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THE OCCURRENCE OF MYCOPLASMAS AND BACTERIA IN LUNGS FROM SHEEP IN SOUTHERN NORWAY

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BAKKE, T.: *The occurrence of mycoplasmas and bacteria in lungs from sheep in Southern Norway.* Acta vet. scand. 1982, *23*, 235—247. — In order to study the occurrence of mycoplasmas among Norwegian sheep, lungs from a great number of different herds were collected at 3 abattoirs in Southern Norway. The presence of fermenting mycoplasmas and bacteria was examined in both normal and pneumonic lungs to determine whether recovery of these agents could be related to pneumonic changes. Pneumonic lungs demonstrated lesions typical of the condition described as subacute or chronic pneumonia.

Mycoplasma ovipneumoniae was found in 87 % of the 126 pneumonic lungs and in 6 % of the 83 normal lungs. Bacteria, mostly Pasteurella haemolytica, were less frequently encountered in the pneumonic lungs, and usually in combination with M. ovipneumoniae. It is concluded that M. ovipneumoniae is widespread among sheep in Southern Norway and can be considered to have etiological significance in subacute or chronic pneumonia, whereas bacteria probably occur mainly as secondary invaders. Changes resulting from moderate invasion by lungworm were found in about half of the lungs, but just as frequently in normal as in pneumonic lungs, and accordingly did not appear to contribute to the pneumonia investigated.

ovine pneumonia; Mycoplasma ovipneumoniae; bacteria; lungworm.

From abroad subacute or chronic pneumonia in sheep is usually known to be mild, and rather common among young animals. Some authors include this pneumonia in the term "enzootic pneumonia", a term which also includes the more acute pneumonic form of pasteurellosis (*St. George & Sullivan* 1973). Subacute or chronic pneumonia is by other authors designated as "atypical pneumonia" (*Jones et al.* 1978) or "chronic non-progressive pneumonia" (*Alley & Clarke* 1980).

The etiology of subacute or chronic pneumonia is still not clear. Both mycoplasmas, viruses and chlamydia have been incriminated as possible primary causative agents (*St. George & Sullivan* 1973, *Winter & Young* 1975, *Davies et al.* 1976); of these Mycoplasma ovipneumoniae seems to be of greatest significance. M. ovipneumoniae was first described and characterized by *Carmichael et al.* (1972) in connection with a mycoplasma strain isolated from ovine pneumonia in Queensland, Australia (*St. George et al.* 1971). This organism appeared to be the same species as those isolated from sheep lungs in Victoria, Australia (*Leach et al.* 1976) and in Scotland (*Jones et al.* 1976). M. ovipneumoniae has later been isolated from sheep in New Zealand (*Clarke et al.* 1974), USA (*St. George & Carmichael* 1975), Hungary (*Stipkovits et al.* 1975), Iceland (*Friis et al.* 1976) and Switzerland (*Nicolet et al.* 1979). There is only 1 report on isolation of mycoplasmas from sheep lungs in Norway (*Mohn & Utklev* 1974). The organism isolated was not further identified.

In the present work the aim was to investigate the occurrence of M. ovipneumoniae and bacteria in sheep lungs from different parts of Southern Norway. Both pneumonic and normal lungs were collected from 3 abattoirs, to see whether the findings of infectious agents were related to pathological lesions.

MATERIAL AND METHODS

Lungs were collected in the autumn from 3 abattoirs located in areas with different climates. Abattoir A and B were both located in the inland, but A was in the highlands and B in the lowlands. Abattoir C was located near the coast.

The material included i) lungs without macroscopic lesions, ii) lungs having only small areas of macroscopic atelectasis (Fig. 1), iii) lungs with lobular (Fig. 2), and iv) lungs with lobar consolidations (Fig. 3) in the anterio-ventral parts. These consolidations had a reddish or grey appearance and were most typically located in the right apical lobe. The lungs were mostly taken from animals about 6 months old. The total number of lungs was 209. Twenty-six lungs were unidentified, the rest represented 118 herds, 58 from A, 28 from B and 32 from C.

Samples for microbiological and histological examinations were taken aseptically from macroscopic lesions; from normal lungs samples were taken from the right apical lobe. The tissue

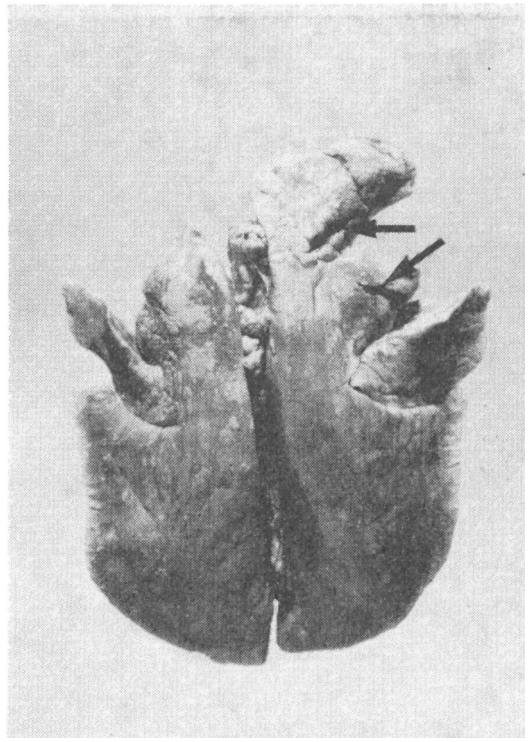

Figure 1. Sheep lung with atelectasis (arrow) in the right apical lobe.

sections were immersed in 10% buffered neutral formalin, processed by conventional paraffin embedding and stained with haematoxyline-eosine and van Gieson. To estimate the numbers of fermenting mycoplasmas present, 0.5 g of lung tissue was ground in a mortar with sterile quartz sand and added to 4.5 ml of a medium originally intended for cultivation of Mycoplasma suipneumoniae (*Friis* 1975), but without antibiotics. This suspension was regarded as a 10^{-1} dilution of lung tissue. Ten-fold dilutions were then made in Friis' medium (with antibiotics) up to a final dilution of 10^{-9}. The dilutions from 1:100 upward were then incubated for 10 days in a rotating drum at 37°C; negative samples were incubated for another 3 days. Dilutions showing an unequivocal drop in pH were recorded as positive, and the highest dilution giving such a change was recorded as the growth titre. Mycoplasmas from one of the highest positive dilutions were subcultured in liquid medium before inoculation on agar

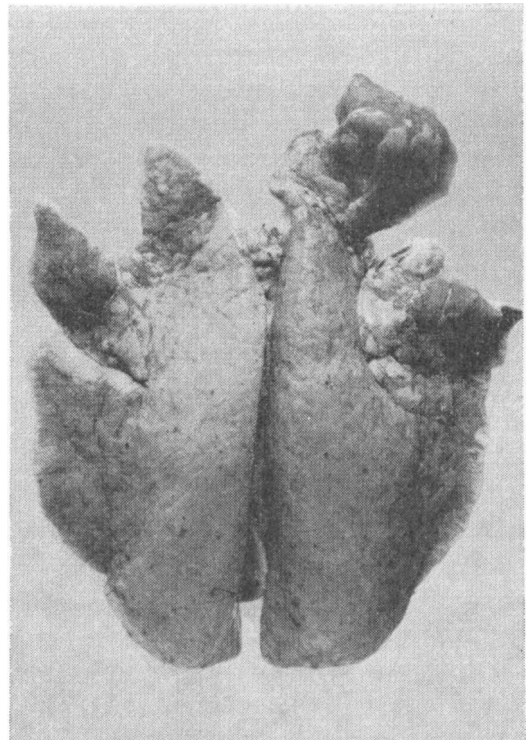

Figure 2. Sheep lung with lobular consolidation in the right apical lobe.

as described by *Friis* (1975) for cultivation at 37°C in ordinary atmosphere for 4—5 days. After examination of agar colonies and cloning once, all mycoplasmas isolated were tested for sensitivity to digitonin. They were further examined serologically using rabbit antiserum against M. ovipneumoniae, strain Y98 (NCTC 10151), in a disc growth inhibition test (g.i. test). From 109 mycoplasma isolates, 24 strains were chosen for more extensive examination. These strains were selected to represent as many herds as possible from all 3 abattoirs. They were passed through a 0.45 μm membrane filter before cloning. In addition to the tests used for all mycoplasma isolates these isolates were tested in g.i. test with antiserum against Mycoplasma dispar, strain 462/2 (NCTC 10125) and examined for production of phosphatase. A few of the strains were tested in a metabolic inhibition test (m.i. test) with M. ovipneumoniae antiserum. The g.i. test and the m.i. test were performed as described by *Friis*

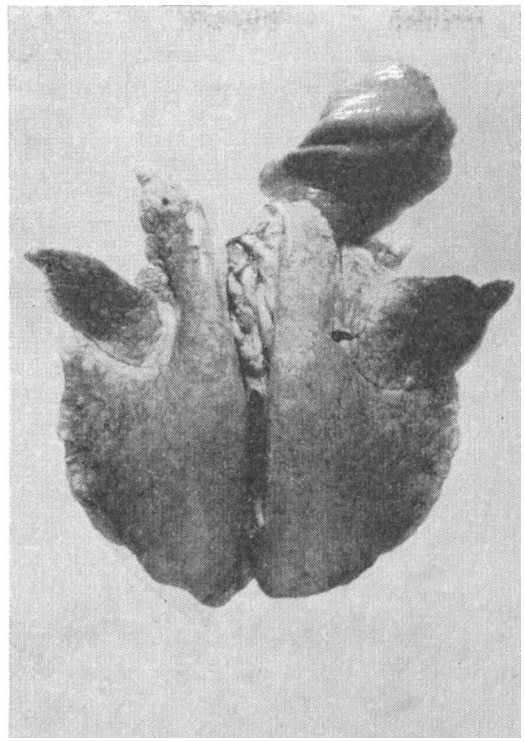

Figure 3. Sheep lung with lobar consolidation in the right apical lobe.

(1974); in the g.i. test agar plates were inoculated by the running drop method (*Freundt et al.* 1979) and placed at 29°C the first day of incubation.

Bacteriological examination was done by inoculating the lung suspension (dilution 1:10) on 2 plates of blood agar, one plate streak-inoculated with Staphylococcus aureus and incubated in CO_2 atmosphere for 2 days, the other incubated anaerobically for 5 days at 37°C. In addition, material was transferred to an enrichment broth containing horse serum. The broth was incubated 1 day at 37°C. This was followed by inoculation on blood agar for incubation in CO_2 atmosphere for 2 days at 37°C.

RESULTS

Changes like cellular exudate in alveolar spaces and bronchies and/or extensive peribronchial lymphoid hyperplasia ("cuffing-pneumonia") were found histologically in 14 (23 %) of 62 lungs

Table 1. Isolations of mycoplasmas from pneumonic and normal lungs from abattoirs A, B and C in Southern Norway.

Lesions	Number of mycoplasma isolations/number of lungs examined (% in brackets)** abattoir A		B		C		total	
Atelectasis*	1/3	(33)	1/4	(25)	1/7	(14)	3/14	(21)
Lobular and lobar pneumonia	73/73	(100)	22/23	(96)	11/16	(69)	106/112	(95)
Pneumonia, total	74/76	(97)	23/27	(85)	12/23	(52)	109/126	(87)
Normal, total	4/22	(18)	1/32	(3)	0/29	(0)	5/83	(6)

* pneumonic lesions histologically.

** there was no significant difference between the threee abattoirs with respect to the presence of mycoplasmas in consolidated lungs (Fischer-Irwin test).

with macroscopic atelectasis. These 14 lungs together with lungs having lobular and lobar consolidations were regarded as pneumonic, making a total of 126 pneumonic lungs (Table 1). None of the histological lesions mentioned were seen in the other 48 atelectatic lungs or in any of the lungs without macroscopic changes, and all these 83 lungs were grouped as normal.

Twenty-one pneumonic lungs were unidentified, the rest represented 68 herds, 45 from A, 6 from B and 17 from C. The normal lungs represented 65 herds, 17 from A, 25 from B and 23 from C. Five normal lungs were unidentified.

Mycoplasmological examination

Fermenting mycoplasmas were recovered from 87 % of the pneumonic lungs (Table 1), representing 55 herds, 43 from A, 5 from B and 7 from C.

Mycoplasmas were isolated from only 6 % of healthy lungs. The mean growth titre from normal lungs with a positive recovery of mycoplasmas was $10^{3.2}$ vs. $\geqq 10^{6.1}$ in pneumonic lungs. There was no significant difference in titre between atelectatic lungs without histological evidence of pneumonia and other normal lungs (Fischer-Irwin test).

The isolated mycoplasmas all grew well in ordinary atmos-

phere producing colonies of the "centreless" type with a granular surface when grown on solid medium. They were all sensitive to digitonin. All strains were identified as M. ovipneumoniae using the g.i. test, the zones varying between 1.5 and 8.5 mm. Broth cultures of 24 isolates tested passed through a 0.45 μm membrane filter. They were not inhibited by M. dispar antiserum, and all were negative in the phosphatase test. The m.i. test performed on 8 of these strains and 9 strains producing small or not unequivocal zones in the g.i. test resulted in titres between 40 and 320. The titre of strain Y98 of M. ovipneumoniae was 640 in the same test. Preimmune sera produced no inhibition.

Bacteriological examination

Bacteria were recovered from 45 % of pneumonic lungs. The number of different bacteria is listed in Table 2. Strains of Pasteurella haemolytica were classified as biotype A and T based on fermentation of trehalose, xylose and salicine and penicillin susceptibility (*Buchanan & Gibbons* 1974). Accordingly 45 strains were classified as biotype A, though 3 strains diverged in the fermentation of 1 carbohydrate. Two strains were classified as biotype T based on the fermentation tests, but were susceptible to penicillin.

Bacteria were recovered from 13 of 83 (16 %) normal lungs. Pleurisy was detected in 8 of these lungs, predominantly as focal, fibrous adhesions. The bacteria isolated were P. haemolytica (4), Pasteurella multocida (1), Corynebacterium pyogenes

Table 2. Occurrence of different bacteria in pneumonic sheep lungs from abbattoirs A, B and C in Southern Norway.

Species	Number of isolations (% in brackets)				
	A	B	C	total	
Pasteurella haemolytica	34	9	4	47	(82)
Pasteurella multocida	2	0	0	2	(4)
Pasteurella-like*	2	0	0	2	(4)
Corynebacterium pyogenes	2	0	1	3	(5)
Other bacteria**	1	0	2	3	(5)
Bacteria, total	41	9	7	57	(100)

* similar to P. multocida according to morphological and cultural properties, but different biochemically.

** Alcaligenes sp., Micrococcus sp., Fusobacterium necrophorum.

(5) and other bacteria (3). Three strains of P. haemolytica were classified as biotype A and 1 as biotype T, though this strain too was susceptible to penicillin.

Relationship between mycoplasmological and bacteriological findings in pneumonic lungs

Table 3 demonstrates the recovery of both mycoplasmas and bacteria in relation to different degrees of macroscopic lesions in pneumonic lungs. The results from each of the 3 abattoirs did not differ from the material as a whole.

Table 3. Occurrence of mycoplasmas and/or bacteria in relation to different degrees of macroscopic lesions in pneumonic sheep lungs from 3 abattoirs in Southern Norway.

Lesions	Number of lungs (% in brackets) with findings of: mycoplasmas and bacteria		mycoplasmas only		bacteria only		with no findings of mycoplasmas or bacteria		total	
Atelectasis*	0	(0)	3	(21)	3	(21)	8	(57)	14	(100)
Lobular pneumonia	9	(20)	29	(66)	0	(0)	6	(14)	44	(100)
Lobar pneumonia	45	(66)	23	(34)	0	(0)	0	(0)	68	(100)
Pneumonia, total	54	(43)	55	(44)	3	(2)	14	(11)	126	(100)
Normal, total	3	(4)	2	(2)	10	(12)	68	(82)	83	(100)

* pneumonic lesions histologically.

Parasitological examination

Macroscopic lesions produced by lungworm migration were seen in a number of lungs, mostly as small (< 1 cm), reddish or grey nodules situated beneath the pleura in the diaphragma lobes as described for Muellerius spp. (*Jubb & Kennedy* 1970). A few lungs had more extensive lesions in the caudal part of the diaphragma lobes indicating Dictyocaulus filaria invasion. Nodules produced by lungworm were seen in 60 % of the lungs from A, in 42 % from B and in 52 % from C. In most cases only 1—3 nodules were found in each lung. There was no significant difference in the occurrence of lesions caused by lungworm between normal and pneumonic lungs (Fischer-Irwin test).

DISCUSSION

M. ovipneumoniae was isolated from 87 % of the total number of pneumonic lungs and from 95 % of lungs with regular consolidations. The organism was found in lungs from 55 of the 68 herds represented in the pneumonic material and in lungs from all 3 abattoirs. These results indicate that M. ovipneumoniae is widely distributed among sheep in Southern Norway. Investigations of this type of lung lesions in slaughtered lambs abroad have given similar results (*Alley et al.* 1975, *Friis et al.* 1976). The hight rate of isolation and the relatively high titre of M. ovipneumoniae in pneumonic lungs as compared with the normal ones indicate that this organism is of etiological significance in subacute or chronic pneumonia, an assumption also made previously (*St. George & Sullivan* 1973, *Jones et al.* 1979, *Alley & Clarke* 1980).

All mycoplasma isolates were identified as M. ovipneumoniae, indicating that other fermenting mycoplasmas rarely or not at all are present in sheep lungs in Southern Norway. This seems in accordance with findings in other countries (*Alley et al.* 1975). One would expect that other mycoplasmas if present would grow in the medium used as this is a complex medium designed for fastidious mycoplasmas as M. suipneumoniae and Mycoplasma flocculare. The isolates possessed the cultural and biochemical characteristics given for the type strain of M. ovipneumoniae (*Leach et al.* 1976, *Cottew* 1979).

P. haemolytica, biotype A, seems to be the bacterium most frequently involved in the pneumonia investigated. This is in accordance with findings from other countries (*Alley* 1975, *Jones et al.* 1978) and results from earlier investigations on sheep pneumonia in Norway (*Mohn & Utklev* 1974, *Andersen & Lutnæs* 1977).

According to Table 3 bacteria were isolated from 45 % of the total number of affected lungs and in 48 % of lungs with lobular and lobar pneumonia, but then always in combination with mycoplasmas. Mycoplasmas, however, were found just as often alone as combined with bacteria. Mixed infection (both mycoplasmas and bacteria) were significantly more frequent in lobar than in lobular consolidations. These findings indicate that mycoplasmas are the primary etiologic organism of the pneumonia in this material, while bacteria are secondary invaders with little significance for the initiation of the disease. The bacteria contribute,

however, to the extension of established lesions. Clues for such an interaction have been found in experimental infection with M. ovipneumoniae and P. haemolytica (*Gilmour et al.* 1979) and in transmission tests with lung homogenate containing M. ovipneumoniae and bacteria with simultaneous suppression of bacterial growth in some of the test lambs (*Alley & Clarke* 1980). However, bacterial superinfection does not seem to be necessary for lesions to include whole lobes of the lung, as mycoplasmas alone were isolated from 34 % of lungs having lobar consolidations. Such extensive lesions are not commonly reported from experimental infection with M. ovipneumoniae (*Sullivan et al.* 1973, *Foggie et al.* 1976, *Alley & Clarke* 1980). The relatively great number of lobar consolidated lungs with growth of mycoplasmas but not bacteria might to some extent be explained by the fact that mycoplasmas have a greater ability to persist in consolidated tissue. The ability of mycoplasmas to persist in lung tissue has been demonstrated in longitudinal studies of experimental infection with M. suipneumoniae (*Whittlestone* 1972).

Distinct atelectatic areas in the apical and cardial lobes are described as a common finding in experimental infection with M. ovipneumoniae (*Sullivan et al.* 1973). However, in the present material most atelectatic lungs (77 %) had no pneumonic lesions histologically and did not differ from lungs without macroscopic lesions concerning microbiological results. Atelectasis probably might have various causes and is an uncertain basis for suspecting pneumonia. Some atelectatic lungs, however, proved to have pneumonic lesions histologically, but the microbiological examination was in many cases (8 of 14 lungs) negative. Nor were microorganisms found in 14 % of the lungs having lobular consolidations. The possibility that other microorganisms than fermenting mycoplasmas and bacteria might be of etiological significance for such small lesions has been pointed out (*St. George & Sullivan* 1973, *Winter & Young* 1975, *Davies et al.* 1976) and will be investigated in later work.

The findings that the occurrence of verminous nodules did not differ between normal and pneumonic lungs indicate that moderate invasion of lungworm is of little significance as a predisposing factor for the pneumonia described.

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SAMMENDRAG

Forekomst av mycoplasmer og bakterier i lunger fra sau i Sør-Norge.

Et utvalg av pneumoniske og normale lunger fra sau, vesentlig lam, fra et større antall besetninger i Sør-Norge, er undersøkt m. h. p. glukoseforgjærende mykoplasmer og bakterier. Mycoplasma ovipneumoniae ble påvist i 87 % av 126 pneumoniske og 6 % av 83 normale lunger. Bakterier, vesentlig Pasteurella haemolytica, ble påvist mindre hyppig i pneumoniske lunger og som regel sammen med M. ovipneumoniae. Det er konkludert med at M. ovipneumoniae er vidt utbredt i den norske sauepopulasjonen, og antas å være av etiologisk betydning ved subakutt eller kronisk pneumoni hos lam, mens bakterier antas vesentlig å opptre som sekundærinfeksjon. Slike bakterielle sekundærinfeksjoner synes å øke utbredelsen av de pneumoniske lesjoner. Forandringer etter moderat invasjon av lungeorm ble påvist i ca. halvparten av lungene, men like hyppig i normale som i pneumoniske lunger, og synes således ikke å disponere for den omtalte pneumoni-type.

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