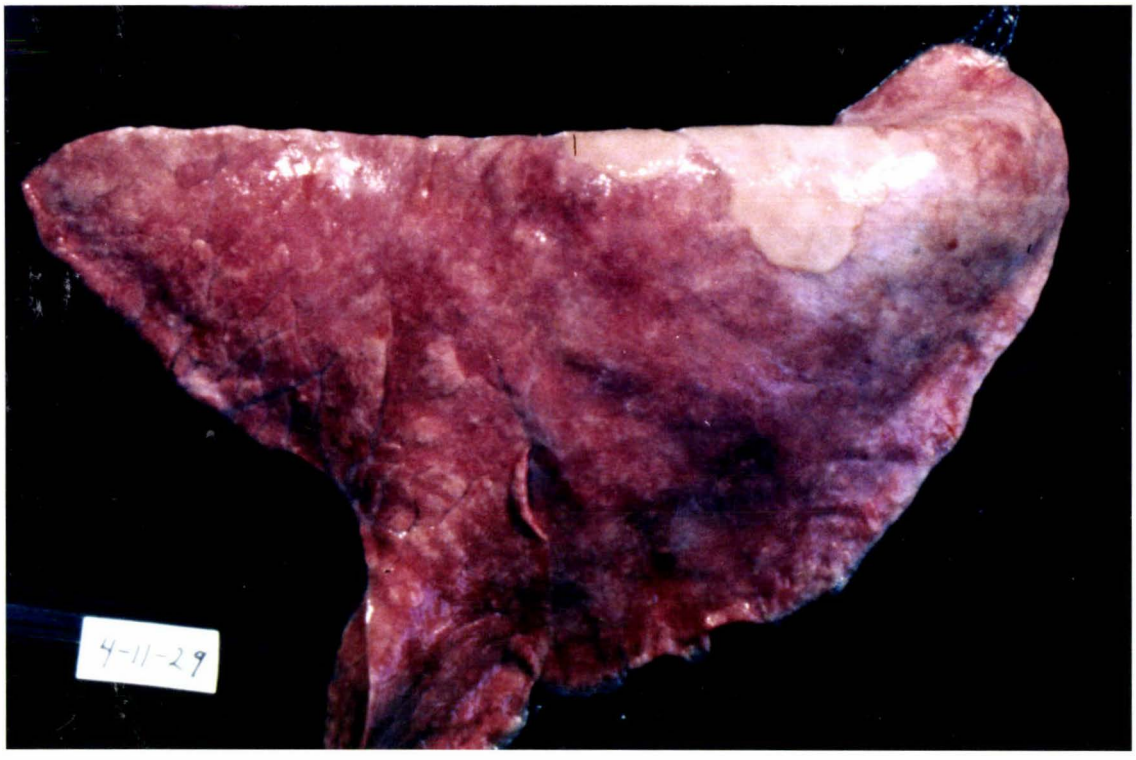

Figure II.19. Fibrous pleural plaques on the surface of caprine lung.

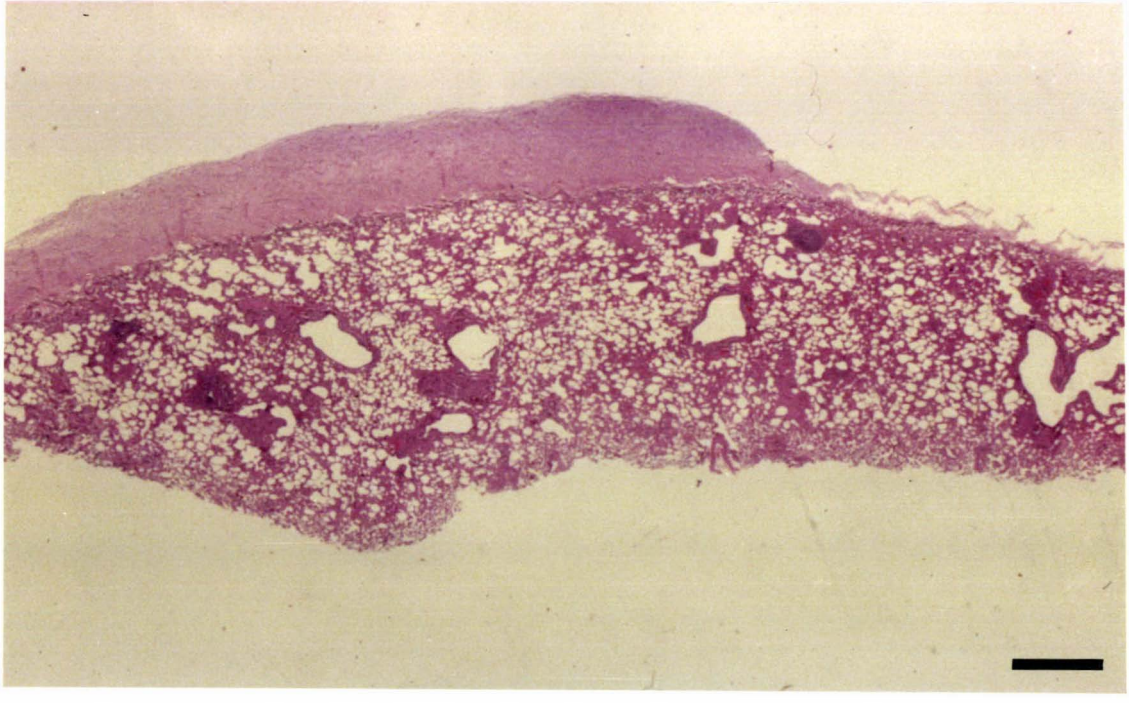

Figure II.20. Fibrous pleural plaque showing a discrete margin. HE stain.
Bar = 1000 µm.

d) Pneumoconiosis

One case had multiple small (<4mm), dark black spots on the cranial apex of the cranial lobe. On microscopic examination, large macrophages with a foamy, dark brown, granular pigment were found within macrophages in the alveolar lumina. The alveoli containing the macrophages had undergone epithelization. Some free pigment was seen in the bronchiolar lumina. A few pigment-laden macrophages were also seen in the subpleural lymphatics. Under polarized light, the pigment was birefringent and appeared as bright orange granules and crystals of varying size and shape.

e) Subpleural Lymph Nodes (**SLN**)

In many lungs, small (1-15 mm), single or multiple (<14) (**Table II.IV**), roughly spherical, pale grey, lumps were found at the periphery. Most were seen in the middle of the ventral border of the caudal lobe, but a few were found on the border of the middle lobe, and one case on the medial side of the mediastinal attachment of the caudal lobe (**Figures II.21 II.22**). In some cases of multiple **SLN** there was a tendency towards bilateral symmetry in their distribuition (**Figure II.22**).

Some flocks of goats had a higher frequency of SLN than others ($p<0.001$). Amongst the groups with a higher rate of SLN were owner codes 39 (13 cases per 48 animals = 27%) and 26 (ten cases in 49 animals = 20%). Because no SLN were observed in the first four groups of owners, the data was diregarded for this part of the study, as this finding was probably due to observer unfamiliarity. Nevertheless, groups 5 to 9 still had a lower frequency of cases than the average (chi squared= 8.1 , 0.59 , 1.89 , 3.25 , 2.20).

Omitting the first four groups of owners resulted in an overall total of 141 cases of SLN in 3201 animals, a 4.4 percent frequency. Assuming a conservative 3% overall frequency, the probability of failure to detect at least one case from a survey of 250 animals would be extremely small ($p<0.001$)provided observers were familiar with these structures (Cannon & Roe, 1982).

The presence or absence of SLN was associated with breed ($p=0.0042$), with ferals having more SLN (77/1393 = 5.5%) than Angoras (2/104 = 2%) or cross-breds (66/1965 = 3.3%). The actual number of SLN per animal was the same for all three breeds ($p=0.53$).

The presence of SLN was mildly associated with age (p=0.0476), but their numbers per animal were not (p=0.13). Kids had less (2.8%) SLN than full-mouthed animals (4.9%).

The presence of SLN was not associated with carcass weight (p=0.35), nor was their number (p=0.63).

Microscopically, these structures consisted solely of lymphoid tissue showing varying degrees of organization into follicles, medulla and cortex. The larger SLN had a more organized appearance (**Figure II.23**), and a stain for reticulin demonstrated the architectural arrangement of lymphoid follicles (**Figure II.24**) and medulla.

Some SLN contained lesions, the most common being parasitic granulomas associated with degenerating lungworm larvae.

Table II.IV. FREQUENCY DISTRIBUTION OF SUBPLEURAL LYMPH NODES

Quantity	Cases	Percent	Histogram
0	4134		
1	65	43.3	|**********
2	33	22.0	|**********
3	20	13.3	|**********
4	17	11.3	|********
5	6	4.0	|***
6	5	3.3	|**
7	2	1.3	|*
10	1	0.6	|*
13	1	0.6	|*
Total	(150)	(3.6)	

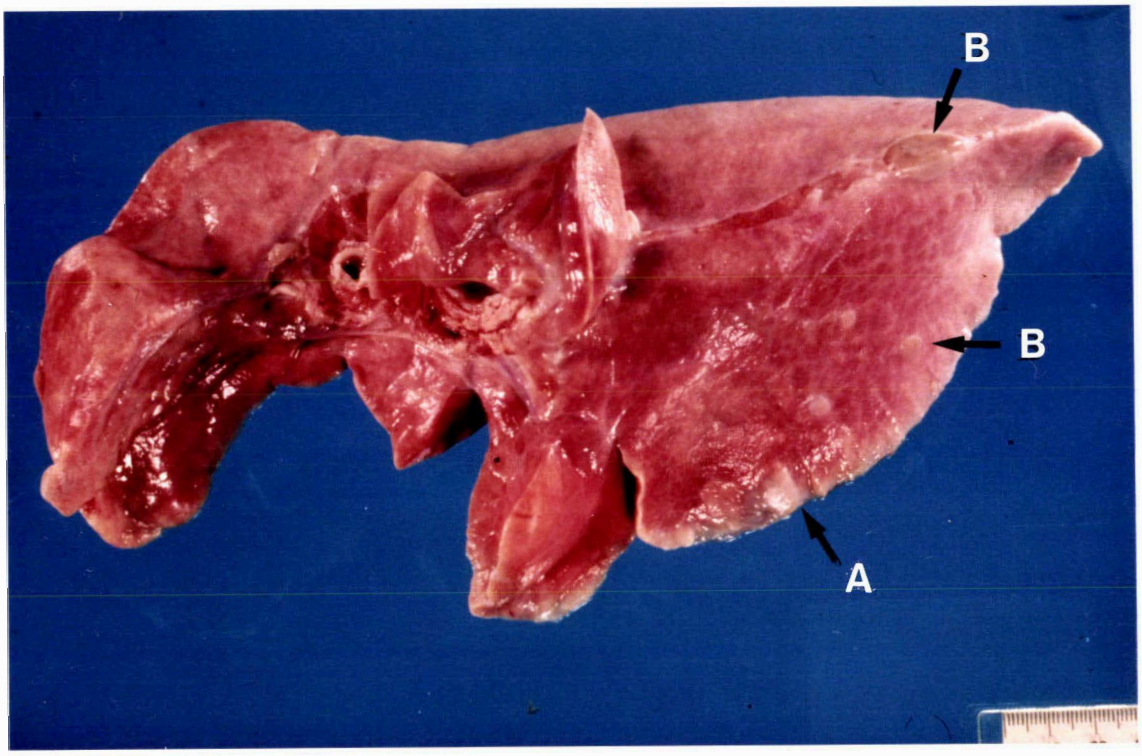

Figure II.21. A caprine lung with subpleural lymph nodes in two positions (A.usual location; B.uncommon locations).

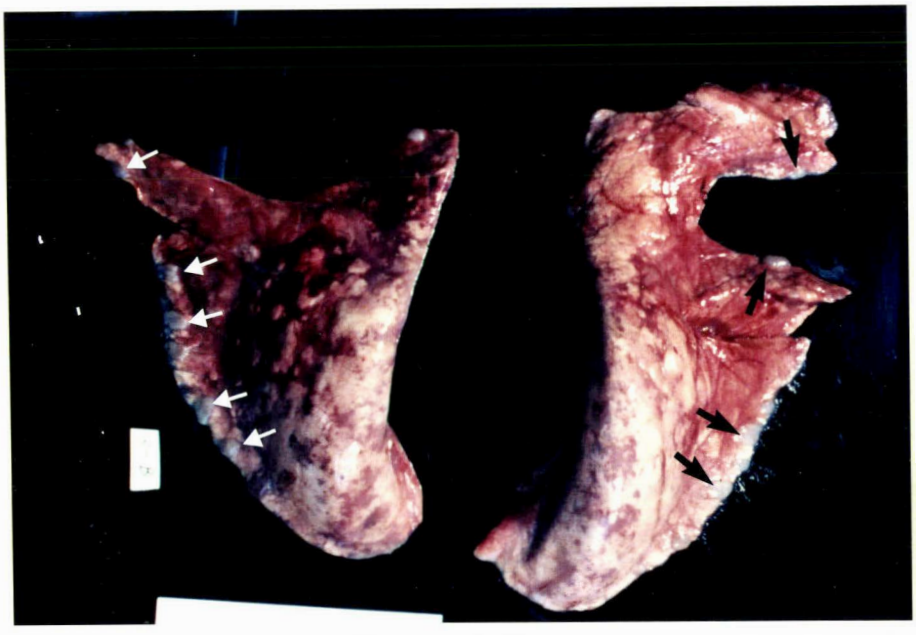

Figure II.22. A caprine lung with thirteen subpleural lymph nodes (arrows) showing bilateral almost symmetrical distribuition.

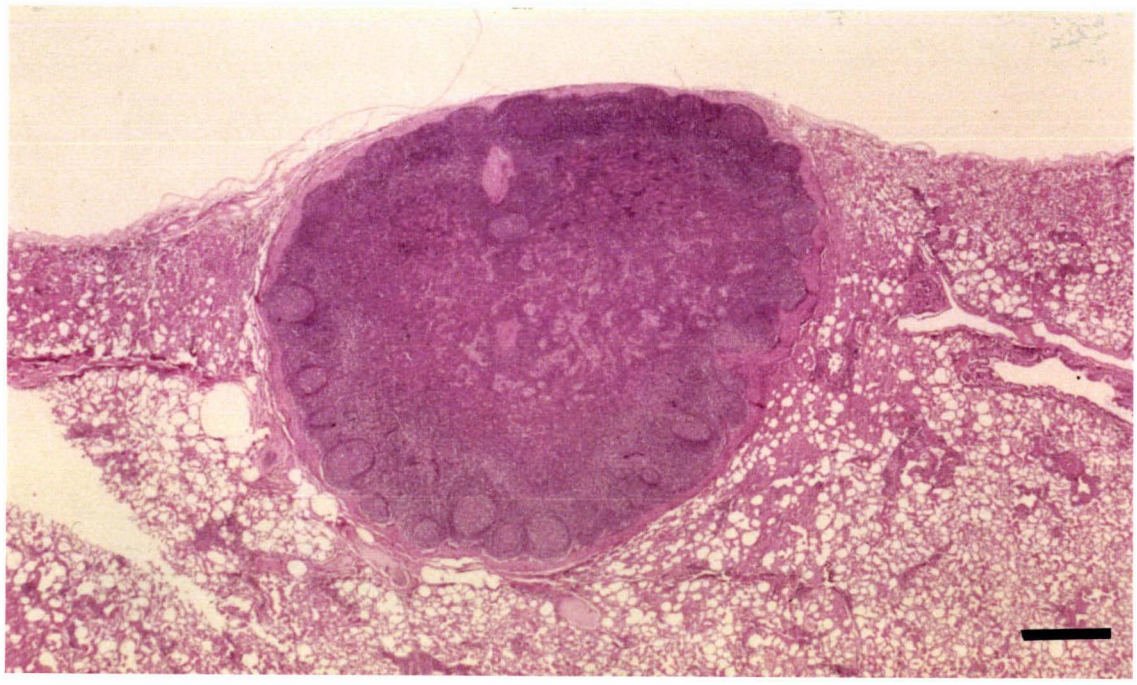

Figure II.23. A caprine lung with a subpleural lymph node showing medulla and cortex with follicular architecture and distinct fibrous capsule. HE stain. Bar = 1000 µm.

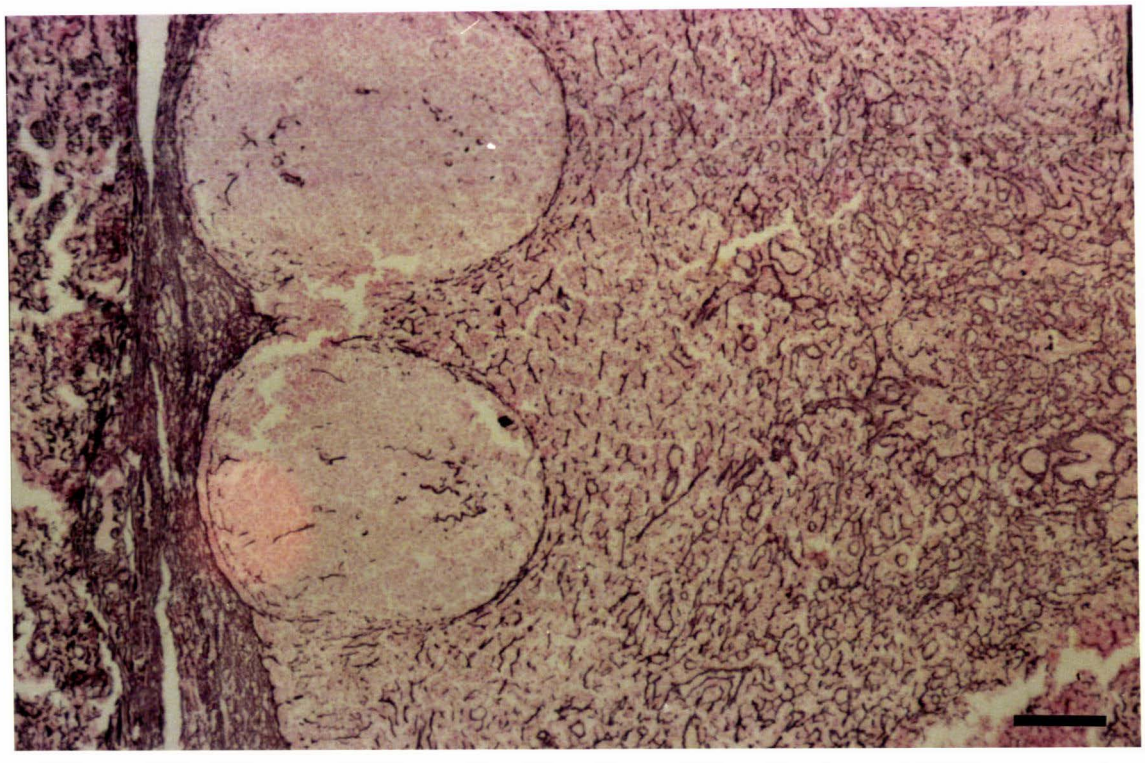

Figure II.24. The cortex of a caprine subpleural lymph node showing well developed follicles. The stain for reticulin demonstrates the internal framework. Bar = 100 µm.

f) Blood Aspiration

There were many cases of blood aspiration. Their frequency seemed to vary according to the presence or absence of certain workers on the chain. Some cases were characteristic in their appearance, but others had a colour, texture, size and distribution which resembled small nodular muellerius lesions. Histopathology in two such cases confirmed that they were blood aspiration.

Carcass Weights

There was a large range of carcass weights; the lowest weight recorded was 4.5 kg and the highest was 30 kg. The average carcass weight was 11.57 kg, but there was a large variation in the average weights of carcasses between lines (flocks) (**Table II.VI**). The median value for carcass weight was 10.5 kg. The frequency distribution of weights is seen in **Table II.V**

The weights of carcasses followed a typical logarithmic normal distribution (**Table II.V**), with a steep left shoulder indicating the minimum weight for slaughter.

A regression analysis showed a significant ($p<0.05$) change in carcass weight was associated with age levels 0, 2 and 6, owners 5, 15, 35 and 41, all levels of dictyocaulus lesions and the presence of >10 small or large nodular muellerius pleural lesions (**Table II.VII**). The relative effect of each of the factors on carcass weight is seen in **Table II.VIII**. Although these 12 factors had a significant association with carcass weight, they accounted for only 40% of the total variation. The remaining 60% variability in carcass weight was not accounted for. No association of either bronchopneumonias or pleurisy with carcass weight was demonstrated.

Table II.V. FREQUENCY DISTRIBUTION OF CARCASS WEIGHTS.

Value	Frequency	Percent	Histogram
4	3	0.1	|
5	48	1.1	|***
6	192	4.4	|**********
7	346	7.9	|**********
8	522	11.9	|**********
9	575	13.1	|**********
10	517	11.8	|**********
11	469	10.7	|**********
12	349	7.9	|**********
13	282	6.4	|**********
14	254	5.8	|**********
15	185	4.2	|**********
16	155	3.5	|**********
17	117	2.7	|********
18	95	2.2	|******
19	74	1.7	|*****
20	75	1.7	|*****
21	39	0.9	|***
22	37	0.8	|**
23	22	0.5	|*
24	17	0.4	|*
25	12	0.3	|*
26	4	0.1	|
27	3	0.1	|
28	1	0.0	|
29	1	0.0	|
30	4	0.1	|
Total	(4398)	(100.0)	

Table II.VI. AVERAGE CARCASS WEIGHTS OF LINES OF GOAT CARCASSES FROM DIFFERENT SOURCES.

Line number	Weight (kg)	Line number	Weight (kg)	Line number	Weight (kg)	Line number	Weight (kg)	Line number	Weight (kg)
01	10.01	11	19.22	21	9.13	31	9.72	41	10.74
02	14.71	12	11.39	22	10.96	32	10.96	42	10.47
03	9.85	13	11.67	23	7.28	33	12.56	43	10.96
04	14.69	14	10.78	24	12.54	34	10.96	44	10.43
05	8.57	15	15.47	25	9.96	35	9.82	45	12.19
06	10.34	16	12.02	26	14.46	36	14.09	mean	11.57
07	12.07	17	10.50	27	12.25	37	10.20		
08	10.48	18	12.55	28	12.43	38	12.42		
09	10.45	19	11.70	29	9.96	39	11.70		
10	21.96	20	8.76	30	10.74	40	11.72		

Table II.VII. VARIABLES WITH A SIGNIFICANT ASSOCIATION WITH WEIGHT OF CARCASS.

Variable and Level	Amount (kg)	T-test value	Probability value
Age 0	-2.5	-3.72	0.0002
Age 2	-1.5	-2.17	0.0297
Age 6	1.5	2.29	0.0220
Owner 5	-3.9	-18.59	<0.0001
Owner 15	3.1	17.54	<0.0001
Owner 35	-2.3	-12.39	<0.0001
Owner 41	-0.73	-3.79	0.0002
Mild dictyocaulus lesions	-0.81	-5.25	<0.0001
Moderate dictyocaulus lesions	-0.85	-5.29	<0.0001
Severe dictyocaulus lesions	-1.52	-5.97	<0.0001
Presence of >10 small nodular lesions	-0.75	-3.52	0.0004
Presence of >10 large nodular lesions	-0.86	-2.57	0.0102

Table II.VIII. ANALYSIS OF VARIANCE OF CARCASS WEIGHT

Source	Individual SS	Cum DF	Cumulative SS	Cumulative MS	Adjusted R-squared
CONSTANT	436330.0				
AGE0	4988.3	1	4988.3	4988.3	0.0991
AGE2	3129.5	2	8117.8	4058.9	0.1612
AGE4	1240.1	3	9358.0	3119.3	0.1857
AGE6	873.7	4	10232.0	2557.9	0.2029
AGE8	101.3	5	10333.0	2066.6	0.2047
BREED1	7.2	6	10340.0	1723.4	0.2046
BREED2	225.8	7	10566.0	1509.4	0.2089
BREED3	32.4	8	10599.0	1324.8	0.2093
OWNER15	5222.0	9	15821.0	1757.8	0.3133
OWNER17	6.3	10	15827.0	1582.7	0.3133
OWNER35	885.3	11	16712.0	1519.3	0.3308
OWNER41	40.9	12	16753.0	1396.1	0.3314
OWNER5	2716.6	13	19470.0	1497.7	0.3855
DICTYO1	94.0	14	19564.0	1397.4	0.3872
DICTYO2	185.9	15	19750.0	1316.6	0.3907
DICTYO3	289.8	16	20040.0	1252.5	0.3964
LM11	50.0	17	20090.0	1181.7	0.3972
LM21	11.0	18	20101.0	1116.7	0.3972
SM11	105.5	19	20206.0	1063.5	0.3992
SM21	15.6	20	20222.0	1011.1	0.3993
SLNTRUE	2.2	21	20224.0	963.0	0.3991
SLN	5.9	22	20230.0	919.5	0.3991
RESIDUAL	29962.0	3336	50192.0	15.0	

CASES INCLUDED	3337	MISSING CASES	1347
DEGREES OF FREEDOM	3314		
OVERALL F	101.	P VALUE	< 0.0001
ADJUSTED R SQUARED	0.399		
R SQUARED	0.403		
RESID. MEAN SQUARE	9.041		

AGE : Number of permanent incisors
BREED: 1=Angora 2=Crossbred 3=Feral
LM11 : Presence of more than 10 large nodules per 10 cm^2
LM21 : Presence of more than 20 large nodules per 10 cm^2
SM11 : Presence of more than 10 small nodules per 10 cm^2
SM21 : Presence of more than 20 small nodules per 10 cm^2
SLNTRUE : Presence or absence of subpleural lymph nodes
SLN : Number of subpleural lymph nodes present in both lungs

Discussion

A low frequency of bronchopneumonias was one of the unexpected features observed in the current survey. This result indicates that goats in New Zealand have a much lower prevalence of pneumonia than sheep, where levels of up to 70% have been reported during the autumm months (Kirton, 1976).

It is possible that pneumonia in goats in the North Island of New Zealand has a seasonal occurrence, and the current sample was obtained in the low frequency phase of this cycle. Such a hypothesis is supported by the higher frequency of cases of pleurisy found relative to the frequency of pneumonia. However, this frequency is considerably lower than the frequency of pleurisy seen in sheep which averages 19% throughout the slaughtering season, and can be as high as 36% in some flocks (McGowan *et al*, 1978).

The very low frequency of bronchopneumonias encountered in the present survey together with the large variability of carcass weights that was seen between groups of animals might have been responsible for the failure to demonstrate any relationship between bronchopneumonia and carcass weight ($p>0.05$).

It was interesting to find that the presence of fibrous tags (indicative of pleural adhesions) on the lungs did not always match the presence of fibrous adhesions on the thoracic pleura. Nevertheless, most of the severe cases of extensive, fibrous, dense adhesions were seen on both the costal and pulmonary pleura together (**Table II.III**).

In sheep, most pleural adhesions are related to current lesions of pneumonia, but some are not (Pfeffer, 1986). The presence of pleural adhesions in the thoracic wall downgrades carcasses for export markets (Alley, 1987). However, no relationship between carcass weight and pleurisy was demonstrated in the current survey.

The cases of bronchiectasis seen in this survey varied from a moderate dilatation of occluded bronchi, which retained an intact epithelium (**Figure II.15**) to large foreign body granulomas where the remaining bronchial outlines were barely discernible (**Figure II.16**).

Bronchiectasis in ruminants is usually a sequel to bronchitis (Jones & Hunt, 1983; Dungworth, 1985). In a small proportion of parasitic bronchitis cases, the bronchi of a lung lobe may fail to clear themselves of exudate and become the site of a low-

grade chronic purulent bacterial infection. The walls of the bronchi are gradually eroded and the lung lobules collapse, so that the bronchus becomes a tube of viscid pus (Urquhart *et al*, 1973; Breeze, 1985).

In roe deer, bronchiectasis, caused by verminous pneumonia and the stagnation of contents in the dilated air passages, predisposes the lung to fungal colonization (Vivovec *et al*, 1972).

The presence of nematode larvae in three cases supports the view that the origin of these lesions was from a verminous bronchitis. However, the scarcity of polymorph leukocytes and the presence of a marked foreign-body granulomatous reaction suggests that the inflammatory reaction was directed against the proteinaceous material comprising the bulk of the inspissated bronchial exudate, rather than being merely a low-grade chronic bacterial infection as suggested by Urquhart *et al* (1973) and Breeze (1985). Thus, lungworm-related bronchiectasis could be analogous to the *Brucella ovis*-related spermatic granulomas in the ovine epididymis, inasmuch as the severe inflammatory reaction is due to a reaction against stagnant exudate, rather than against a causative infectious agent.

Parasitic pneumonia was an extremely common finding in the surveyed animals. There was an association between the severity of the lesions and decrease in carcass weight for both dictyocaulus and nodular (*Muellerius capillaris*) lesions.

The decrease in carcass weight observed here may not, however, be a direct effect of lungworm infection. Management practices such as drenching for gastrointestinal worms with an anthelmintic which is also active against lungworms, would be confounding variables as they would result in the control of dictyocaulosis and muelleriosis as a by-product. It is common to find mixed infections of pulmonary and gastrointestinal parasites (Altaif, 1973; Sangster, 1990; Behnke, 1987) and many goat farmers in the Manawatu area recognize internal parasites as one of their most important health problems (Baticados, 1990). This finding therefore highlights the need for experimental work on controlled lungworm infections in goats.

Both goats (Sangster, 1990) and sheep (Altaif, 1973) affected with lungworms usually have significant infections of gastro-intestinal nematodes, often combined with *Fasciola* (Altaif, 1973), and conditions suitable for the development of lungworm larvae also favour a gastrointestinal burden (Robinson, 1990).

Animals with more than 10 small nodular pleural lesions per 10 cm^2 had a carcass weight 0.75 kg lighter than those with less or no lesions (p=0.0004). Animals with more than 10 large nodular pleural lesions per 10 cm^2 had a carcass weight 0.85 kg lighter (p=0.0102). This suggests that muellerius infections in goats may be of economic importance. A controlled infection of lambs with *Muellerius capillaris* found only a small decrease in live weight gain in infected animals (Rose, 1959), in the absence of clinical signs. However, muellerius infections in goats can be be a severe disease, rather than the mild disease associated with shot-like lung nodules seen in sheep (Sangster, 1990). Since nematodes cause longstanding stable infections in their definitive host, but are less successful in abnormal hosts (Behnke, 1987), it is reasonable to speculate that *Muellerius capillaris* evolved as a goat parasite, with sheep as a newer host.

The amount of carcass weight loss associated with the presence of more than 10 nodules per 10 cm^2 (> 750 g) is substantially smaller than the 9.4 pounds decrease in liveweight found by Rose (1959) in lambs experimentally inoculated with *Muellerius capillaris*. Rose (1959) believed that because his study was not designed specifically to study weight gain, no definitive statement on his data could be made, since liveweight in his study was highly variable. It is unfortunate that he did not publish carcass weights.

No attempt was made to differentiate between *Muellerius capillaris* and *Protostrongylus* spp, *Pneumocaulus* spp, *Cystocaulus* spp, *Capreocaulus* spp, *Neostrongylus* spp, *Varestrongylus* spp, or any other lungworms from the Protostrongyloidea superfamily (Rose, 1961; Soulsby, 1982) as they have a similar appearance in microscopic section (Chitwood & Lichtfels, 1972) and produce similar lesions (Stockdale, 1976). Only *Muellerius capillaris* has been reported in New Zealand.

Some researchers had shown that in goats the probability of muellerius lesions increases with age (McCraw *et al*, 1981). This is supported by the present findings, showing an increase in large nodular lesions characteristic of *Muellerius* with age. A higher infection rate would increase the likelihood of confluent lesions, which would be classified as large.

A significant association between the severity of dictyocaulus lesions and carcass weight was also found. Animals with a mild dictyocaulosis had a carcass 0.81 kg lighter (p<0.0001) than those with no lesions. Animals with a moderate dictyocaulosis were 0.85 kg lighter (p<0.0001) and those with severe lesions 1.52 kg (p<0.0001). It can

therefore be concluded that the severity of dictyocaulosis is negatively associated to the growth rate of goats.

The magnitude of decrease in carcass weight associated with severe dictyocaulus lesions (of unknown duration) is just as large as that resulting from (60 days of) an experimentally induced chronic non-progressive pneumonia in lambs (Alley, 1987). Thus, from the farmer's viewpoint, either disease may be associated with considerable economic loss.

It was interesting to find some distinct large emphysematous lesions in places where there were only a few lungworms in the local bronchi (**Figure II.02**). The finding of just one or two lungworms giving rise to a distinct lesion was reported by Murray (1986). These cases may represent animals in the post-patent phase (Breeze, 1985).

A small but significant negative correlation was found between the presence of dictyocaulus and muellerius lesions. The correlation between the presence (or absence) of dictyocaulus lesions of all levels of severity and the number of either small or large nodular lesions was -0.2271 ($n=4283$ $p<0.001$). This was most noticeable in the cases with moderate dictyocaulosis, in which there was a concurrent presence of >10 muellerius nodules per 10 cm^2 in only ten cases ($p<0.001$). These results suggest that there is an interaction between lungworms of different genera having a different habitat within the lung, whereby the status of infection by one lungworm affects the other. This may be due to shared antigens producing a cross-reaction and affecting the establishment or development of the other species.

Because the pulmonary lesions of *Muellerius* in goats are more commonly of a diffuse type (Nimmo, 1979; Cabaret, 1984) instead of the nodular type seen in sheep (Dungworth, 1985), it is possible however, that there was an overlapping between the lesions attributed to *Dictyocaulus* on gross examination and those caused by *Muellerius*.

Pleural plaques of fibrous tissue have not been reported previously in the veterinary literature. The presence of hyaline fibrous plaques on the diaphragmatic or posterior parietal pleura of humans is seen following exposure to asbestos (Jones & Sheers, 1973; Le Bouffant *et al*, 1973; Walter, 1989). They are commonly seen concurrently with pulmonary fibrosis, diffuse pleural fibrosis and mesothelioma (Jones &

Sheers, 1973). Although asbestos is not the only cause of pleural plaques in humans, it is certainly the most common (Jones & Sheers, 1973).

The microscopic appearance of the pigment found in the single case of suspected pneumoconiosis was different from that of silica, charcoal or asbestos (Dungworth, 1985; Le Bouffant *et al*, 1973). The type II cell hyperplasia seen in the surrounding alveoli suggested that the exogenous pigment had remained within the lung for some time. As the amount of lung tissue involved was small, and there was little inflammatory reaction elicited, and it was most likely of no clinical significance to the animal.

Subpleural Lymph Nodes (SLN) have only been described once previously (Giesel, 1977) as a rare (9 cases per 2526 animals) finding in sheep lungs during routine meatworks inspection. The number of SLN in a pair of lungs followed a Poisson distribution (**Table II.IV**), with a maximum of 10 SLN in each pair. The average number of SLN in those cases which had them was 2.35.

The finding that some flocks of goats had a higher frequency of SLN than others suggests a genetic variability in their frequency from one closed population to another. The possibility is supported by the breed differences in occurrence, with the SLNs present in 5.5% of ferals and only 2% of Angoras.

It was interesting to note that although the presence of SLN was mildly associated with age ($p=0.0476$), their numbers were not ($p=0.13$). They were found with less frequency in kids (2.8%) than in full-mouthed animals (4.9%). The difference in frequencies between these two age groups is less than twofold and it is therefore, unlikely that the SLN develop with age. Rather, it would be logical to assume that they are easier to find when they are larger, as would be the case in full-mouthed animals. On the other hand, neither the presence ($p=0.35$) nor the quantity ($p=0.63$) of SLN were related to carcass weight.

The range of variability in number, size, shape, location, tendency towards bilateral symmetry, and degree of organization of SLN is similar to that of normal lymph nodes (Yao, 1986). In the sheep's lung, lymph vessels form an extensive plexus throughout the serous pleural membrane of all lobes (Albertine *et al*, 1982). The total number of lymphatics is much greater in the caudal pleura (Albertine *et al*, 1982).

Pleural lymphatics drain in a ventral to dorsal direction toward the pulmonary ligament or hilum, depending on lung region (Albertine *et al*, 1982). Communications among pleural and intrapulmonary lymph vessels occurrs frequently (Albertine *et al*, 1982), with lymphatic valves directing lymph flow toward the pleural surface (Albertine *et al*, 1982).

It is useful to compare the SLN described here with the pulmonary lymphoid nodules (PLN) described by Selman *et al* (1973), Breeze *et al* (1975), Urquhart *et al* (1981), and Breeze (1985) in the lungs of cattle with dictyocaulus reinfection syndrome. The PLN are structures of 3-4 mm in diameter, found beneath the visceral pleura of each lung. In the initial stages, these nodules are raised above the lung surface and have a grey-red or green-yellow centre. Histologically, this is composed of a central core of brightly eosinophilic debris surrounded by many proliferating macrophages within a ring of eosinophils, macrophages, plasma cells, lymphocytes and giant cells. In time, these nodules form mature lymphoreticular tissue with germinal centres, and the eosinophilic debris is no longer apparent (Selman *et al*, 1977; Breeze, 1985). In most animals there are no adult lungworms in the tracheobronchial system, and when these are found, they are small, stunted and apparently sterile (Selman *et al*, 1973).

The differences between Breeze's (PLN) description and the (SLN) cases found in this study are:

i) The location of Breeze's PLN are in mid-lung whereas the great majority of SLN were found in the ventral edge of the caudal lobe.

ii) In the present study no case had a grey-red or greenish-yellow center.

iii) The well developed SLN had subcortical sinuses, pigment-laden macrophages and distinct cortical and medullary zones similar to ordinary functional tracheo-bronchial lymph nodes.

iv) The size of SLN varied from 1 mm to more than 30 mm where the PLN were constantly 3-4 mm in diameter.

Althought it seems likely that SLN are, merely cases of ectopic lymphoid tissue, an odd finding of little, if any, clinical significance, it is puzzling that they have been described in only one of the many slaughterhouse surveys of lungs of sheep and not previously reported in goats.

Conclusions

i) The low level of **pneumonia** and the high level of **pleural lesions** found in this survey suggests that enzootic **pneumonia** is a transient seasonal problem of lesser importance in goats than in sheep.

ii) Parasitic lesions are very common in New Zealand goat lungs and show increased prevalence with age.

iii) The **carcasses** of goats with mild to severe *Dictyocaulus filaria* lesions were from 0.81 to 1.52 kg lighter than those of animals without these lesions.

iv) The **carcasses** of goats with more than 10 nodular pleural (*Muellerius capillaris*) lesions were 0.75 kg lighter than those of animals without these lesions.

iii) **Subpleural lymph nodes** were found in more than 4 percent of goat lungs. The frequency varying between flocks from as little as 2% to as much as 27%.

v) Multi-focal **pleural plaques** were rare lesions found in two cases of the over 4000 goat lungs sampled.

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APPENDIX 1
REPEATABILITY OF THE PHOTOGRAPHIC SILHOUETTE MEASURING TECHNIQUE

In order to evaluate how consistent this technique for measuring lung areas was, a duplicate set of measurements were produced on different days from the same sets of photographs. Three pairs of lungs, each of which had four photographs, had their silhouettes drawn, cut and weighed, and the total and pneumonic areas for lateral surface, medial surface, and the sagittal cross sections of both halves were calculated. The results are shown in **Table APPENDIX.I.**

Table APPENDIX.I. DUPLICATE MEASUREMENTS OF LUNG AREAS FROM THE SAME PHOTOGRAPHS *

	Medial area	Lateral area	SAGGITAL CROSSSECTIONS Medial half	Lateral half
31 Left	167/170	218/224	144/154	181/189
32 Right	201/205	251/250	171/170	201/204
33 Right	180/174	142/134	102/128	102/105

* The numerator is the first measurement, the denominator is the second measurement. All measurements are in cm^2.

The correlation of both measurements was 0.9813172 ($P<0.001$). This represents the random measurement error of 0.0186828. The greatest single discrepancy between the first and the second measurements was found in the sagittal cross-section of the medial half of case 33Right (102cm2 *versus* 128cm2). This difference could be due to the difficulty in discriminating the border of the cross-section from the rest of the lung tissue. When this single data pair was removed from the calculations, the correlation coefficient increased to 0.9909 ($p<0.001$), and the random variation decreased to 0.0091, half the previous value. This data suggests that the estimation of lung surfaces from the weight of a paper silhouette drawn from an enlarged photograph has an average error of less than 2 percent, and this error is greater for the measurements of cross-sections as opposed to lateral or medial surfaces.

The paper sheets used for drawing the silhouettes had variable weights (up to 20 % variation). Each sheet was therefore weighed before use and a correction made to the area measurements to allow for this variation.

APPENDIX 2
ASSESSMENT OF ACCURACY OF LUNG DENSITY CALCULATIONS

Twelve ovine pneumonic lungs were obtained from the Oringi meatworks and taken to the laboratory for examination. The lungs were separated into left and right sides, and the bronchi severed at the entrance to the lungs immediately cranial to the carina. The lungs were weighed and the lung volume by water displacement was calculated according to the method of Scherle (1970) using a 10 kg mechanical analogue balance with a 10 grams division. The same measurements were then repeated using a Sartorious digital electronic balance.

The comparison of the measurements from both balances are shown in **Figures APPENDIX.1** and **APPENDIX.2**.

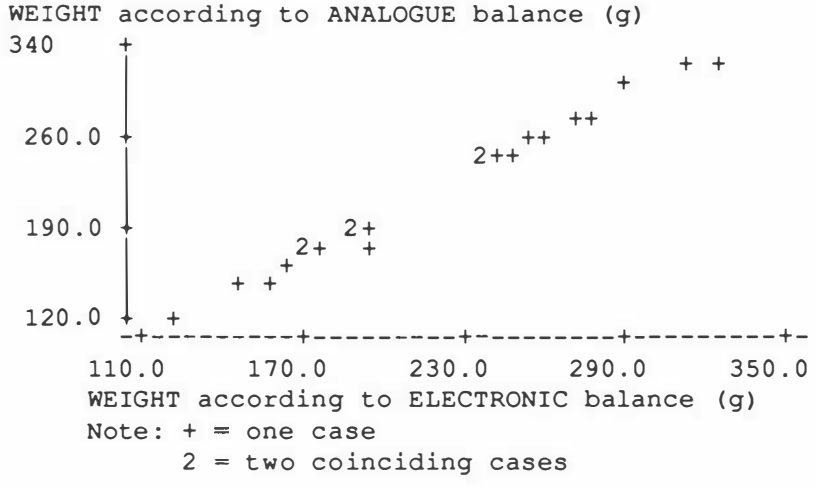

Figure APPENDIX.1. Weights of Ovine Pneumonic Lungs Using Different Balances.

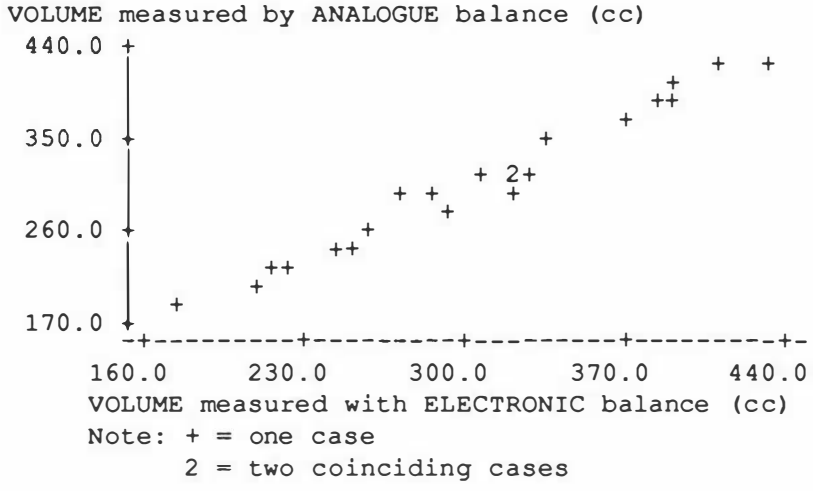

Figure APPENDIX.2. Volume of Ovine Pneumonic Lungs Measured by Different Balances

Although the simple mechanical analogue balance had a restricted accuracy of 10 grams, in all measurements there was a discrepancy of less than 5 % between the two types of balance. The lung weight measurements had a smaller variation between balances than the lung volume measurements.

However, small measurement errors may hamper the use of lung density examinations, and in the present case, the densities obtained by the two techniques for the same portion of lungs were quite different (**Figure APPENDIX.3**) (**Figure APPENDIX.4** *versus* **Figure APPENDIX.5**).

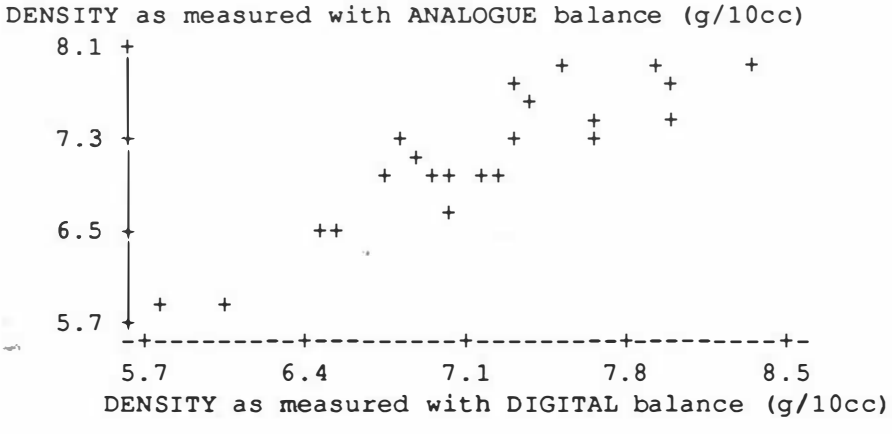

Figure APPENDIX.3. Comparison of Whole Lung Densities Obtained With Analogue and Digital Balances

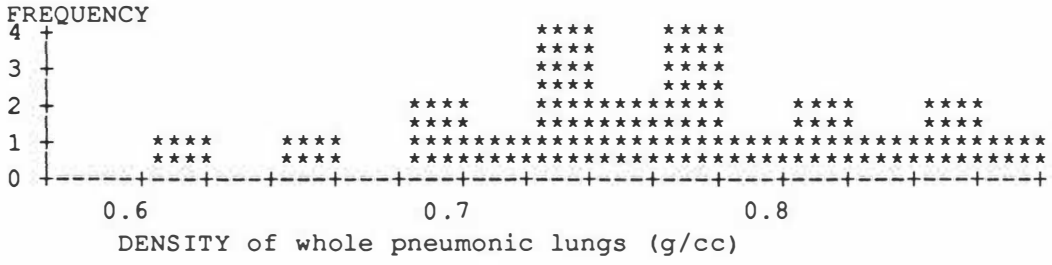

Figure APPENDIX.4. Densities of Whole Pneumonic Lung as Measured by Digital Electronic Balance

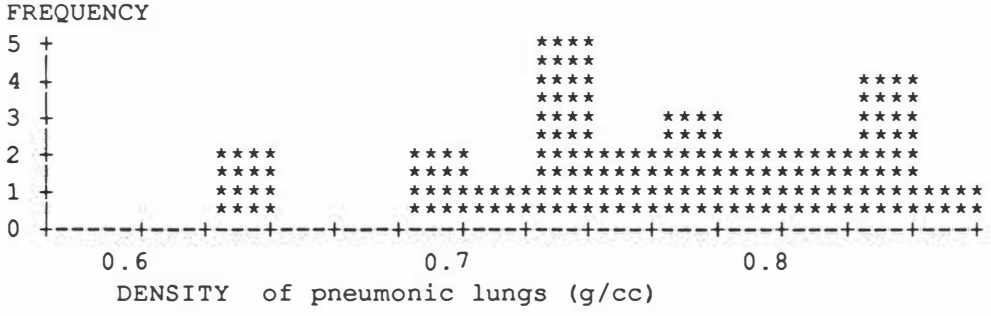

Figure APPENDIX.5. Densities of Whole Pneumonic Lung as Measured by Analogue Balance

Other evidence of the inaccuracy off lung density measurements was seen in the following situations:

i) When a small piece of non-pneumonic lung was measured, it seemingly had a higher density than the whole lung. This proved to be the smallest measurement, and a small error in its volume reading produced an impossible calculated amount of pneumonia of minus 68 cc.

ii) When the scatter plot diagrams are analyzed, three data points are clear outliers (eg: on the bottom right of **Figure IB.5** in Part IB). The likely source of error can be identified when the size of the sample of non-pneumonic lung is examined (**Figure IB.6** in Part IB). The smallest densities (asterisks) are clearly separated from the rest of the group, and these correspond to very small sample sizes. It is probable that a (relatively) large error in the volume measurement was produced in these cases because of the fluctuating readings caused by the manual holding of the submerged specimen.

APPENDIX 3
ASSESSMENT OF THE IMAGE ANALYSIS TECHNIQUE:

Accuracy

When the same image was measured several times, there was little variation from one reading to the next, as seen in **Table APPENDIX.II**. The coefficient of variation was less than one percent.

Table APPENDIX.II. REPEATED MEASUREMENTS OF THE SAME GRABBED IMAGE

	163.43212	203.46816
	165.72063	206.50747
	163.63641	203.61516
	165.40081	204.39410
	165.12154	202.55606
	167.47278	201.26275
	165.80187	203.74585
	166.25896	204.18181
	165.48269	202.59559
	164.53212	205.38865
	164.99104	203.14585
mean	165.411	203.432
standard deviation	1.078	1.209
coefficient of variat	0.69	0.70

The image analysis measures of lung areas were more reproducible than the paper silhouette tracing technique, but some problems emerged with the hardware, the calibration of the equipment, and the differentiation between pneumonia, collapse and background.

Hardware problems encountered

The capture of the video signal from the camera was complicated by an extraneous persistent roughly vertical pattern superimposed on the image. As this vertical pattern was produced when using either the video-camera, or a microscope with a different video-camera, it seemed to be inherent to the frame grabber card in the computer.

The monitor for displaying the digitized image had a moderate cushion-shape distortion, which complicated the calibration of the equipment.

Calibration of Image Size

Several approaches were tried in order to calibrate the area measurements of the analyzed images:

i) Initially, an attempt was made to produce a lifesize image in the computer display monitor, however a moderate cushion-shape distortion was produced on the monitor screen. When the video camera was focused on a 29 cm x 21 cm piece of paper placed perpendicular to the optical axis, the image appearing on the monitor screen was wider at the top and bottom than in the middle. This effect was more pronounced on the right vertical border of the displayed image.

After calibrating the system with this defect, and enlarging the lung images until the ruler included in the photograph appeared lifesize on the screen, lung areas which were 10-24% greater from those measured by the paper silhouette technique were obtained (a lung area which measured 206 cm^2 area by paper silhouette resulted in measures of 254.54 cm^2 , 228.60377 cm^2 and 225.53646 cm^2) (measurement of lung 1 Left at 24 hours resulted in 181.81697 cm^2 and 183.13535 cm^2, *versus* 172 cm^2 using paper silhouette).

ii) In the second approach, the system was re-calibrated using the top horizontal length of the complete photograph as reference, and calculating the vertical size of the photograph from its known proportions (24mm by 36 mm). With this arrangement, the system measured lung 1Right at 48 hours as having 218.05 cm^2, *versus* 198 cm^2 using paper silhouette. Lung 1 Left at 48 hours produced 179.84 cm^2, compared to 172 cm^2 using paper silhouette.

iii) Because of the in-accuracy and unreliability of the former area calibration procedures, a white square of paper of known area was included in all photographs of lungs to be analysed. All area measurements then had a defined calibration area in each photographed lung.

Differentiation between pneumonia, collapse and background

Even though the colour of the areas of grey consolidation, collapse and emphysema were easily distinguished from normal lung in the photographs, the

computer image analysis system could not distinguish them and they appeared as shades of grey. The differentiation between pneumonic and non-pneumonic areas was often not clear-cut and required the simultaneous viewing of the photograph. A few attempts to automate the measurement of grey areas resulted in very different results according to how the threshold of density (shade of grey) was set. The blue background favoured for gross pictures was also difficult to differentiate from the border of some lungs, and was replaced by a black background.

APPENDIX 4
VISUAL ASSESSMENT OF PNEUMONIC AREAS

To test the ability of veterinarians to estimate the severity of pneumonia twelve persons working in veterinary pathology in either the Department of Veterinary Pathology and Public Health at Massey University, or the Batchelar Agricultural Centre Animal Health Laboratory were asked to estimate the percentage of the lung surface that appeared consolidated in eleven *Ektachrome* colour slide transparencies of ovine pneumonic lungs (from Part IC). The transparencies were projected onto a screen and the subjects were requested to record the proportion of the lung area affected. The percentage of pneumonic area in the cases used ranged from 0 to 49%. All experimental subjects had more than one year's experience in veterinary pathology. The results are presented in Table **APPENDIX.III** and **Table APPENDIX.IV**.

Table APPENDIX.III. ESTIMATES OF PNEUMONIC PERCENTAGE BY DIFFERENT PATHOLOGISTS (lungs with less than 30% pneumonic surface area).

Case	A	B	C	D	E	F	G	H	I	J	K	L	Real
3	15	20	20	20	15	15	20	15	20	30	20	20	23
4	15	20	15	15	20	10	28	28	20	30	20	25	18
5	10	15	20	15	15	10	18	10	10	20	18	15	21
6	2	3	5	5	2	5	5	2	1	4	2	5	1
8	10	20	25	10	15	20	18	18	15	25	18	15	20
9	5	1	0	0	2	5	0	5	1	10	4	0	<1
14	20	20	25	20	20	15	20	12	15	25	20	20	28
15	5	9	15	10	10	10	18	10	10	20	12	10	16
18	5	5	5	5	5	5	3	9	1	10	6	0	3

Notes: All values were truncated to integers.
Real = Percentage of area measured with image analysis.
A-L = Experimental subjects

Table APPENDIX.IV. ESTIMATES OF PNEUMONIC PERCENTAGE BY DIFFERENT PATHOLOGISTS (moderately severe pneumonia).

Case	A	B	C	D	E	F	G	H	I	J	K	L	Real
1	20	30	30	20	30	25	40	30	30	30	30	30	34
2	30	27	25	20	25	20	30	28	25	30	28	30	33
7	25	35	40	30	30	30	28	35	35	40	38	30	39
10	20	36	35	30	35	33	30	30	30	35	40	40	49
11	30	31	25	25	25	20	28	32	30	30	30	30	38
12	25	28	30	40	25	25	22	38	30	40	33	30	43
13	35	32	25	35	30	30	38	30	30	70	35	30	43
16	40	38	40	40	35	35	38	38	35	45	48	30	43
17	22	28	35	20	20	25	28	20	20	30	33	25	32
M.	27 bc	31 b	31 b	28 bc	28 bc	27 c	31 bc	31 bc	29 bc	38 a	35 b	30 bc	39 a
sd	6.8	3.9	6.1	8.2	5.0	5.3	6.0	5.5	4.6	12.9	6.2	3.9	5.5

Notes: All values were truncated to integers.
A-L = Experimental subjects
Real = Percentage of area measured with image analysis.
M. = Means
sd = Standard deviation
Means with different subscripts are statistically different (alfa=0.05)

Some subjects complained that they could not estimate the amount of consolidation from a photograph, for they needed to palpate it.

Subjects I and L favoured estimates rounded to the nearest 10%.

The most interesting findings came from analyzing data for cases 1, 2, 7, 10, 11, 12, 13, 16, 17, which were lungs with moderately severe pneumonias in which 32% to 49% of the surface was consolidated (**Table APPENDIX.IV**). For these lungs, all subjects except one (subject J) underestimated the amout of pneumonia ($p<0.05$). Subject J made a very accurate estimate of the proportion of pneumonia, with an average deviation of 0.41% ($p=0.91$). The average deviation of the estimated values from the true values of pneumonia ranged from 4.3 ($p=0.037$) (11% of the actual value) for subject K, to 12.3% ($p<0.0001$) (31% of the actual value) for subject F.

It thus appears that, except for one subject (J), and marginally for another (K), all pathologists underestimated the extent of moderately severe pneumonias.

On the other hand most subjects tended to overestimate the extent of very small lesions (Cases No 6, 9 and 18) (**Table APPENDIX.III**). Case 9 had <1% of

consolidation, but showed multiple petechiae on the lung, which half the subjects interpreted as pneumonia.

It can be concluded from this data that observers tend to be conservative when estimating proportions of pneumonic lungs. Large lesions are consistently underestimated and small lesions are overestimated. There is a range of variation in the amount of underestimation between individual pathologists, from very little to about one third of the real value.