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AN INVESTIGATION OF OVINE PNEUMONIA IN FOUR HERDS FROM CENTRAL NORWAY

I. PREVALENCE OF PNEUMONIA AND MICROBIOLOGICAL FINDINGS *

By

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BAKKE, T. and S. NØSTVOLD: *An investigation of ovine pneumonia in four herds from Central Norway. I. Prevalence of pneumonia and microbiological findings.* Acta vet. scand. 1982, *23*, 248—258. — In a study of 4 sheep herds, 1 apparently healthy and 3 having respiratory problems, lesions typical of subacute or chronic pneumonia were found in 3—36 % of slaughtered lambs. Occurrence appeared to be related to certain environmental factors such as pasture, whereas moderate lungworm invasion was not found to contribute to subacute or chronic pneumonia. Relation between pneumonia and low carcass weight was established only in 1 herd.

Lungs were subjected to microbiological examination. Mycoplasma ovipneumoniae was isolated from both normal and pneumonic lungs from all 4 herds. The prevalence was far higher in pneumonic (98 %) than in normal ones (28 %). Bacteria, mostly Pasteurella haemolytica, were also found in both pneumonic (49 %) and normal (18 %) lungs from all 4 herds. These results confirm the conclusions of a previous study that M. ovipneumoniae is of etiological significance in subacute or chronic pneumonia, whereas bacteria mainly occur as secondary invaders. M. ovipneumoniae appears, however, only to be a potential pathogen. Examinations for Mycoplasma arginini and virus were negative and these agents are considered to be of less significance in subacute or chronic pneumonia under Norwegian conditions.

ovine pneumonia; Mycoplasma ovipneumonia; Mycoplasma arginini; virus; bacteria; lungworm.

A previous work indicated that Mycoplasma ovipneumoniae is widespread among sheep in Southern Norway and is of etiological significance regarding subacute or chronic pneumonia,

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while bacteria most commonly act as secondary invaders (*Bakke* 1982). These findings are in agreement with earlier reports from Australia (*St. George & Sullivan* 1973), New Zealand (*Alley & Clarke* 1980) and Great Britain (*Jones et al.* 1979). Based on the results from this screening for M. ovipneumonia in a limited number of samples from each of a great number of herds, a second investigation on ovine pneumonia was designed. The purpose of this second investigation was to study the prevalence of subacute or chronic pneumonia and M. ovipneumonia within single herds and to search for other possible non-bacterial pathogens which have been associated with this type of pneumonia (*St. George & Sullivan* 1973, *Winter & Young* 1975, *Davies et al.* 1976, *Jones et al.* 1979).

MATERIAL AND METHODS

Based on clinical information and earlier carcass recordings 1 presumably healthy herd (herd A) and 3 herds with a history of respiratory disease (herd B, C and D), all located in the inland of Central Norway, were chosen for the present investigation. The presence of the lung disease Maedi in these herds was unlikely on the basis of previous serological and pathomorphological examinations and geographic location. Some observations of environmental conditions of the 4 herds were recorded.

A few days (1—7) prior to slaughter (autumn season) blood samples were collected for serological examination for antibodies against parainfluenza-3-virus (PIV-3). The serum was stored at —25°C until analyzed using a bovine strain of PIV-3 ("Umeå 23 B") as antigen in a hemagglutination-inhibition test.*

Lungs from all 308 slaughtered animals from these herds were collected at the abattoir for a later macroscopical examination. The majority of animals being slaughtered were lambs about 6 months old, but some lungs from adult sheep were included. Each pair of lungs was put into a separate plastic bag, rapidly cooled to +4°C and brought to the laboratory in a cooled condition within 3—5 h. For further microbiological and histological examination, a selection was made of apparently normal lungs and lungs displaying various degrees of pneumonia.

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* These analysis were performed by J. Krogsrud, Dept. of Virology, National Veterinary Institute, Oslo, Norway.

From each pair of lungs, tissue samples for microbiological and histological examinations were taken aseptically from 3 different locations: 1) the center of pneumonic consolidations, 2) the borderline between consolidated and normal lung tissue, and 3) mediodorsally in the right diaphragmatic lobe. In lungs without any macroscopical changes, two samples were taken from the right apical and the left cardiac lobe, respectively. The procedures for the recovery and identification of fermenting mycoplasmas and bacteria are given elsewhere (*Bakke* 1982). Examination for arginine metabolising mycoplasmas was carried out for 60 % of the lungs (25 pneumonic and 27 normal lungs, representing all 4 herds). The medium used was a modification of Haylick's type, enriched with arginine and mucin (*Friis* 1975). Suspensions of lung tissue were stored at —20°C at the most for 11 weeks before inoculation.

Lung tissue from the 3 different sampling locations was mixed and screened for cytopathogenic or hemagglutinating effect through 2 passages in lamb kidney cell culture.*

Sections for histological examination were taken in the immediate vicinity to the samplings for microbiological investigations. The tissue sections were stained with haematoxyline-eosine and van Gieson. Additionally, a selection of sections were stained according to the Gram and periodic acid Schiff (PAS) methods.

The individual carcass weights of all lambs from the 4 herds were obtained from the abattoir records.

RESULTS

Macroscopical examination

It is evident from Table 1 that the presumed healthy herd A was not completely devoid of gross pneumonic lesions (3 %), but it clearly distinguished itself from the 3 other herds which had a frequency of pneumonia from 16 to 36 % in lambs. All ewes from herds B, C and D examined (1, 8 and 7 animals, respectively) were devoid of macroscopical lung changes. In herd A, 2 ewes had areas of atelectasis, in the other 13 ewes no macroscopical lesions were found.

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* These examinations were performed by J. Krogsrud, Dept. of Virology, National Veterinary Institute, Oslo, Norway.

Table 1. Occurrence of macroscopical lung lesions in lambs from the herds A, B, C and D in Central Norway, fall of 1978.

Lesions	Number of lungs (% in brackets) from herd							
	A		B		C		D	
No lesions	48	(71)	13	(28)	25	(46)	73	(68)
Atelectasis	18	(26)	17	(36)	20	(37)	17	(16)
Lobular* pneumonia	2	(3)	15	(32)	8	(15)	9	(8)
Lobar* pneumonia	0	(0)	2	(4)	1	(2)	9	(8)
Total	68	(100)	47	(100)	54	(100)	108	(100)

* Lungs displaying pneumonic consolidations are subdivided into lobular and lobar pneumonia according to anatomical extension of the lesions.

Mycoplasmological examination

M. ovipneumonia was the only fermenting mycoplasma isolated and was recovered from 57 out of 86 lungs (66 %). Arginine metabolising mycoplasmas were never recovered, while M. ovipneumoniae grew in the arginine medium in 29 out of 52 cultivations.

The isolated mycoplasma strains all grew on solid medium in ordinary atmosphere and developed colonies of the "centreless" type with a granular surface. All isolates were sensitive to digitonin. In growth inhibition test on solid medium using antiserum against M. ovipneumoniae, strain Y98 (NCTC 10151), all isolates showed zones of inhibition varying between 1.5 and 9 mm. Broth cultures of 4 strains tested passed through a 0.45 μm pored membrane and displayed no zone of inhibition in a growth inhibition test using antiserum against Mycoplasma dispar, strain 462/2 (NCTC 10125). The 4 isolates were also negative in the phosphatase test. These 4 strains as well as 2 strains displaying small or unequivocal zones in the growth inhibition test were all positive in the metabolic inhibition test with titres between 80 and 640. The titre of strain Y98 of M. ovipneumoniae was 640 in the same test.

The relative occurrence of mycoplasmas in normal versus pneumonic lungs from the 4 herds is shown in Table 2. Included in the pneumonic group are lungs with macroscopical atelectasis with definite exudative or proliferative inflammatory changes

Table 2. Isolation of mycoplasmas from normal and pneumonic sheep lungs from herds A, B, C and D.

	Number of mycoplasma isolations/number of lungs examined from herd					
	A	B	C	D	total	
Normal	4/19	3/7	2/4	2/9	11/39	(28 %)
Pneumonic	4/4	15/16	9/9	18/18	46/47	(98 %)

histologically. As clearly evident from Table 2, mycoplasmas were recovered from almost all (98 %) lungs displaying pneumonic lesions. The mean growth titre was $10^{5.9}$ in pneumonic lungs compared to $10^{3.7}$ in normal lungs with a positive recovery of mycoplasmas. In lungs from the 7 ewes (3 from A, 1 from B and C and 2 from D, all normal), M. ovipneumoniae was isolated only from 1 animal (herd A) and with a low titre (10^2).

Bacteriological examination

Bacteria were demonstrated in 23 out of 47 pneumonic lungs (49 %) and in 7 out of 39 normal lungs (18 %). Pasteurella spp. represented the majority of the bacterial isolates (83 %). Pasteurella haemolytica was present in lungs from all herds both in pneumonic lungs (1 from A, 6 from B, 4 from C and 9 from D) and normal lungs (1 from each herd). One isolate similar to Pasteurella multocida according to morphological and cultural properties, but differing biochemically, was isolated from 1 normal lung. Other bacteria were Corynebacterium pyogenes (1 pneumonic and 2 normal lungs), Acinetobacter sp. and Staphylococcus aureus (pneumonic lungs).

Virological examination

Two passages in cultures of lamb kidney cells failed to demonstrate cytopathogenic and hemagglutinating agents from any of the 86 lungs being examined.

Serological examination

In 308 blood samples collected just prior to slaughter comprising all animals of the present investigation, antibodies against PIV-3 were not detected.

Parasitological examination

Macroscopical lesions indicative of Muellerius spp. (*Jubb & Kennedy* 1970) were observed in lungs from all 4 herds. However, adult worms or development stages were not detected histologically or in smears from nodules and bronchial epithelium. Verminous nodules were seen in 27, 35, 65 and 4 % of the total number of lungs from herds A, B, C and D, respectively. None of the lungs were heavily invaded. In fact, only a small number of lungs containing more than 3 lungworm nodules were found (herds B and C). Normal lungs were more frequently affected by verminous nodules than pneumonic lungs, the difference, however, not being statistically significant (Chi-square test).

Carcass weight recordings

The mean carcass weights of lambs scored as healthy or pneumonic in the 4 herds are shown in Table 3. Lungs demonstrating atelectasis are listed as normal based on the earlier experience that the majority of such macroscopical lesions are without histological evidence of inflammation (*Bakke* 1982). Besides, even frank pneumonia with such a minor extension would hardly have any obvious influence on growth rate (*Kirton et al.* 1976). Only in herd C a significant reduction of carcass weights was found for pneumonic lambs (Student t-test, $P < 0.05$).

Table 3. Mean carcass weight ± standard deviation (s) for healthy and pneumonic lambs from herds A, B, C and D.

Herd	Healthy lambs			Pneumonic lambs		
	Number of animals	Carcass weight (kg)** mean	s	Number of animals	Carcass weight (kg)** mean	s
A	66	15.5	± 2.9	2	17.6	± 1.4
B	30	17.3	± 2.7	17	17.8	± 1.7
C	45	13.3	± 2.4	9	10.6	± 1.7
D	88*	16.1	± 3.2	18	14.6	± 3.7
Total	229	15.5	± 3.1	46	15.1	± 3.7

* 2 lambs having diarrhoea excluded.

** No significant difference in carcass weight between lambs scored as healthy or pneumonic was demonstrated in the total material and in herds A, B and D. In herd C, a significant reduction of carcass weights was found for pneumonic lambs (Student t-test, $P < 0.05$).

DISCUSSION

As both M. ovipneumoniae and P. haemolytica were demonstrated in the one healthy herd, other factors than the mere presence of these organisms must be of importance for the morbidity and severity of this subacute or chronic ovine pneumonia. When comparing the environmental conditions during the indoor season for the different herds with the frequency of pneumonia at slaughter, no definite conclusions seemed justified. In the one healthy herd the environmental conditions were satisfactory, but the same situation was present in herds B and D. Other investigations have demonstrated a major influence of housing factors on the frequency of ovine pneumonia (*Øverås & Nedkvitne* 1976, *Jones et al.* 1979). There are reasons for assuming that increased humidity, low temperatures and crowding during the indoor season may expose the lambs to more heavy infection during the first month of life before the animals are turned out on pasture. Even though pneumonic lesions usually do not develop in lambs during the first 6—7 weeks of life, most likely due to protection from maternal antibodies (*McGowan et al.* 1978, *Jones et al.*), an establishment of M. ovipneumoniae in the upper respiratory tract shortly after birth has been demonstrated (*Jones et al.*). To what extent the organism later will give rise to pneumonia must largely depend on the environmental conditions of the grazing period. Rough climatic conditions in a mountainous area offer an explanation for the high frequency of pneumonia in herd B. Lungworm migrations were considered as another predisposing factor for the development of pneumonia. However, no such relationship was detected concerning the minor lesions present in this material. This is in agreement with a previous investigation (*Bakke* 1982).

No systematic clinical observations were performed, but coughing was regarded as a problem in both herds B and D. Maedi was no relevant differential diagnosis in these herds, and the coughing was unlikely to be caused by the low grade of lungworm invasion detected at slaughter. Anthelmintic treatment in herd D, ranking highest with respect to coughing during the previous winter, was reported to have no effect on clinical respiratory symptoms. Necropsy of an 11-months old lamb at this time revealed changes typical of subacute or chronic pneumonia and M. ovipneumoniae was found in pure culture, indicating a relation between clinical disease and mycoplasma infection.

Coughing was observed in both lambs and ewes during the previous winter, though no lesions in ewes were found at slaughter the following autumn. Subacute or chronic pneumonia is regarded mainly to be a lamb problem, but coughing in ewes has been reported from herds suffering from the disease (*Sullivan et al.* 1973, *Jones et al.* 1979). This probably reflects a clinical exacerbation of a chronic pneumonia, which is most likely to occur in periods of environmental stress. Herds A, B and C were located in a district where coughing was reported to be a common herd problem in midwinter at a time when very low outdoor temperatures resulted in poor ventilation and high humidity in the sheep houses.

The present material does not justify any conclusion concerning a possible influence of subacute or chronic pneumonia on the growth of lambs. In the one herd (C) where pneumonic lambs had a significant reduction in carcass weights, the mean carcass weights regardless of pneumonic findings were lower than in other herds, most likely due to scanty lowland pastures. This is in agreement with reports from abroad where an effect of pneumonia on gain of weight was evident only when the growth rate was low, i.e. on inadequate pastures (*Kirton et al.* 1976). Low grade of pneumonia and lungworm invasions was then assumed not to influence growth rate and final slaughter weights.

Results obtained through the microbiological examination of the present material support the conclusions of a previous work (*Bakke* 1982) stating that M. ovipneumoniae is of etiological significance for subacute or chronic ovine pneumonia in Norway. When comparing the recovery rate of mycoplasmas from normal lungs in the 2 investigations under consideration, mycoplasmas were found more frequently in normal lungs in the present study. This might in part be explained through the cultivation from 3 locations of each pair of lungs compared to only 1 in the previous examination. Also, the previous investigation comprised a relatively large number of herds virtually without pneumonic lesions at slaughter, and it is possible that many of these herds might be free of M. ovipneumonia. One would expect to find a higher frequency of healthy carriers in affected herds compared to healthy herds where the pathogen might be totally absent. In a corresponding pneumonia of pigs caused by Mycoplasma suipneumoniae, the organism may persist in the bronchial tree

for many months after clinical recovery (*Whittlestone* 1975). Recovery of M. ovipneumoniae from normal lungs has been described by several authors (*Alley et al.* 1975, *Leach et al.* 1976, *Jones et al.* 1979). Presumably the organism may be present in the lung parenchyma without eliciting an inflammatory reaction, thus being only potentially pathogenic.

Mycoplasma arginini was not found. This mycoplasma species is regarded to grow in ordinary mycoplasma growth media (*Cottew* 1979) and the medium used should be optimal for the isolation of this organism. In more than half of the samples screened for M. arginini, acid production due to growth of M. ovipneumoniae occurred in the arginine medium, thus possibly camouflaging a low growth titre of M. arginini. The failure to isolate M. arginini is in agreement with the conclusion drawn by other authors (*Jones et al.* 1979, *Alley & Clarke* 1980), that M. arginini is unimportant in subacute or chronic pneumonia.

Several viruses, especially PIV-3, have been isolated from the respiratory tract of sheep (*Winter & Young* 1975, *Davies et al.* 1976). It remains to be settled, however, what relevance these agents may have to subacute or chronic pneumonia. Cytopathogenic or hemagglutinating viral agents were not found. A possible contribution of viruses in the establishment of pneumonic lesions in the early phase can, however, not be excluded. It has been reported that respiratory viruses, in contradiction to mycoplasmas, are rather quickly eliminated from lung parenchyma (*Hore & Stevenson* 1969, *Winter & Young* 1975). Considering a possible contribution of PIV-3 in initiating the pneumonic lesions of the present study, the negative serological survey for antibodies against this virus makes the existence of PIV-3 in these herds unlikely, even at the early stage of the disease (*St. George & Sullivan* 1973, *Singh & Pathak* 1979). The application of a bovine strain of PIV-3 in the serological test is justified by the close antigenic relationship found between ovine and bovine strains of this virus (*St. George* 1969). The prevalence of PIV-3 antibodies in the Norwegian sheep population is not known.

Screening for chlamydia were not performed in the present investigation. Such agents have been isolated from field cases of ovine pneumonia, but their significance in respiratory infections in sheep is questionable (*Davies et al.* 1975, *Littlejohns* 1976, *Foggie* 1977).

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SAMMENDRAG

En undersøkelse av ovin pneumoni i fire besetninger fra midt-Norge. I. Forekomst av pneumoni og mikrobiologiske funn.

Lunger fra samtlige sauer slaktet om høsten i 3 besetninger med pneumoni-problemer og 1 tilsynelatende frisk besetning ble undersøkt makroskopisk. Pneumoniske fortetninger typiske for subakutt eller kronisk pneumoni ble påvist i alle 4 besetninger, men forekomsten varierte fra 3 til 36 % av lammene. Ingen voksne dyr hadde lesjoner. Forekomsten syntes å ha sammenheng med enkelte miljøfaktorer som beiteforhold, mens en moderat invasjon av lungeorm ikke ble funnet å disponere for denne type pneumoni. Sammenheng mellom pneumoni og lav slaktevekt ble bare påvist i 1 besetning.

Mikrobiologiske undersøkelser ble foretatt for et utvalg av lunger. Mycoplasma ovipneumoniae ble isolert fra såvel normale som pneumoniske lunger fra alle 4 besetninger, men i langt høyere frekvens i pneumoniske (98 %) enn i normale lunger (28 %). Bakterier, vesentlig Pasteurella haemolytica, ble også påvist i såvel pneumoniske (49 %) som i normale lunger (18 %) og i alle 4 besetninger. Resultatet bekrefter tidligere antagelser om M. ovipneumoniae som primært etiologisk agens og bakterier som sekundære patogener ved subakutt og kronisk pneumoni hos sau. Funn av M. ovipneumoniae i lunger uten pneumoniske lesjoner tyder på at organismen er betinget patogen. Undersøkelser m.h.p. Mycoplasma arginini og virus var negative, og disse agens antas å ha mindre betydning ved ovin pneumoni under norske forhold.

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